Geographic patterns of morphological variation in the lizard *Podarcis carbonelli*, a species with fragmented distribution

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Podarcis carbonelli is a lacertid lizard endemic to the western Iberian Peninsula, with a highly fragmented distribution and complex patterns of phylogeographic structure. Here, we investigate intraspecific morphological variability in this species, considering both biometric and pholidotic traits. Our results reveal local patterns of variation in total body size and scalation, but also indicate the existence of gradual, geographically structured morphological variation when size-independent biometry is considered. Total body size is the main factor determining variation across our sample, but this seems to be the result of within-population variability in this trait and is not geographically structured. The southern isolated populations seem highly differentiated in morphological terms, a pattern that also corresponds to singular environmental conditions and distinctive genetic variation, and should therefore be the focus of special attention for future investigation and conservation.

Key words: biometry, scalation, Iberian Peninsula, intraspecific variation

INTRODUCTION

Podarcis carbonelli Pérez-Mellado, 1981 is a lacertid lizard displaying a set of eco- and phylogeographic traits that are unique among the herpetological species of the Iberian Peninsula. Endemic to western Iberia, this species ranges from the Western Central System in Spain and Portugal (Pérez-Mellado, 1981), through the northern coast of Portugal south of the Douro river southwards along the Portuguese coast, where a progressively narrower stripe ends in a line of scattered populations (Sá-Sousa, 1999, 2000, 2001a), and finally, an isolate in Doñana, around the Guadalquivir river mouth in Spain (Sá-Sousa et al., 2001; Harris et al., 2002; Fig. 1). In its ecogeographic affinities, it is associated with Atlantic and sub-Atlantic conditions, with climatic variables such as the number of frost days per year, temperature and precipitation apparently acting as determining factors (Sá-Sousa, 2001a). However, recent studies indicate that such environmental conditions clearly differ among the geographic subranges of the species, when modelled as separate units (Carretero & Sillero, submitted). Based on the peculiar shape of its distribution range, it has been suggested that this species may have previously been more widely distributed and became restricted to its present range due to climatic modifications during the Pleistocene and Holocene (Sá-Sousa, 2001a, 2002). Because of this reduced and fragmented range, *P. carbonelli* was first considered as vulnerable in Portugal (Cabral, 2005) and is now classified as endangered by the IUCN (Sá-Sousa et al., 2008).

First given a separate taxonomic status as a subspecies of *Podarcis bocagei* (Seoane 1884) (Pérez-Mellado, 1981), *P. carbonelli* has been shown to constitute a distinct evolutionary entity, with an independent phylogenetic history (Harris & Sá-Sousa, 2001, 2002; Pinho et al., 2006), and is therefore now treated as a full

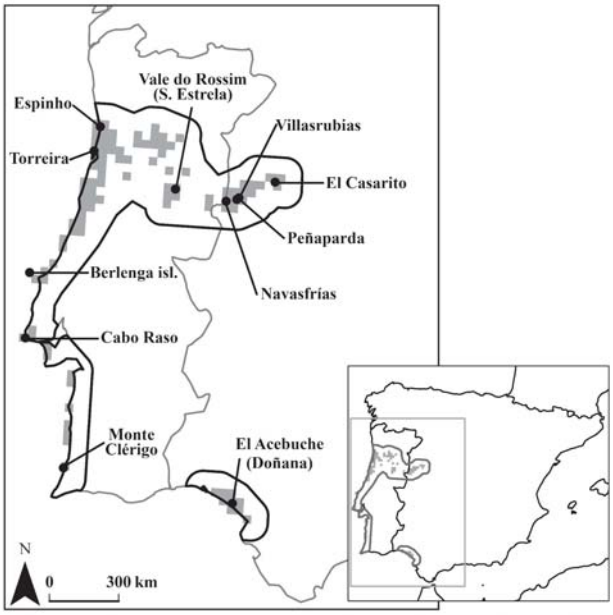

Fig. 1. Map showing the distribution of *P. carbonelli* in 10 x 10 UTM squares (grey), the location of the study populations (see Table 1 for details) and the 20 km buffer used for interpolation of morphological characters. Notice that this buffer does not represent the actual distribution of the species – which is in fact much more restricted – but was used in order to be able to visualize geographical patterns of variation.

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Table 1. Sample localities, geographic coordinates (in WGS1984), altitude in metres, general habitat characterization, sample sizes for males (Nm) and females (Nf) and mean body size as represented by SVL in males and females (SVLm and SVLf).

Locality	Geographic coordinates	Alt.	Habitat	Nm	Nf	SVLm	SVLf
El Casarito	40°31.39'N, 6°8.41'W	1070	Oak forest	8	9	46.67	49.09
Villasrubias	40°20.29'N, 6°38.42'W	850	Oak forest	7	19	51.88	48.71
Peñaparda	40°19.26'N, 6°40.22'W	890	Oak forest	11	15	49.11	52.26
Navasfrías	40°17.69'N, 6°49.29'W	895	Oak forest	7	9	47.64	51.28
Vale do Rossim	40°24.11'N, 7°35.25'W	1392	Mountain shrubland/ granite walls	14	5	54.30	55.11
Espinho	41°1.65'N, 8°38.73'W	0	Coastal dunes	10	10	51.39	46.93
Torreira	40°45.79'N, 8°42.62'W	0	Coastal dunes	10	10	49.92	47.54
Berlenga isl.	39°24.90'N, 9°30.66'W	75	Granite rocks	10	10	57.07	54.23
Cabo Raso	38°42.56'N, 9°29.12'W	0	Human constructions/ coastal dunes	10	10	48.41	46.82
Monte Clérigo	37°20.39'N, 8°51.23'W	0	Human constructions/ coastal dunes	8	11	45.90	48.31
El Acebuche	37°2.87'N, 6°33.94'W	46	Human constructions/ coastal dunes	9	10	47.62	46.96

