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Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex

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Cryptic species complexes represent groups that have been classified as a single species, because of the difficulty in distinguishing its members morphologically. Morphological investigation following the discovery of cryptic diversity is crucial for describing and conserving biodiversity. Here we present a detailed account of morphological variation in a group of Iberian and North African *Podarcis* wall lizards of the family Lacertidae, trying to elucidate the morphological patterns observed between known mitochondrial lineages. Our results reveal very high morphological variation within lineages, considering both biometric and pholidotic traits, but also indicate that lineages are significantly different from each other. The main sources of variation, both globally and between lineages, arise from body size, head dimensions, and limb length, possibly pointing to underlying ecological mechanisms. A combination of body size, body shape, and continuous pholidotic traits allows a relatively good discrimination between groups, especially when comparing one group with the rest or pairs of groups. However, ranges of variation greatly overlap between groups, thereby not allowing the establishment of diagnostic traits. The high morphological variation observed indicates that external morphology is not particularly useful for species delimitation in this group of lizards, as local adaptation seems to play a major role in within- and between-group differentiation.

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INTRODUCTION

Cryptic species are a very interesting puzzle for systematists and evolutionary biologists, as they represent cases in which distinct species are very difficult – or even impossible – to distinguish morphologically and have consequently been classified as a single species (Beheregaray & Caccone, 2007; Bickford *et al*., 2007). The above definition inevitably leads to the question of how species are defined and delimited. This question precedes Darwin (Hey, 2006) and has caused extensive debate in recent years, leading to the main conclusion that the problem is not one of species concept – as most biologists share a common view of evolutionary lineage, related to the philosophical definition of a species – but rather of the tools and criteria used for species delimitation (de

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Queiroz, 2005; Hey, 2006). A review of the different 'species concepts' reveals that each of the available species definitions includes – implicitly or explicitly – a methodology that should be followed for species delimitation (de Queiroz, 1998). And in a sense it is precisely the development of new tools and criteria for species delimitation that open the way to the discovery and investigation of cryptic species. For centuries systematics (the discipline that aims at studying diversity and at determining phylogenetic relationships) has worked on the basis of morphological characters (Wiens, 2007). The development of molecular phylogenetics, and mitochondrial DNA (mtDNA) phylogenies in particular, changed the way biologists view and explore organismal diversity and evolution (Avise, 1986; Avise & Wollenberg, 1997).

Cryptic species are at the centre of this conceptual shift. The number of cryptic species reported, and of studies on cryptic species and/or species complexes, has dramatically increased after the introduction of molecular methods (Bickford *et al*., 2007). The use of molecular phylogenetics for investigating evolutionary relationships between organisms has often revealed high levels of cryptic diversity in a very wide variety of organisms, previously classified as single species using morphological criteria. Although posterior examination of morphological diversity in a molecularly informed framework has in many cases revealed the existence of corresponding morphological differentiation, the existence of 'true' cryptic diversity, where sister species cannot be identified using morphological traits, is also very frequent. This observation leads to two important conclusions: that morphological diversification does not always accompany speciation and that the human sensory machine is not always primed for species recognition (Fritz *et al*., 2006). Independent evidence on the evolutionary relationships between organisms provided by molecular phylogenetics put the basis for extensive research on morphological evolution, which has revealed that morphological change frequently emerges without reproductive isolation or a genetic basis in general (for example in the form of phenotypic plasticity or local adaptation; i.e. DeWitt & Scheiner, 2004). On the other hand, comparative phylogenetic methods have allowed biologists to explore the relationship between speciation and morphological divergence, revealing that species diversification is not always coupled to morphological evolution (Bickford *et al*., 2007; Adams *et al*., 2009; but see Ricklefs, 2004). Simultaneously, the discovery of phylogenetic variation within groups that were morphologically classified as a single species, has led to the realization that human perception is probably not sufficiently sensitive to capture natural complexity (Beheregaray & Caccone, 2007). Nevertheless, species are still described based on one or several quantitative characters that do not overlap with other species (Wiens, 2007), and after the discovery of cryptic phylogenetic variation morphological evidence should be put together to enhance species delimitation (Schlick-Steiner *et al*., 2007), and definitively test whether the suggested cryptic species can be distinguished on the basis of morphological characters (Sáez & Lozano, 2005).

Reptiles are no exception to the increasing discovery of cryptic species. Similarly to what has happened in other animal groups, reports of cryptic diversity in reptiles have increased in recent years (Uetz, 2009). This encompasses a wide variety of different groups, including for example worm lizards (Albert & Fernández, 2009), geckos (Harris *et al*., 2003; Oliver *et al*., 2007), chameleons (Raxworthy *et al*., 2003), slow worms (Gvoždík *et al*., 2010), skinks (Greaves *et al*., 2007), *Liolaemus* lizards (Morando *et al*., 2007), and colubrid snakes (Rodríguez-Robles & De Jesús-Escobar, 2000). Moreover, several reptile groups have been used as model systems for developing new tools and approaches for species delimitation, and for exploring relationships between phylogenetic and morphological variation (see for example Puorto *et al*., 2001; Wiens & Penkrot, 2002; Morando, Avila & Sites, 2003; Carretero *et al*., 2005; Raxworthy *et al*., 2007). Among reptile groups, the lizards of the family Lacertidae represent an intriguing case: the family includes about 300 recognized species distributed throughout Africa and most of Eurasia, and its generic systematics have suffered numerous revisions until very recently (Harris, Arnold & Thomas, 1998; Arnold, Arribas & Carranza, 2007). Interestingly, among lacertids there are numerous genera with very complex patterns of phylogenetic and morphological variation, which result to a high number of cryptic species complexes, including for instance *Acanthodactylus* (with at least eight species groups, Salvador, 1982; Arnold, 1983; Harris & Arnold, 2000), *Mesalina* (Arnold, 1986; Kapli *et al*., 2008), *Darevskia* (Fu, Murphy & Darevsky, 1997), *Iberolacerta* (Mayer & Arribas, 2003; Carranza, Arnold & Amat, 2004; Crochet *et al*., 2004), and *Podarcis* (Harris & Sá-Sousa, 2002; Poulakakis *et al*., 2003, 2005; Harris *et al*., 2005), to mention a few characteristic examples.

Podarcis wall lizards from the Iberian Peninsula and North Africa represent a characteristic case of a cryptic species complex. Long recognized as a monophyletic clade (Harris & Arnold, 1999), this group of lizards could probably be considered a cryptic species complex even before the description of its phylogenetic structure (Harris & Sá-Sousa, 2002). Indicative of this is its long history of taxonomic revisions and instability at the specific and subspecific level

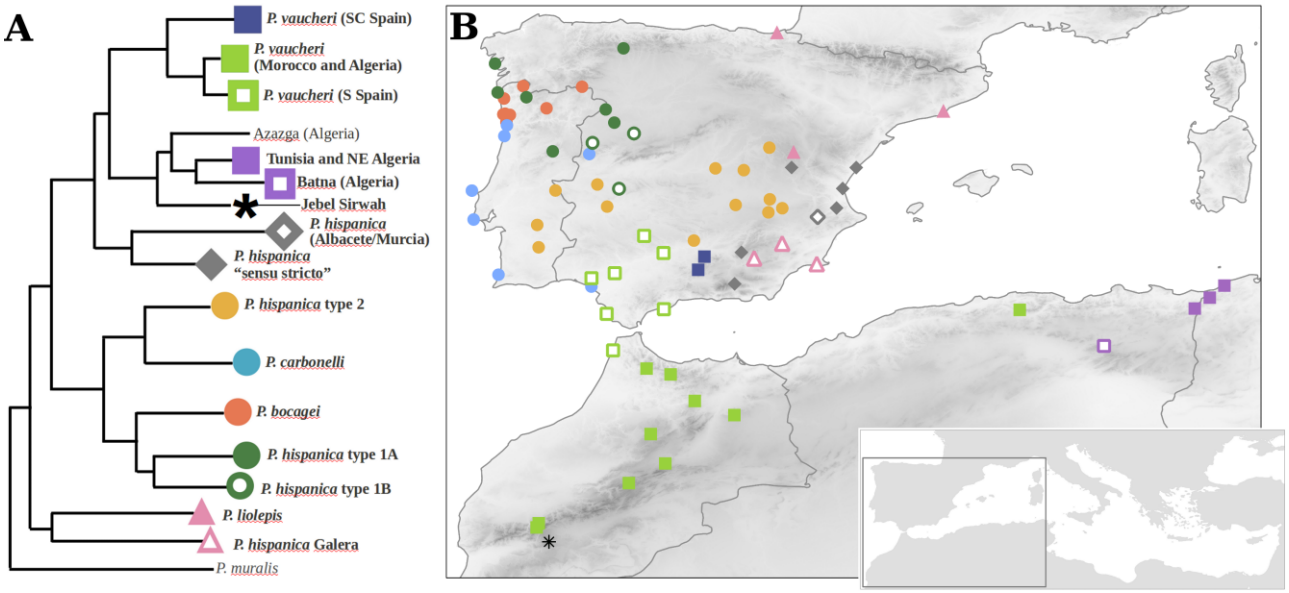

Figure 1. Mitochondrial DNA lineages sampled, maximum likelihood tree of phylogenetic relationships between them (A, modified from Kaliontzopoulou *et al*., 2011), and map of the localities from which the samples analysed morphologically were obtained (B).

