

# Inferring evolutionary scenarios with geostatistics and geographical information systems for the viperid snakes *Vipera latastei* and *Vipera monticola*

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The present study aimed to infer evolutionary scenarios for *Vipera latastei* and *Vipera monticola* in the Iberian Peninsula and the Maghreb through the identification of spatial patterns in morphological character variation and biogeographic patterns in morphological variability distribution. Ten morphological traits from 630 vipers were analysed with geostatistic and ecological niche modelling in a geographical information system. Interpolation by Kriging was used to generate surfaces of morphological variation, which were combined with spatial principal components analysis (SPCA). Putative morphological differentiated groups generated by SPCA maps were tested with discriminant function analysis (DFA). Maximum entropy modelling and nine environmental variables were used to identify factors limiting the distribution of groups and areas for their potential occurrence. Groups supported by DFA were: Western Iberia, Eastern Iberia, Rif plus Middle Atlas, Algeria, and High Atlas. Their distribution is influenced by common environmental factors such as precipitation. Areas of probable sympatry between Iberian groups matched the morphological clines observed by geostatistics tools. Geographic variation patterns in *V. latastei-monticola* are probably due to vicariant separation of Iberian and African populations during the opening of the Strait of Gibraltar, and population refugia during the Quaternary glaciations with secondary contact. The taxonomic status of northern Morocco and Algerian groups should be further investigated. We conclude that geostatistics and niche-modelling tools are adequate to infer morphological variability across wide geographic ranges of species. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 95, 790–806.

**ADDITIONAL KEYWORDS:** evolution – geographic variation – Gibraltar Strait – Iberian Peninsula – morphology – systematics – viper.

## INTRODUCTION

The Western Mediterranean Basin is part of one of the world's biodiversity hotspots (Myers, Mittermeier

& Mittermeier, 2000) and has received great attention focusing in its biogeographical complexity. Two major paleogeographical events shaped current biodiversity patterns. The first comprised the opening of the Strait of Gibraltar (approximately 5.33 Mya) that ended the land-bridge connection between Europe and Africa,

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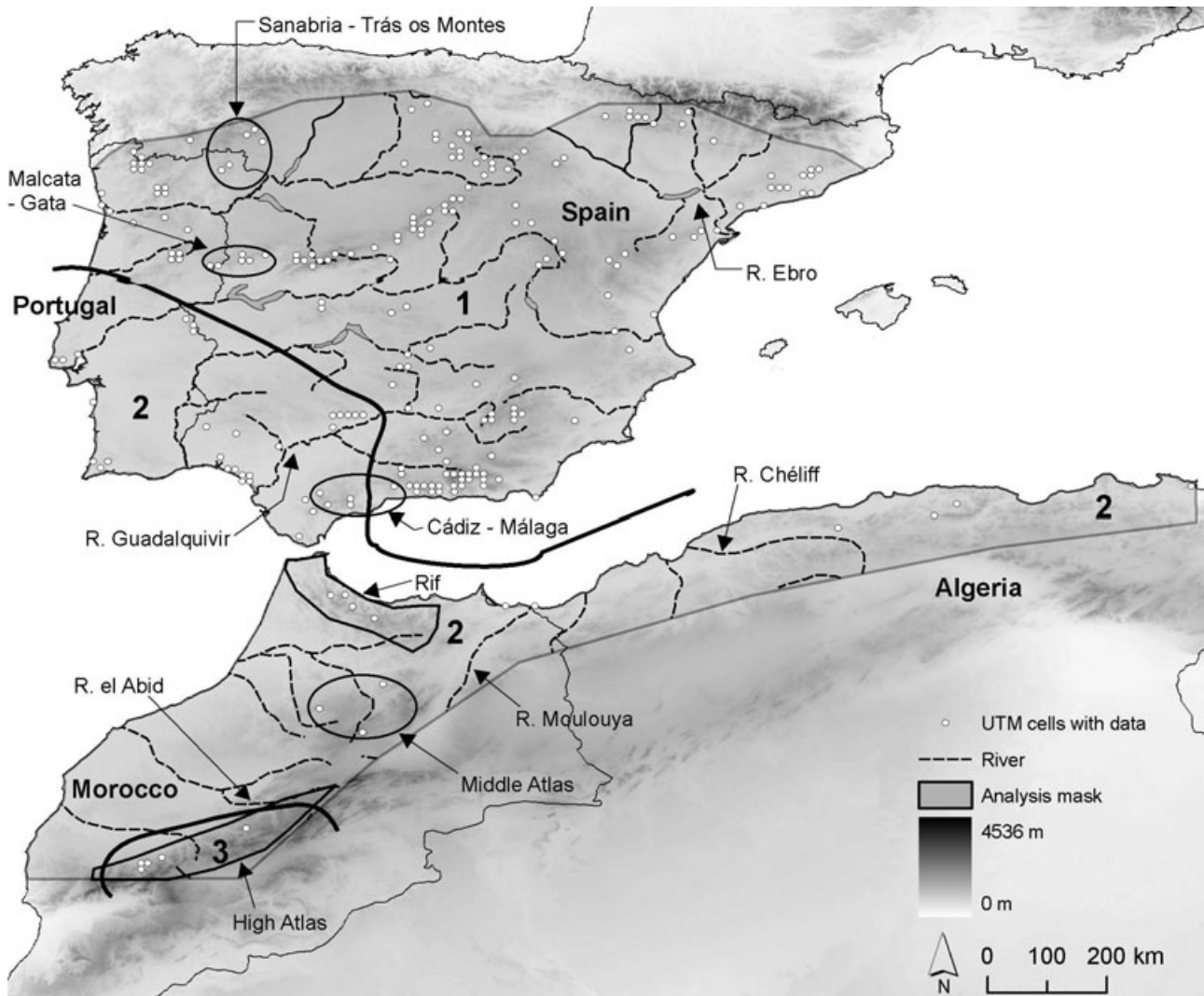
which had been in place for approximately 0.63 Myr during the Messinian Salinity Crisis (Duggen *et al.*, 2003); this event separated faunas inhabiting southern Iberian Peninsula from those in the Maghreb and induced considerable differentiation between allopatric taxa, which evolved in fairly diverse climatic and ecological scenarios (De Jong, 1998). The second comprised Pleistocene climatic cycles that involved repeated global cooling and warming periods over the last 700 kyr (Dawson, 1996). These dynamic cycles caused great changes in species ranges and induced allopatric speciation processes in southern European refugia for many species ancestors (Hewitt, 1996, 2000). When climatic conditions ameliorated, northwards expansion followed but was later hampered by ecological barriers or competing species (Hewitt, 1999).