species (Sá-Sousa & Harris, 2002). In fact, numerous phylogenetic studies have shown that *P. carbonelli* is not directly related to *P. bocagei*, but is instead a sister taxon to the mitochondrial lineage denominated "*P. hispanica* type 2" (Harris & Sá-Sousa, 2002; Pinho et al., 2006). Additional analyses of intraspecific genetic variability have corroborated previous biogeographic hypotheses since mtDNA markers show a shallow but evident phylogeographic differentiation, the Doñana isolate being quite divergent (Pinho et al., 2007). On the other hand, examination of allozyme and microsatellite markers has revealed the existence of local variation, indicating a complex but recent history of fragmentation and high levels of variability remaining in Doñana (Pinho, 2007; Pinho et al., in press).

In contrast to detailed investigations of phylogeographic patterns, little is known of the intraspecific morphological differentiation of *P. carbonelli*. Previous studies have mainly focused on and comprehensively documented the morphological differentiation between this species and *P. bocagei* (Pérez-Mellado, 1981; Pérez-Mellado & Galindo, 1986; Sá-Sousa, 2001b; Harris & Sá-Sousa, 2001; Sá-Sousa & Harris, 2002; Kaliontzopoulou et al., 2005), as a result of the initial taxonomic adscription of both species and their overall morphological resemblance when compared to the remaining Iberian *Podarcis*. In such studies, *P. carbonelli* is only represented by a few populations from a part of the distribution range. Additionally, the isolate of Doñana was only detected in 2001 (Sá-Sousa et al., 2001) and definitely ascribed to *P. carbonelli* in 2002 (Harris et al., 2002), and its morphological properties have never been examined. In contrast, various studies have explored the morphological differentiation of the population of Berlenga island, usually treated as a separate subspecies (*P. c. berlengensis*) (Vicente, 1985; Sá-Sousa et al., 2000; Harris & Sá-Sousa, 2001). Nevertheless, an extensive morphological characterization of different populations of *P. carbonelli* is still lacking.

Here, we examine biometric and pholidotic variation among eleven populations of *P. carbonelli* from across the whole distribution range of the species, in order to 1) investigate patterns of morphological variability, 2) detect characters that contribute to intraspecific morphological patterns and 3) analyse the geographical consistency of such patterns.

MATERIALS AND METHODS

Specimens examined

In order to quantify morphological differentiation in *P. carbonelli* we examined specimens belonging to 11 populations from across the distribution range of the species (Fig. 1, Table 1). Some of the specimens were captured by noose and examined directly in the field, while others came from museum collections (see Acknowledgements for details). Although it has been suggested that preservation may have some influence on morphological analyses (Vervust et al., 2009), preliminary analyses did not indicate a significant effect of this factor in our sample (nested ANOVA design controlling for preservation, $P>0.05$ in all cases); we therefore performed subsequent analyses not taking this factor into account.

Morphological characters quantified

We examined morphological variation taking into account both biometry and scalation. We measured a total of 10 biometric characters to the closest 0.01mm using electronic callipers, including snout–vent length (SVL), trunk length (TRL), head length (HL), head width (HW), head

height (HH), forelimb length (FLL), femur length (FL), tibia length (TBL), hind foot length including the fourth toe and the nail (4TL) and hind limb length (HLL). All measurements were taken by the same person (AK) to minimize measurement error. For scalation we quantified five pholidotic characters including the number of collar scales (CSN), gular scales (GSN), transversal rows of ventral scales (VSN), femoral pores (FPN) and supratemporal scales (StSN). For all bilateral characters, both biometric and pholidotic, we always considered the right side of the body.

Statistical analyses

All variables were log-transformed prior to analyses to ensure normality (Lilliefors test, $P>0.1$) and homogeneity of variances (Levene's test, $P>0.05$). We performed preliminary MANOVA analyses for biometric and pholidotic variables separately and considered the effects of SEX, SITE (capture locality) and their interaction (SEX*SITE) to gain a first impression of patterns of intra- and interpopulational variation. Since most variables showed a significant sexual dimorphism (see Results for details), data from each sex were further analysed separately. We performed principal components analyses (PCA) and canonical variates analyses (CVA) in order to explore patterns of morphological variability in our sample. PCA searches for the direction of higher variability within the sample, by examining individual morphologies and without considering information on population membership. In this way PCA detects those variables responsible for variability at the individual level. In contrast, CVA examines variation among populations compared to the variability within populations and, therefore, detects those variables responsible for population differentiation. By comparing both types of analyses we can examine whether population differentiation works in the same direction as general variation, or if components of variation within populations are different from those among populations. Both analyses were performed for biometric and pholidotic patterns separately, since the two sets of variables were expected to show different patterns.

In order to examine relationships among populations under a multivariate perspective, we first calculated Euclidian distances (ED). When considering biometry, EDs were based on each of the first three principal components separately. When considering scalation, EDs were based on all the first three principal components combined. Only the three first PCs were retained for this analysis, since they were the only ones that each represented more than 5% of the variance in the sample. Additionally, we calculated generalized distances (GD) based on the canonical variates, for biometric and pholidotic traits separately. We then used an unweighted pair group method using arithmetic averages (UPGMA) algorithm of clustering to obtain a phenogram of morphological similarity between populations (Sokal & Rohlf, 1995). To visualize patterns of geographic variation for biometric and pholidotic characters, we performed an interpolation of PCA and CVA scores observed at the sample points and obtained continuous surfaces of variation along the distribution range of the species. For this purpose, we used an inverse distance weighted (IDW) exact algorithm with a power of two. All spatial analyses were performed with the Geostatistical Analyst extension of ArcMap 9.3 (ESRI, 2008).