(Pérez-Mellado, 1998; Carretero, 2008), which persists (Geniez *et al*., 2007). The group, including all (non-introduced) *Podarcis* wall lizards from the continental Iberian Peninsula and North Africa, except for *Podarcis muralis* (Laurenti, 1768), was formally described as a species complex by Harris & Sá-Sousa (2002), who were the first to put together phylogenetic evidence that *Podarcis hispanica* (Steindachner, 1870) was paraphyletic in relation to *Podarcis bocagei* (Seoane, 1884) and *Podarcis carbonelli* Pérez-Mellado, 1981. Since then, extensive research using molecular techniques both for phylogenetic inference and phylogeographic analyses have corroborated this observation, providing a robust mtDNA phylogeny for the group (Pinho, Ferrand & Harris, 2006). Furthermore, the mtDNA groups examined are confirmed by allozyme data (Pinho, Harris & Ferrand, 2007), but nuclear markers fail to recover the units supported by mtDNA and allozymes, although such a pattern is probably the result of incomplete lineage sorting, rather than extensive gene flow between different forms (Pinho, Harris & Ferrand, 2008). On the other hand, although numerous authors have studied morphological variation within this group of lizards, such studies usually focused on certain members of the group or parts of its distribution (Gosá, 1985; Galán, 1986; Harris & Sá-Sousa, 2001; Sá-Sousa, Vicente & Crespo, 2002; Busack, Lawson & Arjo, 2005; Renoult *et al*., 2009). The two studies available that studied morphological variation in the entire Iberian Peninsula (Geniez *et al*., 2007) or the Iberian Peninsula and North Africa (Pérez-Mellado & Galindo-Villardón, 1986), suffered in terms of operational taxonomic unit (OTU) definition, as in both cases OTUs were defined on the basis of habitat or general range, instead of using some independent criterion for OTU delimitation (Carretero, 2008).

Here we provide a detailed account of patterns of morphological variation in the Iberian and North African group of *Podarcis*, considering both body size and shape, as well as pholidosis. We specifically focus on mtDNA lineages, using the independent evidence provided by phylogenetic studies to define groups for comparisons, and use an extensive sampling scheme to capture morphological variability from the population level upwards. Although the evolutionary history of this group is not yet fully understood and mtDNA lineages may not fully coincide with evolutionary lineages (see for example Renoult *et al*., 2009), mtDNA lineages provide an independent criterion for group assignment, and allows us to partially test the evolutionary significance of these groups. We include genetically identified populations of 15 out of the 16 distinct mitochondrial lineages presently identified in the Iberian Peninsula and in the North of Africa (Fig. 1A; Kaliontzopoulou *et al*., 2011) to answer the following questions.

1. Can mtDNA lineages be effectively distinguished from each other based on morphological traits?
2. Which morphological traits contribute the most to lineage differentiation?

3. Can we detect diagnostic traits for each lineage that are useful for species delimitation?

With this extensive investigation of morphological variation across this cryptic species complex, using uniform methods for data acquisition and treatment, we intend to increase the existing knowledge on the morphology of these lizards and shed light on the morphological properties of these evolutionary entities.

MATERIAL AND METHODS

Examined material

In order to investigate morphological variability patterns in the *P. hispanica* species complex, we examined a total of 1291 adult males and 1162 adult females from 75 different localities (Appendix S1). To effectively capture morphological variation between different mtDNA forms, while at the same time including information on the population variation of each form, we sampled as many different localities as possible and tried to examine at least ten adult males and females from each. Although distribution patterns and population densities did not always allow us to fulfil this objective, in most cases we managed to obtain at least five individuals of each sex per locality (Appendix S1; Table 1), covering 15 of the 16 presently known mitochondrial lineages of the group, and spreading throughout the known distribution range of each of them (Fig. 1). Prior to morphological analyses, at least two individuals from each population were genetically analysed in order to independently assign them to one of the known mitochondrial lineages, using diagnostic mtDNA fragments (Kaliontzopoulou *et al.*, 2011). The vast majority of populations was sampled directly by fieldwork, examined in the field, and released back to the locality of capture. In some cases (five populations out of 75), specimens from museum collections were included in order to complete the sampling of certain lineages. Exploratory analyses taking into account the effect of specimen origin (fieldwork versus museums) did not indicate a significant effect for this factor. Further analyses were therefore conducted on all specimens together.

Characters recorded

We examined a total of 12 linear biometric, seven continuous pholidotic, and ten categorical pholidotic characters. Biometric variables were recorded using electronic callipers to the nearest 0.01 mm, always by the same person (AK), and included: HL, head length;

Table 1. Number of populations sampled from each mitochondrial (mt)DNA lineage and corresponding sample size obtained for females (N_f) and males (N_m)

mtDNA lineage	Populations	N_f	N_m	Code
Podarcis vaucheri SC Spain	2	20	17	PVSCSp
Podarcis vaucheri Morocco and Algeria	10	194	214	PVMA
Podarcis vaucheri S Spain	7	96	99	PVSSp
Podarcis hispanica Tunisia and NE Algeria	3	36	46	PHTA
Podarcis hispanica Batna	1	17	20	PHBat
Podarcis hispanica Jebel Sirwah	1	20	20	PHJS
Podarcis hispanica Albacete/Murcia	1	10	10	PHAM
Podarcis hispanica s.s.	6	72	73	PHSS
Podarcis hispanica type 2	13	132	125	PH2
Podarcis carbonelli	8	147	180	PC
Podarcis bocagei	11	229	288	PB
Podarcis hispanica type 1A	7	98	101	PH1A
Podarcis hispanica type 1B	3	27	30	PH1B
Podarcis liolepis	3	48	47	PL
Podarcis hispanica Galera	3	16	21	PHGal

Code: the abbreviation used for group annotation. The names of the lineages are after Kaliontzopoulou *et al.* (2011). See Figure 1 and Appendix S1 for a detailed account of the populations sampled.

PL, pileus length; HW, head width; HH, head height; ESD, eye–snout distance; MO, mouth opening; TRL, trunk length; FLL, forelimb length; FL, femur length; TBL, tibia length; 4TL, hindfoot length; and HLL, hindlimb length (for a detailed description of the way in which measurements were taken, see Kaliontzopoulou, Carretero & Llorente, 2007: fig. 2). Continuous pholidotic characters included: CSN, colaria; FPN, femoral pores; GSN, gularia; SCGN, supraciliary granules; SDLN, subdigital lamellae under the fourth toe; STSN, supratemporal scales; VSN, number of transversal raws of ventral scales. Categorical pholidotic characters and recorded states included: IN_F, contact between the internasal and frontal scales (0, no; 1, yes); 3rdIN_F, presence of a third scale between the internasal and frontal scales (0, no; 1, yes); MASS, presence of the masseteric scale (0, absent; 1, present); O_IP, contact between the occipital and interparietal scales (0, no; 1, yes); and 3rdO_IP, presence of a third scale between the occipital and interparietal (0, no; 1, yes); R_IN, contact between the rostral and internasal scales (0, no; 1, yes); 3rdR_IN, presence of a third scale between the rostral and internasal scales (0, no; 1, yes); SL_SUBOC, the number of supralabial scales in front of the subocular (four or five); TYMP, presence of the tympanic scale (0, absent; 1, present); TYMPfr, state of the tympanic scale (0, not fragmented; 1, fragmented). All bilateral characters were considered on the right side of the body.

Statistical analyses

Biometric variables were log-transformed prior to analyses. To obtain a general estimate of total body size, while taking all examined linear traits into account, we projected the log-transformed raw measurements on an isometric vector to calculate a multivariate representation of the isometric size of each specimen (mSIZE). We then regressed each biometric trait on this size vector and used the residuals obtained as size-corrected variables that represent body shape (Kaliontzopoulou, Carretero & Llorente, 2010b). To examine patterns of morphological variation in continuous traits (i.e. size, shape, and continuous pholidotic characters), we used a factorial multivariate analysis of variance (MANOVA), with mitochondrial lineage (mtDNA), locality (SITE), as a factor nested into mtDNA, SEX, and interaction terms (mtDNA × SEX and SITE × SEX), as well as ANOVA comparisons with the same design on univariate characters. Because of the unbalanced nature of our sampling design, we used non-parametric (M)ANOVA procedures based on 1000 permutations of Euclidean distance matrices between group means. As (M)ANOVA comparisons always indicated a highly significant effect of SEX on the examined variables (see Results; Table 2), and as sexual dimorphism is not the focus of this study, we performed further analyses on males and females separately. We performed principal components analyses (PCAs) on size-corrected shape variables and continuous pholidotic traits separately to investigate main sources of variation in our sample. Because some continuous pholidotic characters could not be recorded on all of the populations examined (see Table 3), PCAs on these traits were conducted on the full set of individuals with a reduced set of variables that did not include SCGN and SDLN.