Finally, in order to evaluate whether biometric or pholidotic variables contributed the most to general and between-sites variation, we performed a PCA and CVA with both sets of variables combined and examined the structure of the first axes constructed by each analysis. All statistical analyses were performed using NTSYSpc 2.21c (Rohlf, 2009).

Table 2. Results of the ANOVAs conducted on biometric and pholidotic variables. df: Degrees of freedom for each effect, *F*: value of the statistic, *P*: corresponding *P*-value. Significant *P*-values (at α=0.05) are marked in italics. See Materials and Methods for variable abbreviations.

		SEX	SITE	SEX*SITE
Biometry*	df	1	10	10
SVL	*F*	0.10	8.62	2.43
	P	0.757	*<0.001*	*0.009*
TRL	*F*	60.93	9.25	2.31
	P	*<0.001*	*<0.001*	*0.014*
HL	*F*	164.26	9.21	1.75
	P	*<0.001*	*<0.001*	0.072
HW	*F*	221.01	16.61	1.19
	P	*<0.001*	*<0.001*	0.297
HH	*F*	165.12	12.79	1.99
	P	*<0.001*	*<0.001*	*0.036*
FLL	*F*	227.49	8.73	1.77
	P	*<0.001*	*<0.001*	0.067
FL	*F*	223.49	14.22	1.90
	P	*<0.001*	*<0.001*	*0.046*
TBL	*F*	195.38	27.31	1.99
	P	*<0.001*	*<0.001*	*0.036*
4TL	*F*	369.22	18.76	1.80
	P	*<0.001*	*<0.001*	0.062
HLL	*F*	333.43	15.39	1.81
	P	*<0.001*	*<0.001*	0.060
Scalation**	df	1	10	10
CSN	*F*	1.88	3.17	0.87
	P	0.173	*0.001*	0.559
GSN	*F*	2.67	5.69	0.76
	P	0.105	*<0.001*	0.666
VSN	*F*	283.51	7.64	1.57
	P	*<0.001*	*<0.001*	0.120
FPN	*F*	49.39	8.09	1.09
	P	*<0.001*	*<0.001*	0.370
StSN	*F*	0.28	5.25	0.86
	P	0.598	*<0.001*	0.569

* Additional df: 1 for slope, 200 for error terms
** Additional df: 1 for slope, 157 for error terms

Table 3. Results of principal components and canonical variates analyses on biometric variables in male and female *P. carbonelli*. PC: principal component, CV: canonical variate, EV: eigenvalue, % exp.: percentage of variation explained, Cum. %: cumulative percentage explained. Only the components that explained >5% of variance in the sample are shown. Variables with the higher correlations with each PC or CV are marked in italics. See Materials and Methods for variable abbreviations.

Males				Correlations with variables									
PC	EV	% exp.	Cum. %	SVL	TRL	HL	HW	HH	FLL	FL	TBL	4TL	HLL
1	0.017	73.116	73.116	0.953	0.865	0.840	0.912	0.898	0.888	0.815	0.689	0.783	0.895
2	0.002	9.215	82.331	0.130	0.108	0.038	*0.270*	0.192	–0.059	0.082	*–0.705*	0.061	*–0.275*
3	0.001	5.986	88.317	0.162	*0.420*	–0.182	–0.068	0.105	–0.110	*–0.424*	0.047	*–0.406*	–0.037
				Correlations with variables									
CV	EV	% exp.	Cum. %	SVL	TRL	HL	HW	HH	FLL	FL	TBL	4TL	HLL
1	4.998	64.327	64.327	0.204	0.079	0.142	*0.404*	*0.333*	0.076	*0.333*	*–0.525*	0.286	–0.183
2	1.386	17.834	82.161	0.545	0.420	0.675	0.646	0.522	0.605	*0.720*	*0.637*	*0.889*	*0.773*
3	0.540	6.952	89.113	0.476	*0.662*	0.163	0.417	*0.652*	0.403	0.172	0.399	0.140	0.314

Females				Correlations with variables									
PC	EV	% exp.	Cum. %	SVL	TRL	HL	HW	HH	FLL	FL	TBL	4TL	HLL
1	0.011	60.533	60.533	0.921	0.859	0.766	0.809	0.881	0.737	0.741	0.612	0.405	0.732
2	0.002	13.214	73.747	0.275	0.283	–0.037	*0.294*	0.125	0.026	–0.288	*–0.721*	0.012	*–0.446*
3	0.002	10.937	84.684	0.113	*0.379*	–0.284	–0.329	0.023	–0.216	*–0.408*	0.298	*–0.691*	–0.274
				Correlations with variables									
CV	EV	% exp.	Cum. %	SVL	TRL	HL	HW	HH	FLL	FL	TBL	4TL	HLL
1	3.742	45.950	45.950	0.235	*0.415*	0.087	–0.091	*0.318*	0.034	0.152	*0.823*	*–0.439*	0.290
2	1.935	23.762	69.712	0.215	0.038	*0.636*	0.509	0.426	0.551	*0.721*	0.446	0.556	*0.805*
3	1.180	14.494	84.206	*0.746*	*0.751*	0.533	*0.763*	0.603	0.469	0.340	0.010	0.034	0.043

RESULTS

MANOVA analyses showed that there were significant effects of SEX, SITE and their interaction for biometric variables (SEX: F=162.6, df=10, P<0.01; SITE: F=8.5, df=100, P<0.01; SEX*SITE: F=1.6, df=100, P<0.01). For pholidotic variables the effects of SEX and SITE were also significant (SEX: F=80.33, df=5, P<0.01; SITE: F=5.47, df=50, P<0.01), but this was not the case for the interaction term (SEX*SITE: F=1.03, df=50, P=0.42). Univariate ANOVA comparisons showed that sexual variation is significant for all biometric variables except SVL (Tables 1 and 2). Males are bigger than females for all body parts (head, limbs), except for trunk length, which is longer in females. All biometric variables show significant between-site variation (Table 2). As far as pholidotic variables were concerned, the effect of SEX was only significant for VSN and FPN, while the effect of SITE was always significant (Table 2).

Patterns of biometric variation

The results obtained from PCA and CVA on biometric variables for each sex in *P. carbonelli* are shown in Table 3. PCA results are consistent between the sexes: the first principal component presents correlations of the same sign and magnitude for all biometric variables, thus giving a multivariate representation of size variation (Burnaby, 1966). The second principal component represents variation in TBL, HLL and HW, while the third correlates with 4TL, FL and TRL. Variation among populations as represented by canonical variates is consistent but not completely coincident with general variation in the sample as revealed by PCA. Again, results are consistent between sexes in terms of variable importance: the first three canonical variates obtained summarize information related to TBL, HH, HW, TRL, FL and 4TL (Table 3).

As far as major patterns of biometric variation among populations are concerned, these are visibly affected by size. The UPGMA clustering based on ED obtained from the first PC scores shows a high differentiation of the populations of Berlenga island and Serra da Estrela and a lack of geographical structuring for the remaining populations in both sexes (Fig. 2A, C). However, when examining the second principal component, which is "size-free" (since it is orthogonal to the first, which represents multivariate size), an evident geographical structure emerges, the two main clusters representing northern and southern subranges (Fig. 2E, G). The UPGMA clustering of GD obtained from CVA also shows an evident geographic structure of biometric variation. In this case, both sexes show two differentiated groups corresponding to northern and southern subranges. The population of Serra da Estrela is the only exception, grouping with the southern populations and specifically showing a high similarity to the population of Berlenga island, as in PCA-retrieved results (Fig. 3D, H).

The analysis of geographic variation in biometric characters using IDW interpolation gives more insights into

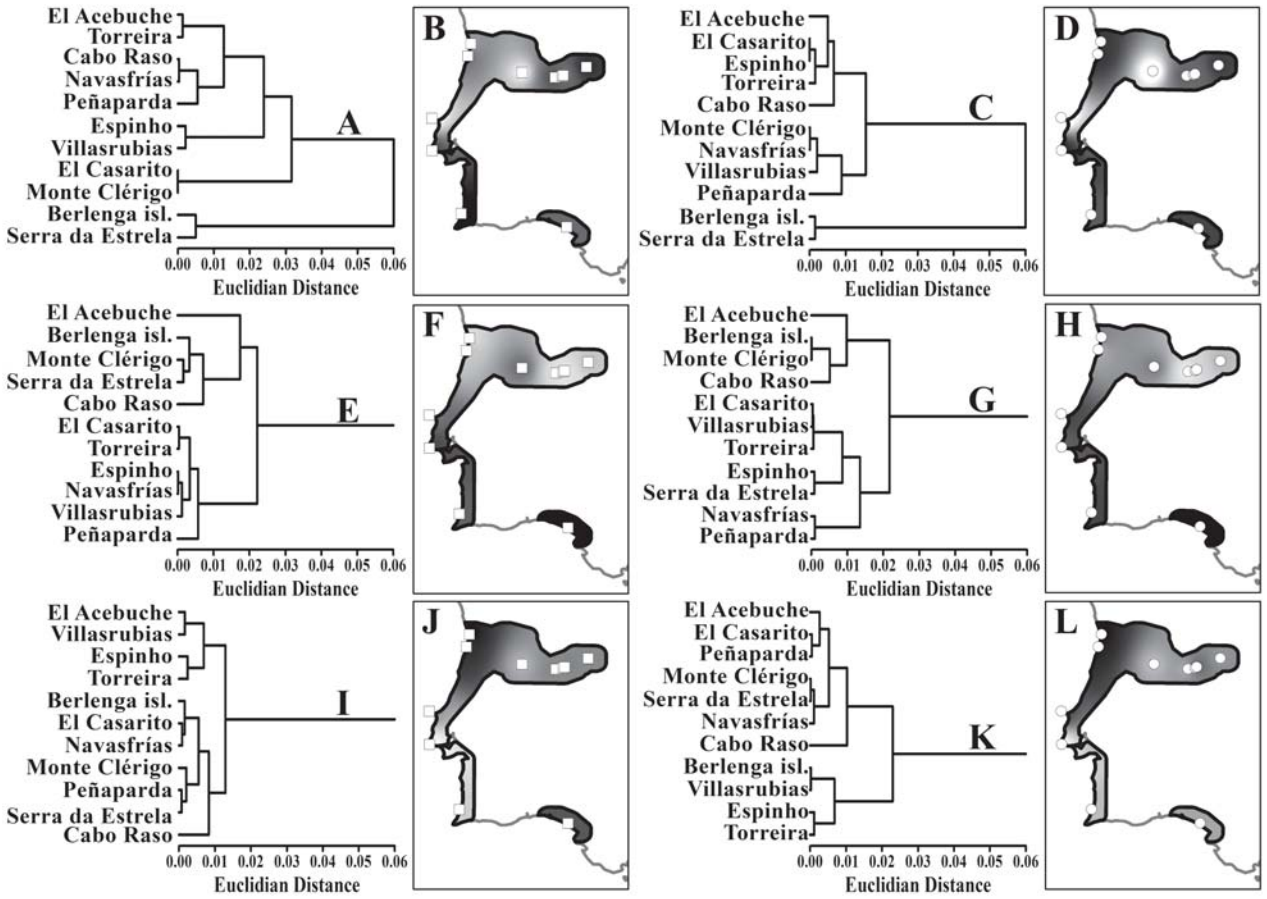

Fig. 2. UPGMA phenograms and IDW interpolation maps produced for the first three principal components of biometric variation for male (left) and female (right) *P. carbonelli*. A–D: first principal component, E–H: second principal component, I–L: third principal component. In interpolation maps, lighter colours represent higher values and darker colours, lower values.