Further on, we performed canonical variates analyses (CVAs) on size, shape variables, and continuous pholidotic traits, considering biometry and pholidosis, both separately and in combination, to investigate multivariate discrimination of mtDNA lineages and detect the characters that contribute the most. Because considering 15 different groups simultaneously in CVA is subject to both conceptual and statistical limitations (asking the question 'is it possible to discriminate all groups from each other simultaneously?'), we performed two additional sets of CVA tests: one considering each individual lineage versus the remaining lineages grouped together (i.e. 'is it possible to discriminate each lineage from the rest?') and another considering all possible pairs of lineages (i.e. 'is it possible to discriminate pairs of lineages?'). To exclude potential effects of the sampling design on CVA we used equal prior probabilities for all the groups examined and applied a leave-one-out bootstrap procedure with 1000 replicates to calculate levels of correct classification.

For categorical pholidotic traits we first examined the observed distribution of character-state frequencies to obtain a preliminary idea of variation. Some of the characters examined were almost fixed in one of the recorded states, not presenting sufficient variation across the sample. These included TYMP (1 in 99.7% of the examined individuals), 3rdR_IN (0 in 99.55% of individuals), IN_F (1 in 96.2% of individuals), and 3rdIN_F (1 in 97.1% of individuals). These variables were therefore dropped from further analyses. For the remaining categorical pholidotic traits, we examined the observed frequencies of the different character states in the mtDNA lineages analysed, and used Fisher's exact test with a Monte Carlo simulation of 1000 replicates on the *P* value (Agresti, 2002) to evaluate differences between mtDNA groups within sexes and between sexes of each mtDNA group. To review relationships between mtDNA groups considering categorical pholidotic traits in a multidimensional space, we first calculated Manly's overlap index for percentage data between groups (Manly, 2005), and then calculated a multidimensional scaling (MDS) on this distance (one-overlap) matrix (Legendre & Legendre, 1998).

Table 2. Results of the non-parametric (M)ANOVAs applied to multivariate size and size-corrected biometric characters

	mSIZE			mSHAPE			HL			PL		
	SS	*F*	*P*	Pillai	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
mtDNA	8.11	125.05	0.001	18.7	1.34	0.001	0.45	16.36	0.001	0.13	14.48	0.001
SEX	14.5	3132.16	0.001	16.26	16.26	0.001	0.14	69.51	0.001	0.16	239.16	0.001
SITE	4.99	16.84	0.001	21.69	0.34	0.001	1.06	8.46	0.001	0.35	8.29	0.001
mtDNA × SEX	0.22	3.47	0.001	0.67	0.05	0.001	0.06	2.09	0.016	0.03	3.45	0.001
SITE × SEX	0.39	1.32	0.051	2.5	0.04	0.001	0.23	1.87	0.001	0.04	1	0.483
	HW			HH			ESD			MO		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
mtDNA	1.45	59.21	0.001	5.45	127.42	0.001	0.19	16.79	0.001	0.24	15.65	0.001
SEX	0.22	124.68	0.001	0.03	8.33	0.006	0.03	39.93	0.001	0.15	140.05	0.001
SITE	1.76	15.77	0.001	1.88	9.64	0.001	0.35	6.71	0.001	0.47	6.85	0.001
mtDNA × SEX	0.05	2.08	0.012	0.12	2.81	0.001	0.02	1.92	0.026	0.03	1.82	0.031
SITE × SEX	0.15	1.37	0.033	0.36	1.86	0.001	0.08	1.47	0.006	0.08	1.19	0.154
	TRL			FLL			FL			TBL		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
mtDNA	3.64	39.36	0.001	0.52	23.67	0.001	0.57	13.15	0.001	3.93	86.53	0.001
SEX	14.75	2233.1	0.001	0.03	21.7	0.001	0.11	36.07	0.001	0.12	36.71	0.001
SITE	5.44	12.86	0.001	0.52	5.27	0.001	2.03	10.22	0.001	4.34	20.9	0.001
mtDNA × SEX	0.16	1.69	0.046	0.02	1	0.433	0.08	1.81	0.030	0.05	1.12	0.339
SITE × SEX	0.66	1.55	0.007	0.14	1.39	0.029	0.23	1.15	0.211	0.26	1.24	0.104
	4TL			HLL								
	SS	*F*	*P*	SS	*F*	*P*						
mtDNA	1.1	29.13	0.001	1.04	68.94	0.001						
SEX	0.29	108.91	0.001	0.23	210.65	0.001						
SITE	2.38	13.78	0.001	1.11	16	0.001						
mtDNA × SEX	0.04	1.03	0.419	0.02	1.06	0.419						
SITE × SEX	0.18	1.03	0.402	0.09	1.32	0.06						

Column headings: SS, explained sums of squares; Pillai, Pillai's trace for MANOVA; *F, F*-statistic value; *P*, resampling *P* value; mSIZE, multivariate body size; mSHAPE, multivariate body shape (see Material and methods). Degrees of freedom are 14 for mtDNA, one for SEX, 64 for SITE, 14 for mtDNA × SEX, 64 for SITE × SEX, and 2295 for residuals in all cases. Abbreviations: HL, head length; PL, pileus length; HW, head width; HH, head height; ESD, eye–snout distance; TRL, trunk length; FLL, forelimb length; FL, femur length; TBL, tibia length; 4TL, hindfoot length; HLL, hindlimb length.

All statistical analyses were performed using R 2.11.0 (The R Foundation for Statistical Computing, 2010).

RESULTS

Biometric variation

Analysis of size and shape biometric variation using (M)ANOVA indicated significant effects for all main factors (mtDNA, SEX, and SITE) and in some cases for interaction terms (Table 2). Post-hoc comparisons indicated that males were always bigger than females (considering mSIZE, $P < 0.001$ in all cases; Appendix S2; Fig. 2). Across-lineage patterns were more complex, but size variation was concordant in both sexes, with the main patterns including a remarkably smaller body size for the mitochondrial lineages PHAM and PHGal, and a larger body size for the lineage PVSSp (Appendix S2; Fig. 3). Body shape

Table 3. Correlations between the first five principal component axes and initial variables as obtained from the principle components analyses (PCAs) applied to size-corrected biometric and continuous pholidotic variables for each sex separately

	Males						Females				
	PC1	PC2	PC3	PC4	PC5		PC1	PC2	PC3	PC4	PC5
						Size-free BIOMETRY					
HL	–0.10	0.06	–0.24	–0.07	–0.71	HL	–0.19	0.08	–0.15	–0.75	0.07
PL	0.01	0.10	–0.29	0.20	–0.48	PL	–0.21	0.22	–0.18	–0.41	0.19
HW	–0.04	**0.54**	–0.01	–0.34	–0.20	HW	–0.27	**0.43**	0.10	–0.43	–0.19
HH	–0.07	**0.80**	**–0.38**	0.09	0.42	HH	–0.16	**0.78**	**–0.38**	0.45	–0.05
ESD	0.12	0.26	–0.29	0.09	–0.43	ESD	–0.05	0.38	–0.15	–0.24	0.10
MO	–0.06	0.13	–0.17	0.18	–0.42	MO	–0.10	0.23	–0.05	–0.22	0.17
TRL	**0.94**	0.06	0.31	0.05	0.08	TRL	**0.97**	0.10	0.20	0.04	0.04
FLL	–0.30	–0.38	0.27	0.28	–0.04	FFL	–0.27	–0.32	0.25	0.16	0.37
FL	–0.41	–0.02	**0.43**	–0.74	0.18	FL	–0.20	–0.26	**0.35**	0.14	–0.85
TBL	0.20	**–0.80**	**–0.48**	–0.15	0.22	TBL	0.27	**–0.71**	**–0.61**	0.07	–0.07
4TL	**–0.63**	–0.15	**0.44**	0.39	0.24	X4TL	**–0.58**	–0.28	**0.47**	0.26	0.29
HLL	–0.38	**–0.52**	0.20	0.41	0.09	HFL	–0.34	**–0.47**	0.17	0.32	0.37
% exp.	25.80	24.20	12.80	9.50	8.88	% exp.	30.60	20.14	12.12	9.50	9.06
Cum. %	25.80	50.00	62.77	72.30	81.16	Cum. %	30.60	50.77	62.89	72.38	81.44
						PHOLIDOSIS (continuous)					
CSN	0.49	0.39	**0.53**	–0.55	–0.12	CSN	0.54	–0.19	**–0.66**	–0.40	–0.25
GSN	**0.75**	–0.17	–0.11	0.00	0.63	GSN	**0.73**	0.17	0.05	–0.12	0.65
VSN	0.28	**0.83**	–0.23	0.43	–0.03	VSN	0.39	**–0.75**	0.02	0.53	0.03
FPN	**0.60**	–0.19	**–0.61**	–0.25	–0.41	FPN	**0.58**	–0.05	**0.66**	–0.29	–0.36
STSN	**0.56**	–0.34	0.45	0.54	–0.28	STSN	**0.50**	0.60	–0.14	0.55	–0.27
% exp.	31.30	20.30	18.30	16.90	13.30	% exp.	31.40	19.80	18.00	17.00	13.80
Cum. %	31.30	51.60	69.90	86.80	100.00	Cum. %	31.40	51.20	69.20	86.20	100.00