the specific patterns of differentiation among the populations examined. The examination of geographic variation in the first PC (Fig. 2B, D), representing multivariate body size, corresponds to an important local variation in the populations of Berlenga island and Serra da Estrela, which show higher PC values (and therefore a bigger body size) compared to the remaining populations. The second PC (Fig. 2F, H), presents a north–south, counterclockwise developing gradient, with extreme (minimum) values in the population of El Acebuche (Doñana), which is therefore highly differentiated from the rest by lower HW and higher TBL and HLL values. Finally, the third PC also shows a north–south gradient (Fig. 2J, L), but with important local variation, especially related to the populations of Espinho and Torreira. The image is simpler when examining geographical patterns of CVA retrieved results (Fig. 3A–C and 3E–G). The first CV presents north–south gradients of variation in both sexes, resulting in a visibly extreme differentiation of the population of El Acebuche (Doñana). A surprising and consistent pattern emerges once again concerning the population of Serra da Estrela, which repeatedly appears as an outlier within the northern subrange and shows results more similar to southern populations.

Patterns of pholidotic variation

The results obtained from PCA and CVA on pholidotic variables of each sex of *P. carbonelli* are shown in Table 4. As for biometric characters, PCA reveals main sources of variability consistent between sexes: the first PC correlates with GSN and FPN, the second with VSN and StSN and the third with CSN. CVA results are also consistent between sexes, at least for the first two canonical variates. The first CV summarizes information about GSN, VSN and FPN; the second correlates to VSN, FPN and StSN; the third shows different structures between sexes, relating to CSN, FPN and StSN in males and to CSN, GSN and VSN in females (Table 4).

Contrasting with the evident geographic variation observed in biometric characters, the results of the UPGMA clustering of pholidotic variables do not reveal any geographical structure, either for PCA (Fig. 4D, H) or for CVA (Fig. 5D, H) retrieved distances (ED and GD respectively). IDW interpolations of PC and CV scores do not reveal the existence of gradual variation, but rather highlight local variation, concordant between sexes. Both the first PC (Fig. 4A, E) and the first CV (Fig. 5A, E) emphasize the differentiation of the population of El Acebuche (Doñana);

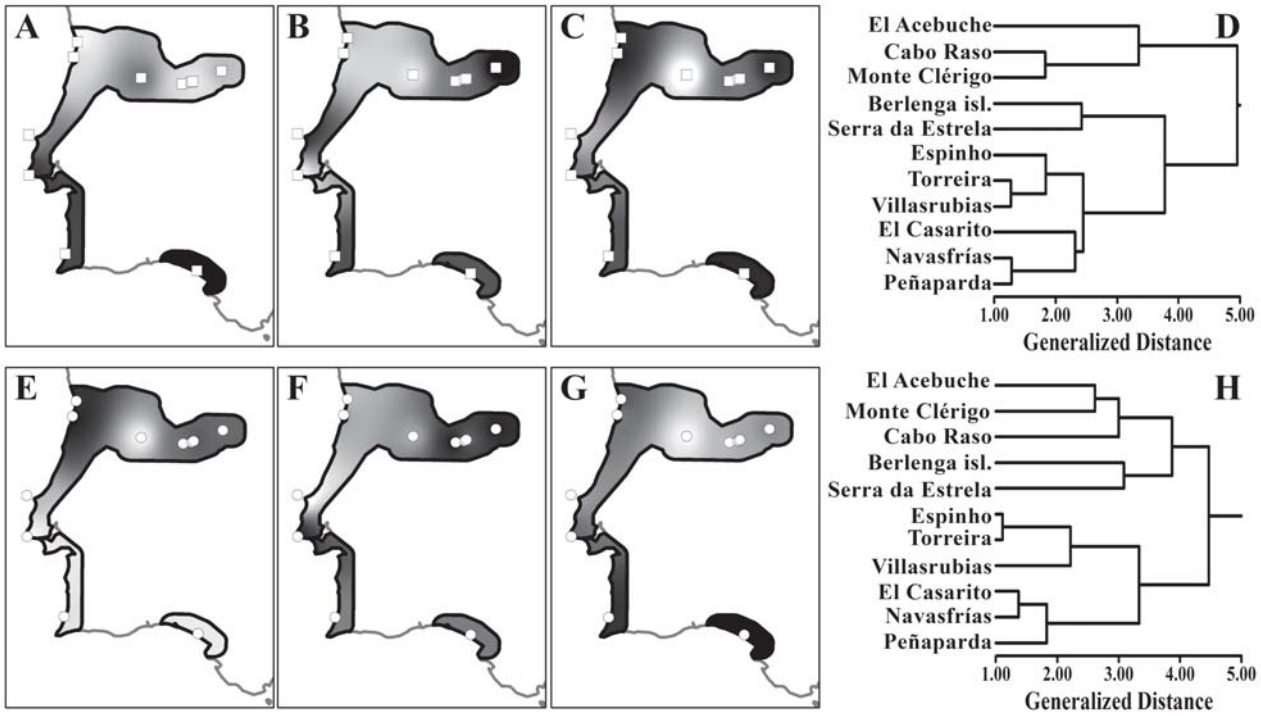

Fig. 3. IDW interpolation maps produced for the first three canonical variates of biometric variation and UPGMA phenograms based on the corresponding generalized distances between populations for male (top) and female (bottom) *P. carbonelli*. A, E: first canonical variate, B, F: second canonical variate, C, G: third canonical variate. In interpolation maps, lighter colours represent higher values and darker colours, lower values.

the second PC (Fig. 4B, F) highlights the Espinho and Torreira populations; the third PC (Fig. 4C, G) and the second and third CVs (Fig. 5B, F and Fig. 5C, G respectively) represent the differentiation of the Monte Clérigo population.

Contrasting biometric and pholidotic characters

The PCA conducted on all variables combined revealed that biometric and particularly size variation are the main source of variability in both sexes, as revealed by high correlations of the first PC with these variables in both sexes (Table 5). On the other hand, differentiation between populations as analysed through a combined CVA is also under the influence of certain biometric variables (namely TBL, TRL and 4TL in females and TBL in males), but is additionally related to variation in pholidotic characters, namely StSN in females and FPN in males (Table 5).

DISCUSSION

The analysis of biometric and pholidotic variability among 11 populations of *P. carbonelli* revealed important morphological differentiation across the distribution range of the species. Patterns were generally consistent between both sexes, suggesting a population-level component, but biometric and pholidotic characters revealed different levels of structure in their variability. While size-free components of biometric variation show a gradual north–south and counterclockwise emerging geographical structure, multivariate body size and scalation represent rather local components of variation.

The contrast between biometry and scalation highlights an interesting difference of scale in the variation of both character sets at the intraspecific level. When these are compared in terms of their contribution to the general morphological variance (PCA), biometric variation is certainly more prominent. On the other hand, both kinds of trait are important concerning their relevance for population-level differentiation through a combined CVA (Table 5). This discordance deserves particular attention. While biometric characters – and particularly body size – are highly variable across the whole sample, interpopulational differentiation rather involves the modification of specific body parts and pholidotic traits, namely trunk, tibia and foot length and supratemporalia in females, and tibia length and femoralia in males. In fact, scalation characters have been extensively used in the taxonomy of the genus *Podarcis* (Arnold, 1973; Pérez-

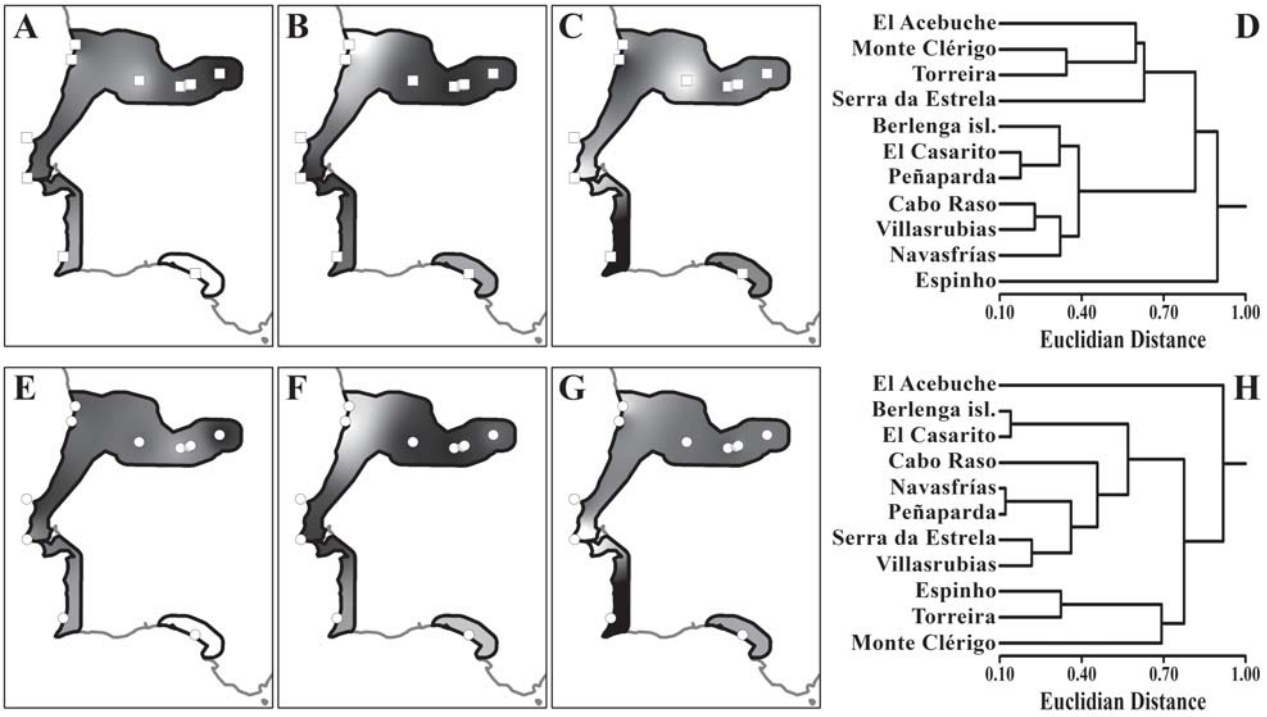

Fig. 4. IDW interpolation maps produced for the first three principal components of pholidotic variation and UPGMA phenograms based on the corresponding Euclidian distances between populations for male (top) and female (bottom) *P. carbonelli*. A, E: first principal component, B, F: second principal component, C, G: third principal component. In interpolation maps, lighter colours represent higher values and darker colours, lower values.