Abbreviations: HL, head length; PL, pileus length; HW, head width; HH, head height; ESD, eye–snout distance; MO, mouth opening; TRL, trunk length; FLL, forelimb length; FL, femur length; TBL, tibia length; 4TL, hindfoot length; HLL, hindlimb length; CSN, collar scales number; GSN, gular scales number; VSN, transversal rows of ventral scales; FPN, number of femoral pores; STSN, supratemporal scales number.
% exp.: the percentage of variation explained by each axis.
Cum. %: the cumulative percentage of variation explained.
The most contributing variables are set in bold.

was also always significantly different between both sexes (considering mSHAPE, $P < 0.001$ in all cases; Appendix S2; Fig. 2), but patterns of shape sexual dimorphism varied across mitochondrial lineages (significant mtDNA × SEX interaction term for mSHAPE; Fig. 2; Table 2). Body shape differed almost always between mitochondrial lineages (Fig. 2), with the only exceptions being the pairs PHAM–PHGal, PH1B–PHBat, PH1B–PVSCSp, and PL–PHJS in females, and PHAM–PHGal, PVSCSp–PHGal, PHAM–PVSCSp, and PVMA–PHJS in males. PCA on individuals of each sex separately indicated that global variation across the sample mainly arose from HW, HH, TRL, FL, TBL, 4TL, and HLL (Table 3). Both the structure of PC axes and the relative positions of different lineages across them were concordant between both sexes (Fig. 3; Table 3). CVAs on size-corrected biometric traits provided low levels of correct classification, with mean correct percentages of 37.61% in males and 37.86% in females (Appendix S4; Table 4). The size-corrected variables that contributed the most in group discrimination were HW, HH, TRL, FLL, TBL, and HLL (Table 4).

Continuous pholidotic traits

Analysis of variation in continuous pholidotic traits through ANOVA revealed significant effects of all main factors (mtDNA, SEX, and SITE) in all cases, except for STSN, for which the effect of SEX was not significant (Table 5). Contrary to what was observed for biometric variation, interaction terms were not significant in most cases for continuous pholidotic traits, with the exception of VSN, SCSN, and SDLN (Table 5). Males of

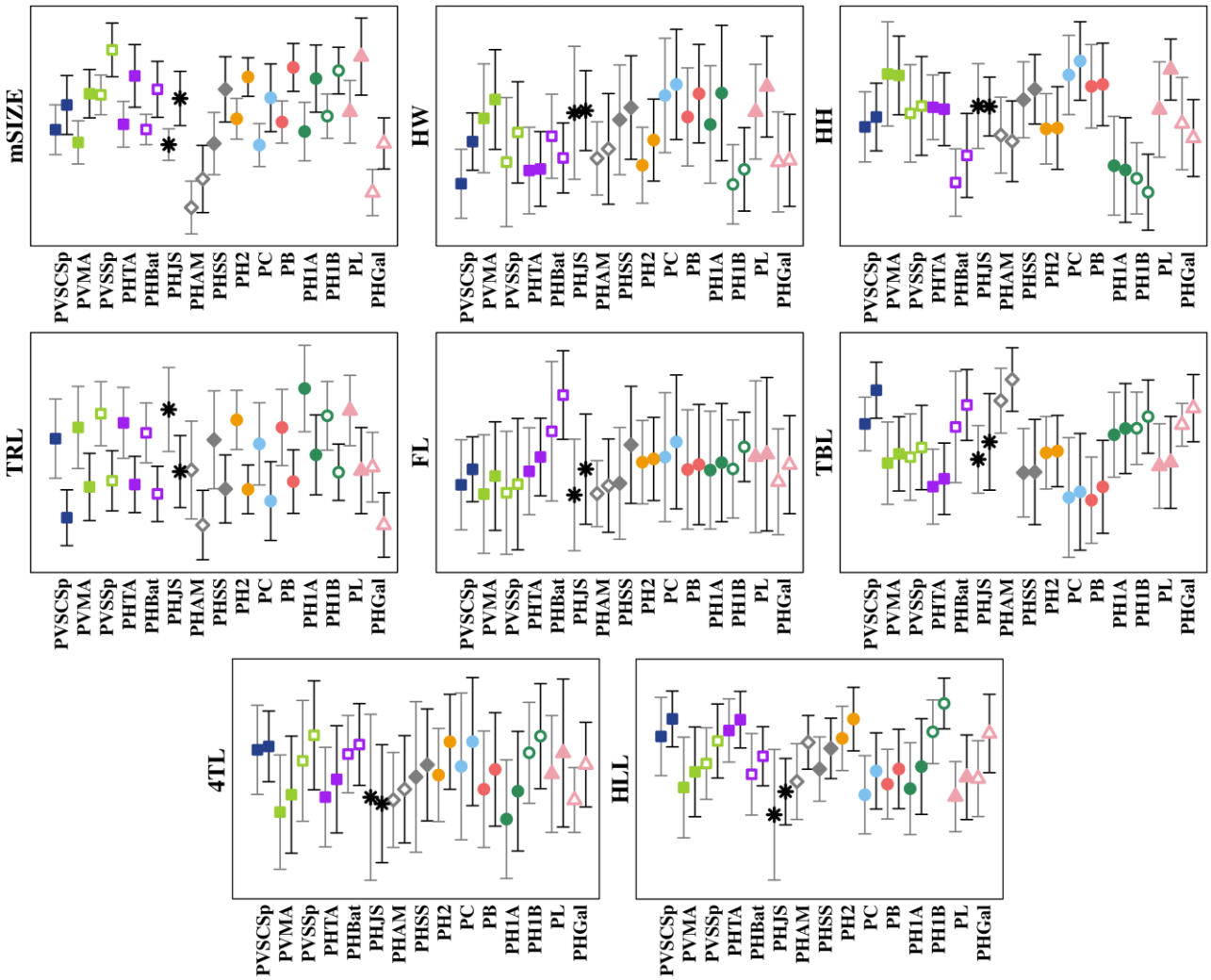

Figure 2. Least-squares means for multivariate body size and size-corrected biometric variables in the different mitochondrial lineages examined. Only the characters most relevant for global biometric variation and group discrimination (after principle components analysis and canonical variates analysis, respectively; see Results) are presented. Error bars denote ± standard deviation. Females of each group are always presented first, denoted with a grey vertical bar, and males are in black. See Table 1 for group codes, Material and methods for variable abbreviations, and Figure 1 for symbols used to represent each lineage.

all lineages presented higher scale counts than respective females for all variables, except for VSN, which was higher in females ($P < 0.01$ in all cases; Appendix S3; Fig. 4). PCAs on individuals of each sex separately indicated that global pholidotic variation mainly resulted from GSN, STSN, FPN, and VSN, a pattern that was concordant in both sexes (Table 3). Across PCA axes, the lineage of PHJS was clearly differentiated by a lower number of GSN, FPN, and STSN (Figs 4 and 5), but variation across the remaining lineages was quite complex, with the observed ranges of continuous pholidotic characters highly overlapping between different groups (Fig. 4). This overlap is reflected in CVA performed on continuous pholidotic traits, which gave very low levels of correct classification, corresponding to a mean of 29.43% in males and 25.36% in females (Appendix S4; Table 4). The characters that contribute the most in group discrimination were GSN, STSN, FPN, and VSN, with concordant patterns between both sexes (Table 4).

Combined analysis of continuous traits

The combined CVAs performed using mSIZE, size-corrected biometric variables, and continuous pholidotic traits, in combination, yielded a better discrimination between mitochondrial lineages in both sexes, but percentages of correct classification were still relatively low,