Mellado, 1998; Geniez et al., 2007) and of lacertids in general (Arnold, 1989; Arnold & Ovenden, 2002), while biometric ones have relatively lower value in this context, with the exception of broad body-size differentiation (Sá-Sousa & Harris, 2002).

The examination of emerging geographical patterns in biometric variation reveals an important influence of body size, with a rather local character. The trend to gigantism, common in the Berlenga and Serra da Estrela populations (Fig. 2A–D), is striking since it cannot be attributed to any evident common causal factor. On one hand, bigger body size in the Berlenga population is consistent with an insularity effect (Meiri, 2007, 2008) and has been evidenced before (Sá-Sousa et al., 2000). On the other hand, bigger body size in Serra da Estrela could be related to adaptation to a higher altitude (Bergmann, 1847, but see Ashton & Feldman, 2003). However, such a hypothesis cannot be confirmed at present due to the uniqueness of this locality in terms of altitude. When size variation is put aside, the second most important direction of general biometric variation is geographically structured, as is evident from both the interpolation maps and the cluster analysis on the second principal component (Fig. 2E–H). The same pattern emerges when differentiation at the population level is examined (Fig. 3). In both cases, variation is related to longer hind limbs or hind limb parts (See Table 3) and may therefore be a result of latitude and temperature effects (Allen's rule: Ray, 1960; Serrat et al., 2008), but such a hypothesis cannot be tested at present.

Although a detailed analysis of possible underlying causation factors is beyond the scope of this study, it is interesting to examine our results in the light of the genetic background and the geographical distribution of the populations in question. Detailed analyses of phylogeographic structure in *P. carbonelli* indicate a clear, though shallow, geographical substructure in terms of mitochondrial DNA (Pinho et al., 2007), but rather local patterns when allozyme and microsatellite markers are considered (Pinho, 2007; Pinho et al., in press). This indicates that genetic and morphological patterns are mostly decoupled, since the mtDNA groupings described in Pinho et al. (2007) are not recovered based on morphological data. In some cases (i.e. Berlenga, Serra da Estrela) morphological differentiation without genetic correspondence is observed. In others, however, morphological distinctiveness seems to parallel genetic differentiation. The Doñana population is highly differentiated in terms of biometric (Fig. 2E–H, Fig. 3A, E), pholidotic (Fig. 4A, D, H, Fig. 5A, C, E, G) and coloration (Sá-Sousa et al., 2001; pers. obs. by the authors) traits and is also quite unique genetically, being characterized by private mtDNA haplotypes (Fig. 2 in Pinho et al., 2007). Similarly, the population of Monte Clérigo is an important source of pholidotic variation within *P. carbonelli* (Fig. 4C, G, Fig. 5B, C, F, G, H), which is also true for allozymes and microsatellites, since low genetic variability in this population has been associated with low population size and/or a possible bottleneck effect (Pinho, 2007; Pinho et al., submitted). It should also be noted that these two populations, but especially Doñana, are currently isolated from the others (Fig. 1, Sá-Sousa et al., 2008), a fact that may have contributed to their differentiation. Addition-

Table 4. Results of principal components and canonical variates analyses on pholidotic variables in male and female *P. carbonelli*. PC: principal component, CV: canonical variate, EV: eigenvalue, % exp.: percentage of variation explained, Cum. %: cumulative percentage explained. Only the components that explained >5% of variance in the sample are shown. Variables with the higher correlations with each PC or CR are marked in italics. See Materials and Methods for variable abbreviations.

Males				Correlations with variables				
PC	EV	% exp.	Cum. %	CSN	GSN	VSN	FPN	StSN
1	1.583	31.667	31.667	0.405	*0.748*	–0.149	*0.740*	0.566
2	1.162	23.245	54.912	0.196	0.218	*–0.852*	–0.039	*–0.593*
3	0.954	19.079	73.990	*0.879*	–0.036	0.205	–0.401	0.015
4	0.671	13.423	87.414	0.034	*–0.391*	*–0.472*	–0.106	*0.537*
5	0.629	12.586	100.000	0.210	*–0.523*	0.054	*0.583*	–0.235
				Correlations with variables				
CV	EV	% exp.	Cum. %	CSN	GSN	VSN	FPN	StSN
1	1.310	59.117	59.117	0.230	*0.593*	*–0.677*	*0.724*	0.097
2	0.491	22.163	81.280	0.045	–0.141	*0.448*	*0.552*	*0.786*
3	0.296	13.381	94.661	*0.638*	0.197	–0.181	*–0.462*	*0.466*
Females				Correlations with variables				
PC	EV	% exp.	Cum. %	CSN	GSN	VSN	FPN	StSN
1	1.513	30.251	30.251	0.422	*0.639*	0.395	*0.744*	0.498
2	1.201	24.021	54.271	0.018	0.530	*–0.678*	0.248	*–0.639*
3	0.955	19.107	73.378	*0.905*	–0.014	0.010	–0.351	–0.156
4	0.772	15.448	88.826	0.143	*–0.265*	*–0.634*	0.138	*0.520*
5	0.559	11.174	100.000	0.125	*–0.521*	0.073	*0.507*	–0.316
				Correlations with variables				
CV	EV	% exp.	Cum. %	CSN	GSN	VSN	FPN	StSN
1	1.053	45.816	45.816	0.203	*0.684*	*–0.578*	*0.625*	–0.164
2	0.603	26.237	72.053	–0.238	–0.076	*0.376*	*0.696*	*0.693*
3	0.349	15.186	87.238	*0.703*	*0.480*	*0.651*	0.103	0.171
4	0.211	9.201	96.440	*0.517*	–0.247	–0.355	–0.244	*0.578*

ally, the population size of Monte Clérigo is notably small, as has been observed during visits to the site in five consecutive years (pers. obs. by the authors).

Taken together, our results indicate that *P. carbonelli* is a species with signs of both gradual morphological variation with a geographical structure and local, population-level differentiation. This, together with the species' peculiar distribution, the multiple ecological optima (Carretero & Sillero, submitted) and its complex, but to some extent geographically structured, genetic variation (Pinho, 2007; Pinho et al., 2007, in press) highlight the necessity for particular attention in terms of both future investigation and conservation. In this context, the highly isolated populations of Doñana, and probably Monte Clérigo, display distinctive genetic and morphological traits which may make them not interchangeable (*sensu* Crandall et al., 2000) in evolutionary and ecological terms, suggesting that they should be considered separate evolutionarily significant units (ESUs) for conservation purposes.

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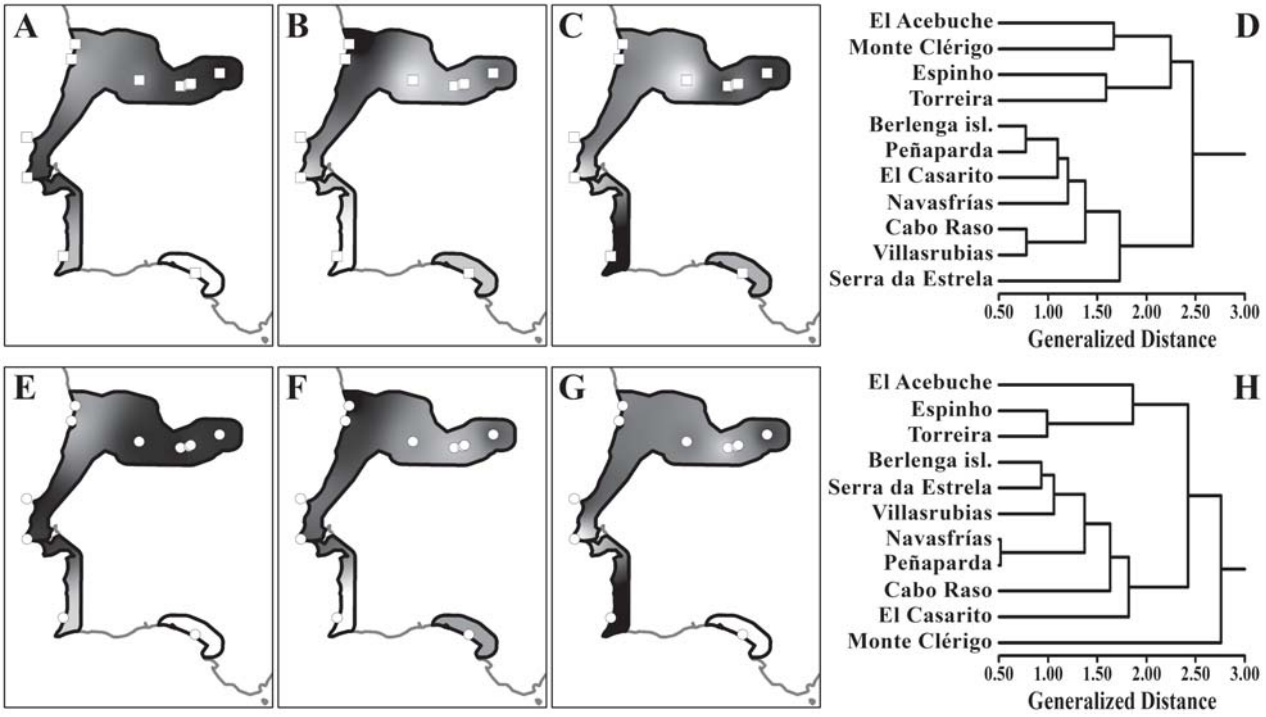

Fig. 5. IDW interpolation maps produced for the first three canonical variates of pholidotic variation and UPGMA phenograms based on the corresponding generalized distances between populations for male (top) and female (bottom) *P. carbonelli*. A, E: first canonical variate, B, F: second canonical variate, C, G: third canonical variate. In interpolation maps, lighter colours represent higher values and darker colours, lower values.

Table 5. Eigenvalues (EV), percentage of variance explained (% exp.) and correlations with original variables for the first principal component (PC1) and first canonical variate (CV1) of analyses conducted with biometric and pholidotic variables combined. Variables with the higher correlations with each PC or CR are marked in italics. See Materials and Methods for variable abbreviations.

	Females		Males	
	PC1	CV1	PC1	CV1
EV	6.044	3.896	7.445	5.329
% exp.	40.293	37.243	49.635	53.547
SVL	*0.889*	0.282	*0.949*	0.194
TRL	*0.775*	*0.460*	*0.851*	0.078
HL	*0.818*	0.115	*0.850*	0.115
HW	*0.841*	–0.050	*0.915*	0.384
HH	*0.842*	0.361	*0.883*	0.320
FLL	*0.788*	0.058	*0.904*	0.055
FL	*0.785*	0.176	*0.828*	0.307
TBL	*0.563*	*0.819*	*0.682*	*–0.537*
4TL	*0.513*	*–0.419*	*0.804*	0.260
HLL	*0.786*	0.296	*0.904*	–0.204
CSN	0.094	0.037	0.159	–0.246
GSN	–0.033	–0.133	0.059	–0.210
VSN	0.025	0.119	–0.049	0.208
FPN	0.080	0.088	0.091	*–0.470*
StSN	–0.100	*0.437*	0.053	–0.345

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