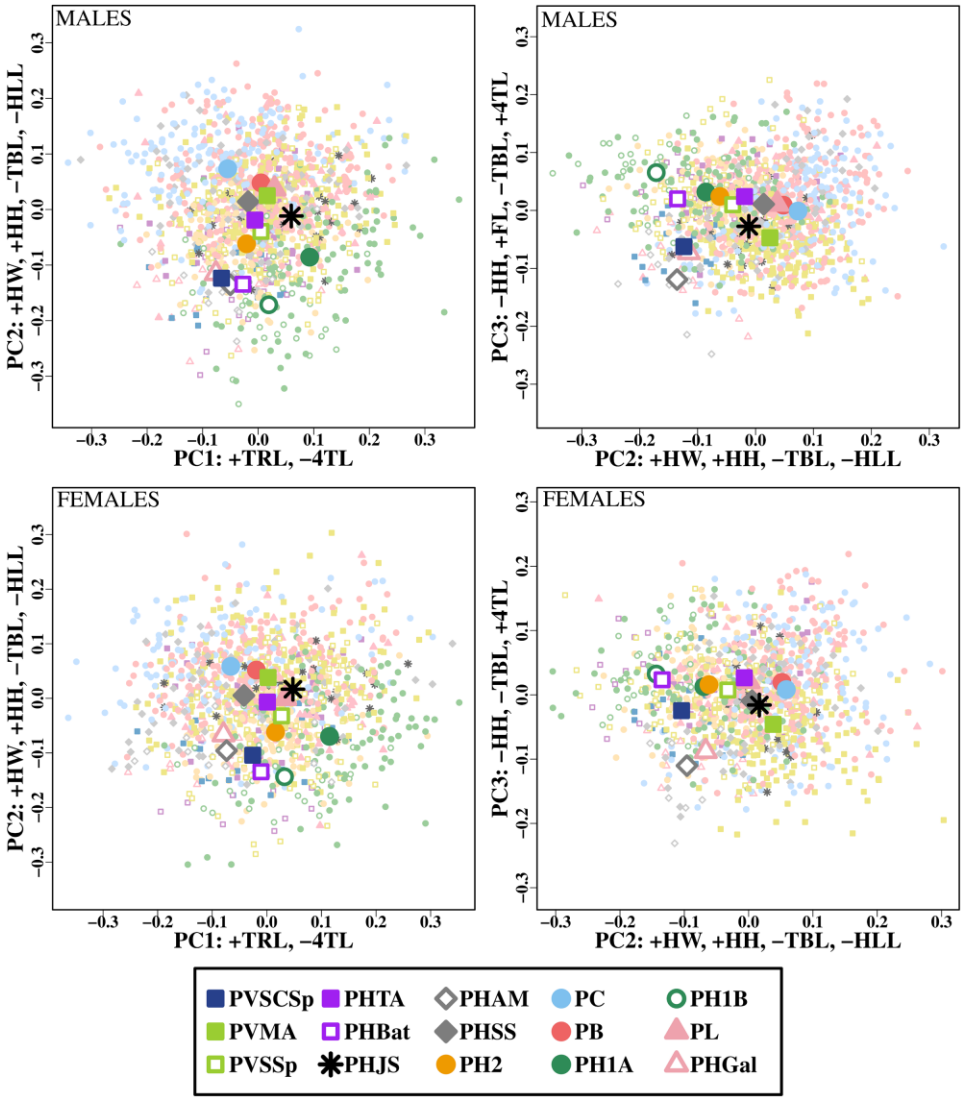

Figure 3. Scatter plots of individual scores (small symbols) and group means (big symbols) of the first three principal components of body shape variation for the mitochondrial lineages examined, considering males (top) and females (bottom) separately. The most highly (+, positively; –, negatively) contributing variables (Table 4) are indicated next to each axis. See Table 1 for group codes and Material and methods for variable abbreviations.

with a mean of 56.54% in males and 51.73% in females (Appendix S4; Table 4). mSIZE was the main variable contributing to group discrimination, followed by HW, HH, HLL, GSN, and STSN, with common patterns between both sexes (Table 4). Alternative schemes of CVAs gave a much better discrimination of different groups. The analyses considering each lineage as compared with the rest grouped resulted to 82.84 and 81.20% of the mean correct classification for males and females, respectively, whereas the pairwise CVAs provided an even better discrimination between pairs of groups compared, with a mean of 91.23 and 91.31% correctly classified in males and females, respectively (Appendix S4).

Categorical pholidotic traits

A Fisher's exact test on the observed frequencies of different character states for categorical pholidotic traits that presented sufficient variation (see Material and methods) indicated a significant effect of mtDNA ($P < 0.001$ for all variables), but not of SEX ($P > 0.1$ for all variables). An examination of the observed frequencies was therefore carried out, grouping both sexes. Considering categorical pholidotic traits, the lineages PHTA and PHBat were differentiated from the rest by high frequencies of five supralabial scales before the subocular (SL_SUBOC) and also, together with PHGal and PHAM, by a frequent (in some cases fixed) absence

Table 4. Correlations between examined variables and the three first canonical axes (CVs) produced by canonical variates analyses on different data sets, i.e. size-corrected biometric variables, continuous pholidotic traits, and the complete data set of multivariate size (mSIZE), shape, and pholidosis

	size-corrected BIOMETRY					
	Males (37.61%)			Females (37.86%)		
	CV1	CV2	CV3	CV1	CV2	CV3
HL	–0.19	0.28	0.21	0.09	–0.39	0.23
PL	–0.10	0.29	0.01	0.20	–0.36	–0.38
HW	**0.51**	0.07	**0.53**	**–0.45**	**–0.42**	0.35
HH	**0.84**	**0.58**	–0.10	**–0.71**	**–0.52**	**–0.44**
ESD	0.13	0.37	0.01	–0.20	–0.34	–0.25
MO	–0.15	0.31	–0.17	0.16	–0.39	–0.25
TRL	0.10	**–0.64**	0.27	–0.11	**0.46**	**0.42**
FLL	–0.29	–0.25	**–0.50**	0.23	0.37	–0.34
FL	0.00	–0.16	0.04	0.01	0.11	0.12
TBL	**–0.71**	0.06	0.22	**0.71**	0.13	0.24
4TL	0.02	–0.14	–0.27	0.03	0.18	–0.03
HLL	–0.43	–0.20	**–0.75**	0.29	**0.59**	**–0.54**
	Pholidosis					
	Males (29.43%)			Females (25.36%)		
	CV1	CV2	CV3	CV1	CV2	CV3
CSN	0.47	0.30	–0.04	0.35	0.33	0.04
GSN	**0.68**	–0.05	–0.40	**0.70**	0.32	0.32
VSN	0.19	0.44	**–0.66**	–0.24	**0.87**	0.39
FPN	0.22	**0.78**	0.37	0.31	**0.52**	**–0.73**
STSN	**0.80**	–0.22	0.31	**0.74**	0.01	0.13
	mSIZE, size-corrected BIOMETRY, PHOLIDOSIS					
	Males (56.54%)			Females (51.73%)		
	LD1	LD2	LD3	LD1	LD2	LD3
SIZE	**–0.76**	0.24	0.32	**–0.79**	0.17	0.32
HL	0.30	–0.08	0.09	0.25	–0.17	0.09
PL	0.24	–0.06	–0.01	0.23	0.06	–0.41
HW	–0.19	**–0.48**	0.17	0.09	**–0.53**	0.07
HH	–0.32	**–0.70**	**–0.55**	–0.09	**–0.66**	**–0.63**
ESD	0.10	–0.25	–0.07	0.06	–0.26	–0.32
MO	0.22	0.02	–0.14	0.28	–0.03	–0.23
TRL	–0.23	0.10	**0.59**	–0.20	0.06	**0.51**
FLL	–0.03	0.42	–0.11	–0.14	0.38	–0.12
FL	–0.01	0.10	–0.02	–0.05	0.09	0.09
TBL	0.43	0.31	0.17	0.26	**0.44**	0.35
4TL	–0.18	0.15	–0.23	–0.18	0.12	–0.05
HLL	0.05	**0.54**	–0.28	–0.22	**0.57**	–0.21
CSN	–0.00	0.34	–0.25	–0.07	0.31	–0.04
GSN	0.09	**0.58**	–0.08	–0.06	**0.57**	–0.02
VSN	–0.14	0.23	0.08	–0.06	0.06	0.30
FPN	–0.23	0.15	**–0.46**	–0.30	0.17	**–0.41**
STSN	0.19	**0.54**	–0.15	0.05	**0.48**	–0.08

Abbreviations: HL, head length; PL, pileus length; HW, head width; HH, head height; ESD, eye–snout distance; MO, mouth opening; TRL, trunk length; FLL, forelimb length; FL, femur length; TBL, tibia length; 4TL, hindfoot length; HLL, hindlimb length; CSN, collar scales number; GSN, gular scales number; VSN, transversal rows of ventral scales; FPN, number of femoral pores; STSN, supratemporal scales number.

% exp.: the percentage of variation explained by each axis. Percentages of mean correct classification after 1000 leave-one-out bootstrap cycles are given in parentheses.

The most contributing variables are marked in bold letter. See Material and methods for definition of variables.

Table 5. Results of the non-parametric ANOVAs applied to continuous pholidotic characters

	CSN			GSN			VSN			FPN		
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
mtDNA	183.54	11.28	0.00	3 901.5	57.27	0.00	652.38	33.27	0.00	1 062.86	40.57	0.00
SEX	37.93	32.63	0.00	261.94	53.83	0.00	6 761.38	4 827.19	0.00	599.29	320.27	0.00
SITE	322.38	4.33	0.00	2 958.32	9.5	0.00	493.23	5.5	0.00	1 040.19	8.69	0.00
mtDNA × SEX	10.5	0.65	0.85	78.69	1.16	0.27	44.54	2.27	0.01	45.15	1.72	0.05
SITE × SEX	85.36	1.15	0.20	421.21	1.35	0.04	108.43	1.21	0.12	138.93	1.16	0.20
Residuals	2 668.14			11 168.1			3 214.58			4 294.36		
TOTAL	3 307.85			18 789.8			11 274.5			7 180.79		
	STSN			SCGN*			SDLN*					
	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*			
mtDNA	379.77	32.22	0.00	742.68	19.68	0.00	1 329.7	47.74	0.00			
SEX	2.23	2.64	0.12	24.79	8.54	0.01	209.23	97.65	0.00			
SITE	393.15	7.3	0.00	877.17	5.4	0.00	1 275.97	11.03	0.00			
mtDNA × SEX	13.5	1.15	0.30	61.33	1.63	0.07	30.46	1.09	0.36			
SITE × SEX	61.07	1.13	0.22	219.36	1.35	0.04	186.12	1.67	0.00			
Residuals	1 932.19			5 675.53			3 974.65					
TOTAL	2 781.91			7 600.86			7 006.13					

Abbreviations: CSN, collar scales number; GSN, gular scales number; VSN, transversal rows of ventral scales; FPN, number of femoral pores; STSN, supratemporal scales number.

Column headings: SS, explained sums of squares; *F, F*-statistic value; *P*, resampling *P* value.

Degrees of freedom: 14 for mtDNA; 1 for SEX; 64 for SITE; 14 for mtDNA × SEX; 64 for SITE × SEX; and 2295 for residuals.

See Material and methods for the variable abbreviations.

*Degrees of freedom for these variables: 13 for mtDNA; 1 for SEX; 54 for SITE; 13 for mtDNA × SEX; 54 for SITE × SEX; and 1855 for residuals.

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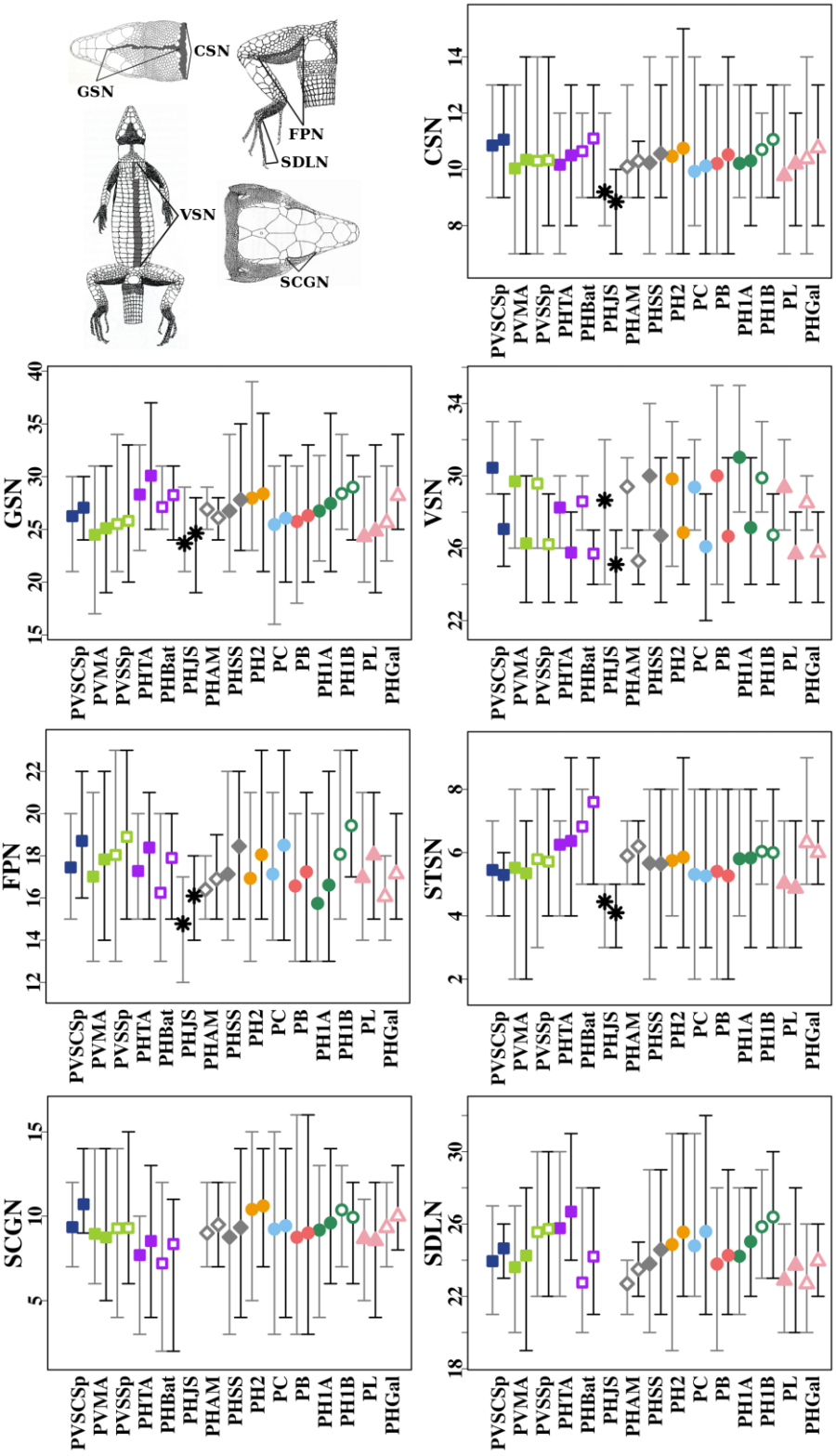

Figure 4. Least-squares means for continuous pholidotic traits in the different mitochondrial lineages examined. Vertical bars denote the observed range. Females of each group are always presented first, denoted with a grey vertical bar, and males are in black. See Table 1 for group codes, Material and methods for variable abbreviations, and Figure 1 for the symbols used to represent each lineage. Notice that no data are available for SCGN and SDLN in the PHJS lineage (Table 3).

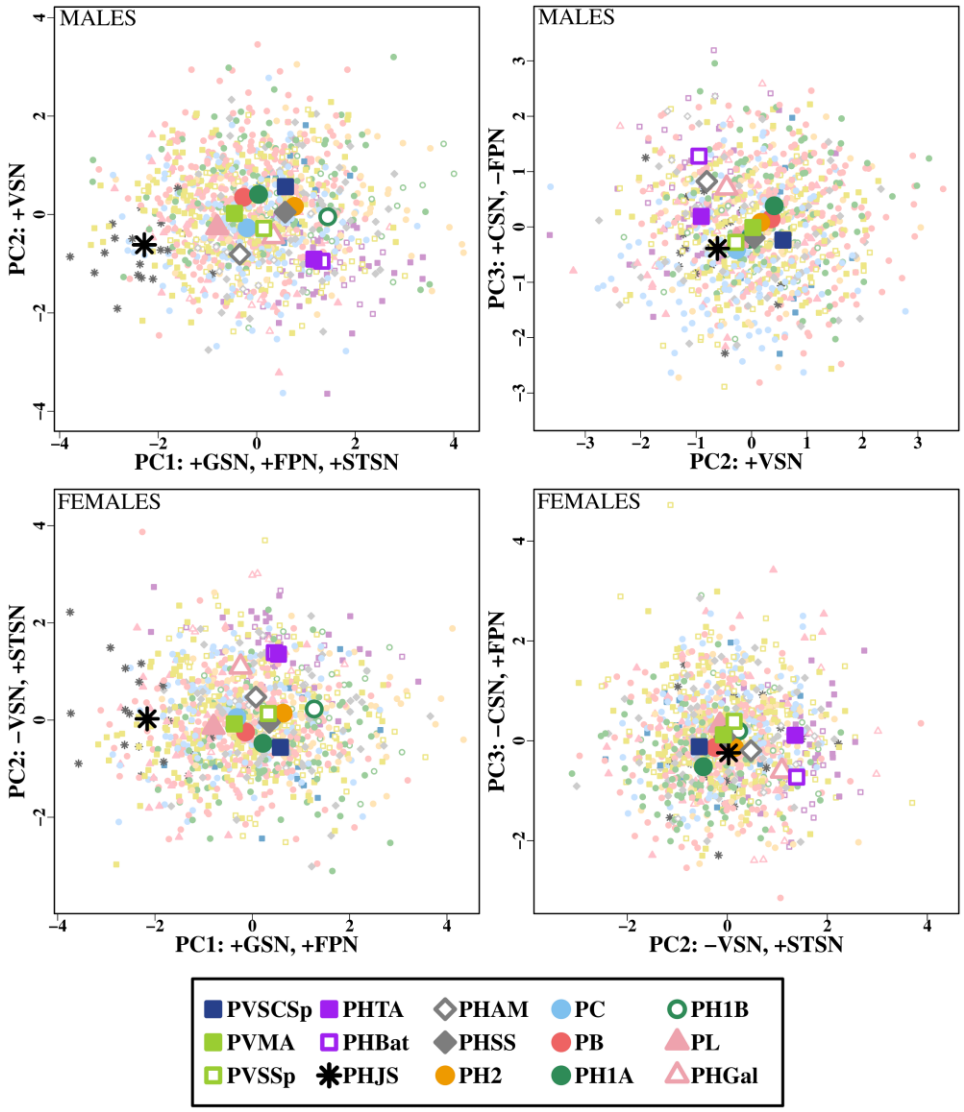

Figure 5. Scatter plots of individuals scores (small symbols) and group means (big symbols) of the first three principal components of variation in continuous pholidotic traits for the mitochondrial lineages examined, considering males (top) and females (bottom) separately. The most highly (+, positively; −, negatively) contributing variables (Table 4) are indicated next to each axis. See Table 1 for group codes and Material and methods for variable abbreviations.

of the masseteric scale (MASS) (Appendix S3; Fig. 6). On the other extreme of variation, lineages PB, PC, PVSCSp, and PVSSp were distinguished by an almost fixed presence of the masseteric scale (MASS; Fig. 6).

DISCUSSION

Morphological investigation following the discovery of cryptic species complexes is crucial for correctly classifying and conserving biodiversity (Beheregaray & Caccone, 2007), but also for understanding the evolutionary mechanisms involved in morphological evolution during group differentiation (Adams *et al.*, 2009). The first thorough analysis of external morphology in the *P. hispanica* species complex carried out here indicates high levels of variation both within and between the existing mitochondrial lineages, giving evidence for their morphological differentiation, but being non-conclusive in terms of their diagnosis. Sexual dimorphism is revealed as the main source of morphological variation in the examined populations. Mitochondrial lineages are also significantly different, considering both body size and shape, as well as different pholidotic characters, but effective discrimination between them is complicated

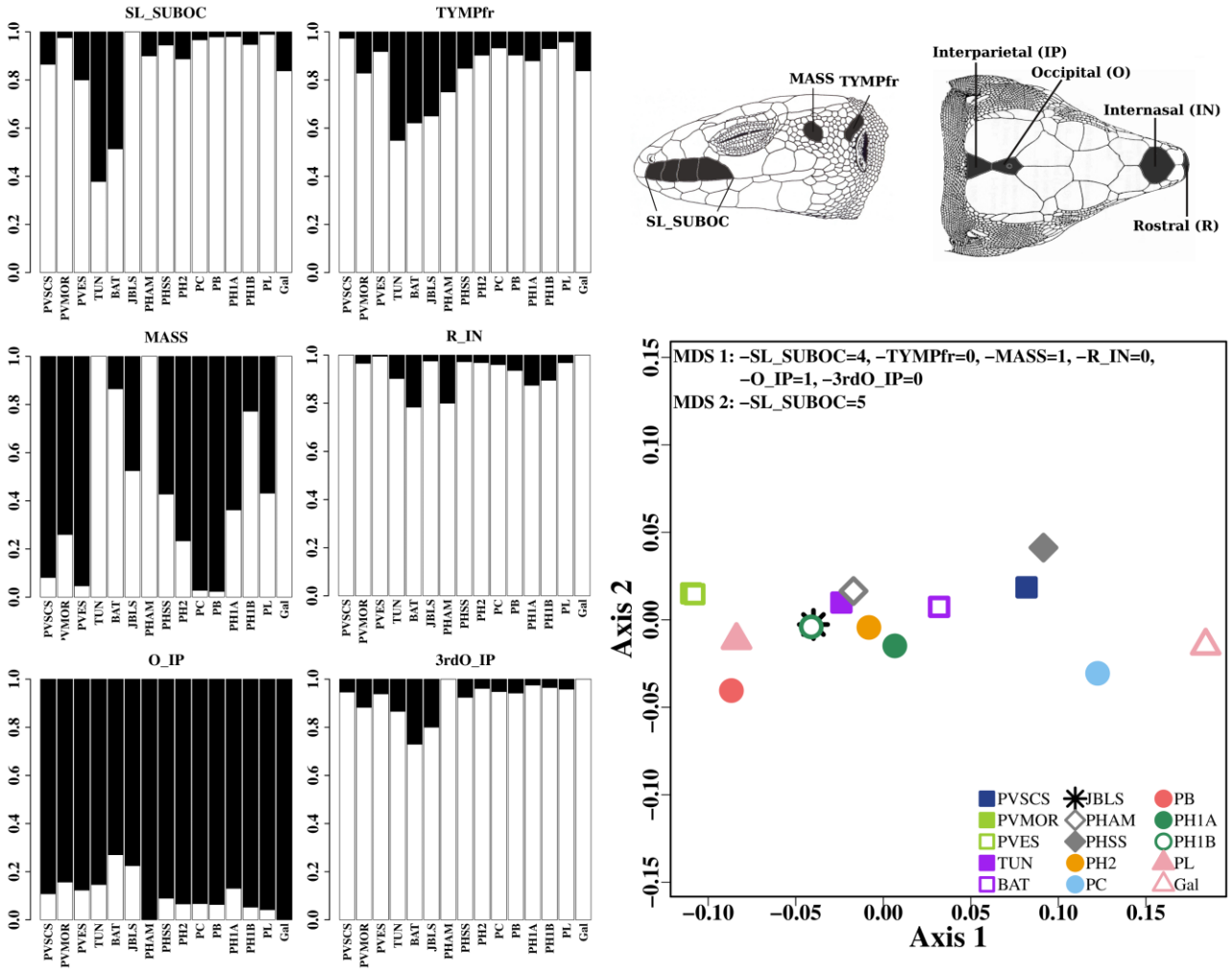

Figure 6. Observed frequencies for the different character states of the categorical pholidotic characters presenting sufficient variation across the sample examined, and multidimensional scaling (MDS) scatter plot of Manly's overlap index between lineages. White always represents character state 0 and black represents character state 1, except for SL_SUBOC, in which white represents state 4 and black represents state 5. See Table 1 for group codes, Material and methods for variable abbreviations, and Figure 1 for the visual symbols used to represent each lineage.

by the elevated intralineage variation observed, and the characters examined here are far from diagnostic in terms of taxonomy. Interestingly, neither biometric nor pholidotic traits show – at least superficially – variation with phylogenetic cohesiveness. Instead, general patterns of biometric variation are primarily linked to body traits with ecomorphological significance, possibly indicating that local adaptation is of major significance in driving morphological evolution in this group of lizards.

Patterns of biometric variation

Podarcis wall lizards from the Iberian Peninsula and North Africa are no exception to the elevated morphological variation characteristic of this genus (Arnold, 1973, 2004). Analysis of variation considering sex, mtDNA lineage, and location of capture as factors revealed that sexual dimorphism is a major component of biometric differentiation, dominating other factors in terms of explained variation in both body size and shape (as expressed by sums of squares; Table 2). This observation is not novel, as sexual dimorphism in both body size and shape have been extensively explored in these lizards (Herrel, Van Damme & De Vree, 1996; Kaliontzopoulou *et al.*, 2007, 2008, 2010a), and will not be further considered here. Interestingly, whereas significant differences existed between the mtDNA lineages examined, the effect of capture locality (examined as nested into

mtDNA lineage) was also significant, and in many cases explained as much variation as mtDNA (Table 2). This fact indicates that although mtDNA lineages are distinct considering body size and shape, variation between populations of the same lineage is also very high, particularly considering body shape (Fig. 3; Table 2).

Multivariate analysis of body shape (PCA) indicated that the overall variation observed is mainly the result of variation in relative head width and height (HW and HH), relative trunk length (TRL), and the relative length (both in total and of different parts) of the hindlimb (TBL, 4TL, and HLL). These variables are also the most relevant for discrimination between mtDNA lineages, as explored through CVA (Table 4). Importantly, the variables involved in both global variation and lineage differentiation are of high ecomorphological relevance, being related to both habitat use (Vitt *et al*., 1997; Vanhooydonck & Van Damme, 1999; Herrel, Meyers & Vanhooydonck, 2001) and to escape from predators through locomotion (Arnold, 1998; Aerts *et al*., 2000; Van Damme *et al*., 2003; Kaliontzopoulou *et al*., 2010a). Additionally, both relative head dimensions and limb length have been shown to vary as a response to habitat type within *P. bocagei* wall lizards (Kaliontzopoulou *et al*., 2010b), a pattern that is expected to persist both within other related lineages and between the different lineages of the Iberian and North African groups of *Podarcis* wall lizards. Remarkably, biometric traits do not seem to vary in a phylogenetically structured manner, although only through a formal phylogenetically informed analysis could one confirm this observation. For example, body size, the main component of biometric variation, is smallest in the lineages PHAM and PHGal, and biggest in the lineages PVSSp and PH3 (Appendix 2; Fig. 2), whereas members of both pairs are quite distant in phylogenetic terms (Fig. 1A). Similarly, PHAM and PHGal, as well as PVSCSp, are similar in terms of body shape (Appendix S4; Fig. 3), not being directly related phylogenetically (Fig. 1A). Interestingly, the groups mentioned above inhabit the southeastern part of the Iberian Peninsula, an area of very particular bioclimatic characteristics (Rivas-Martínez, Penas & Díaz, 2004; Sillero *et al*., 2009), which is also a centre of diversification for other reptile groups (Brito *et al*., 2006; Blain, Bailon & Agustí, 2008; Perera & Harris, 2008; Sillero *et al*., 2009). The application of phylogenetic comparative methods could highly enhance our understanding of the relative importance of historical factors (phylogenetic inertia) versus local adaptation in shaping biometric variation in this group of lizards.

Pholidosis and its usefulness for group delimitation

Pholidotic traits have long been used for taxonomy and field identification of lizards in general, and lacertids in particular (Boulenger, 1920; Salvador, 1998; Arnold & Ovenden, 2002). Our results indicate that although pholidotic traits may be used to distinguish the mtDNA lineages examined, their usefulness in terms of diagnosis of the different groups is limited. In fact, as also observed for body shape, mtDNA lineages differ considering continuous pholidotic traits. However, within-lineage variation is also very high (Table 5), with different groups presenting in most cases highly overlapping ranges (Fig. 4), and assignment to the correct mtDNA lineage is very low when considering these traits alone (Appendix S4; Table 4). Categorical pholidotic traits also differ significantly between mtDNA lineages, and are in some cases 'fixed' in one character state in some of them (Fig. 6). However, such cases are rare, and differences between lineages are mostly related to a variation in the frequencies of occurrence of different character states (Appendix S3; Fig. 6), without being diagnostic (*sensu* Wiens & Servedio, 2000). Considering the extensive use of categorical pholidotic traits for species delimitation in lacertids in the past (Boulenger, 1920; Salvador, 1998; Arnold & Ovenden, 2002), two – not mutually exclusive – reasons might be responsible for the observed patterns: either the examined mtDNA lineages do not represent evolutionary units equivalent to the ones delimited in the past using such traits, or the extensive sampling scheme used here has captured extreme intragroup variability. Whereas the question of whether the examined mtDNA lineages represent species or not is an extensive one that still remains open (see further on), the global image given by our analyses is that we are dealing with a group of extreme morphological variability, with respect to both biometric and pholidotic traits. Although such variability may be of high evolutionary relevance and should be taken into account during group delimitation (Wiens, 1999), it does at present prevent the proposal of traits useful for the taxonomical recognition of different *Podarcis* forms (Kaliontzopoulou, Carretero & Llorente, 2005).

Is *Podarcis hispanica* a species complex?

When describing phylogenetic variation in Iberian and North African *Podarcis* for the first time, Harris & Sá-Sousa (2002) suggested that *P. hispanica* is a species complex. Phylogenetic studies ever since have treated this group of lizards as such, uncovering high levels of cryptic diversity (Pinho *et al*., 2006, 2007). However, the assumption that the Iberian and North African clade of *Podarcis* constitutes a complex of

cryptic species has never been tested from a morphological perspective until now. Our investigation of a large number of external morphological characters in 15 of the 16 mitochondrial lineages present in this group paves the way for a formal evaluation of this question. However, the above question incorporates two aspects that should be distinguished, because they represent different biological issues: (1) are mtDNA lineages morphologically distinct, and can they be identified on the basis of morphological characters; and (2) do the evolutionary units corresponding to mtDNA lineages constitute species or not? The first half of the question is related to evaluating how 'cryptic' these lineages are (Sáez & Lozano, 2005), whereas the second half corresponds to whether they are a 'species complex', and concerns the systematic decision of whether such units should be described as separate species (Schlick-Steiner *et al*., 2007).

Considering the question of whether mtDNA lineages are cryptic, the answer is probably not. Our results indicate that although high levels of variability are present both at the population and lineage level, mtDNA lineages are statistically different considering body size, body shape, and pholidotic traits (Tables 2 and 5). Whereas each of the examined data sets in isolation does not provide a good discrimination between lineages, the combined analysis using size, shape, and continuous pholidotic traits provided a much better classification (Table 4). Moreover, when trying to ask questions that are more realistic in practical terms, such as whether we can discriminate one lineage from the rest, or whether we can discriminate between pairs of lineages, discrimination is visibly higher, exceeding 90% of correct classification (CVA; Appendix S4). In this sense, then, mtDNA lineages are morphologically different, and can be identified on the basis of morphological characters. However, the procedures to attain this objective are in practice very complicated: a very large number of individuals should be sampled to include the variation present in each group, and a large number of characters should be quantified (totalling 25 in this study). This makes the working scheme quite unrealistic for field identification of different lineages, but still some general lines can be drawn on the basis of characters most relevant for lineage differentiation. For example, the PHAM and PHGal lineages are distinguished from the rest by a remarkably smaller body size (Fig. 2): PH1A, PH1B, and PHBat are visibly flatter (lower relative HH), and PVMA, PC, and PB are higher (Fig. 2); PHJS has less femoral pores and supratemporal scales (FPN and STSN; Figs 4 and 5); PHTA, PHAM, and PHGal normally do not have a masseteric scale, whereas PB, PC, PVSCSp, and PVSSp normally have one (MASS; Fig. 6); and so on. Interestingly, our results indicate a much higher variability and morphological overlap between mtDNA lineages than that observed in another cryptic species complex of *Podarcis* investigated, in which different species could be effectively delimited and diagnosed on the basis of body size and pholidotic traits (Lymberakis *et al*., 2008). The geological history of the two areas (Iberian Peninsula and Greece) may have played a role in determining this difference, as the geographical isolation between Greek taxa may have enhanced their morphological differentiation (Lymberakis & Poulakakis, 2010), as is common for insular populations (Meiri, 2007).

Considering the morphological identification of different lineages within Iberian and North African *Podarcis*, we should also note that one major type of external morphological characters has not been considered here. Colour variation is frequently used in lizard taxonomy, and can provide useful characters for both group delimitation and field identification. Both empirical observations (A. Kaliontzopoulou, M.A. Carretero & G.A. Llorente, pers. observ.) and the data available for some of the lineages examined here indicate that traits related to colour pattern could in fact be useful for identifying different groups (Sá-Sousa *et al*., 2002; Geniez *et al*., 2007). Moreover, colour characters are known to be used in partner recognition between overlapping mtDNA lineages in this group of lizards (Barbosa *et al*., 2008). However, variation is again extreme (A. Kaliontzopoulou, M.A. Carretero & G.A. Llorente, pers. observ.), and the description of colour pattern using empirically constructed categorical variables would instead increase the already complex image. Novel methods for capturing colour pattern involving quantitative image analysis (Anderson *et al*., 2003; Todd *et al*., 2005; Costa *et al*., 2009) could enhance the description of colour pattern variation in this group of lizards. Additionally, the implementation of techniques for quantifying colour characters invisible to the human eye, such as ultraviolet reflectance (Font & Molina-Borja, 2004; Molina-Borja, Font & Mesa Avila, 2006; Font, Pérez i de Lanuza & Sampedro, 2009), could be very relevant for species delimitation, as they might function for intraspecific communication and may eventually be involved in reproductive isolation between different lineages. However, additional caution should be taken when examining colour traits, as these are known to vary ontogenetically, seasonally, and with reproductive stage (Galán, 1995, 2000, 2008), and are frequently altered by specimen preservation in museum collections (Geniez *et al*., 2007).

But, do the mtDNA lineages of the Iberian and North African group of *Podarcis* correspond to different species? Our results indicate that morphological investigation as traditionally approached cannot answer this question. Traditionally, marked morpho-

logical differences between groups of organisms have been used as indicators to define species and infer their phylogenetic relationships (Wiens, 2007). Our results indicate that morphological variation is extensive both between and within lineages of Iberian and North African *Podarcis*, thereby entangling the detection of diagnostic characters (Wiens & Servedio, 2000). Maybe in this sense we are at the limits of what the human eye can perceive, and what the human brain can register and describe (Beheregaray & Caccone, 2007). Whereas most of the sensory information processed by the human brain is visual, other traits such as chemical or auditory might be more relevant for species delimitation if they are involved in mechanisms promoting reproductive isolation (Sáez & Lozano, 2005; Bickford *et al*., 2007). Future research on the systematics of this group would benefit from focusing on the variation of such characters. For example, behavioural evidence already exists that chemical recognition mechanisms may be playing a crucial role in individual, intra-, and interspecific recognition, and may therefore be involved in reproductive isolation between the Iberian and North African lineages of *Podarcis* (López & Martín, 2001; Barbosa *et al*., 2005, 2006; Martín & López, 2006). Additionally, the question of whether the mtDNA lineages of Iberian and North African *Podarcis* constitute different species should be approached through the investigation of the contact zones between them (de Queiroz, 1998, 2005). The available evidence indicates that although signs of past introgression can be found, present hybridization is rare and does not affect the genetic and morphological cohesiveness of the species involved, at least as far as *P. bocagei* and *P. carbonelli* are concerned (Pinho *et al*., 2009).

Put together, our results confirm that Iberian and North African *Podarcis* wall lizards are characterized by an extremely high level of morphological variation, but also indicate that such variation is not aleatory. Different mitochondrial lineages are morphologically distinct, although the high overlap of character ranges greatly increases the number of traits needed for correct identification. From a historical point of view, our analysis examining biometric and pholidotic traits routinely used in the past for species delimitation in lacertids (Boulenger, 1920; Salvador, 1998; Arnold & Ovenden, 2002) indicates that when such traits are quantified in a large number of individuals, representing a large number of populations within each targeted group, the usefulness of these characters for direct species identification is overwhelmed by local variation. The recent development of molecular tools for studying phylogenetic relationships between organisms, as well as the increased capacity of sampling large areas and gaining access to large numbers of individuals, certainly change the way we explore and understand (morphological) diversity. This does not mean that morphological characters are useless for species delimitation, but rather that a shift of framework is necessary. In this sense, understanding how and why morphological traits evolve in closely related groups may shed more light on the evolutionary meaning and position of such groups than simple morphological comparisons between them.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed list of the populations examined.
Appendix S2. Descriptive statistics for the raw biometric characters in males (M) and females (F) of the 15 mitochondrial lineages examined.
Appendix S3. Descriptive statistics for pholidotic characters in males (M) and females (F) of the 15 mitochondrial lineages examined.
Appendix S4. Case-classification tables for canonical variates analyses.

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