Complex patterns of morphological variation across the Iberian Peninsula, the Strait of Gibraltar and Maghreb have been described for numerous taxa (*Ephippiger*: Kidd & Ritchie, 2001; *Genetta*: Gaubert *et al.*, 2004; *Galerida*: Guillaumet *et al.*, 2006). Amphibians and reptiles have received considerable attention under this cue because they are adequate taxa to highlight evolutionary processes in this region: they appear regularly in the fossil record, their populations are still important, and they have supported recent man-induced changes without large extinctions (Mateo *et al.*, 2003). Patterns of morphological variability across the Western Mediterranean basin have been reported in several amphibians and reptiles (*Discoglossus*: Busack, 1986; *Lacerta*: Perera *et al.*, 2007) and they demonstrate the biogeographic complexity of the region.

The generalized application of molecular techniques in recent years to the study of Western Mediterranean amphibians and reptiles has confirmed the importance of the opening of the Strait of Gibraltar and the Pleistocene Ice Cycles as a source of genetic variability. Several studies have reported genetic differentiation between allopatric taxa inhabiting southern Iberian Peninsula and the Maghreb (*Pelobates*: García-París *et al.*, 2003; *Pleurodeles*: Carranza & Arnold, 2004) and complex patterns of genetic variation resulting from single or multiple colonization events (*Malpolon monspessulanus* and *Hemorrhoids hippocrepis*: Carranza, Arnold & Pleguezuelos, 2006a; *Psammotromus algirus*: Carranza *et al.*, 2006b). Recently, molecular studies suggested the Messinian Salinity Crisis as inductor of old refugia for species within the Iberian Peninsula (Martínez-Solano *et al.*, 2006; Santos *et al.*, 2008). Likewise, they have identified Pleistocene refugia and secondary contact among differentiated lineages that led to the formation of hybrid zones in the Iberian Peninsula (Weiss & Ferrand, 2007).

From the late 1980s onward, several studies have used advanced statistical multivariate analysis to illustrate geographic patterns of variation in morphological characters (Thorpe & Báez, 1987; Brown & Thorpe, 1991). Some of them highlighted the relationship between sharp transition zones and steep clines in morphological traits with the occurrence of hybrid zones due to secondary contact (Thorpe, 1987a, b) and tried to disentangle the effects of ecological adaptation and phylogeographic processes in current morphological patterns (Thorpe *et al.*, 1991; Thorpe, 1996). The irruption of geographical information system (GIS) software allowed the combination of classical statistics with GIS to emphasize the importance of geography in evolutionary biology (Kidd & Ritchie, 2006). Indeed, GIS provides very useful tools to analyse geographic-related processes and has allowed new insights into morphological and genetical patterns of variation (Kidd & Ritchie, 2000; Hoffmann *et al.*, 2003), the location of hybrid zones (Swenson, 2006; Arif, Adams & Wicknick, 2007; Martínez-Freiría *et al.*, 2008), and the dynamics of gene flow therein (Spear *et al.*, 2005). More recently, the combination of ecological niche modelling with molecular phylogenies expanded the horizons of evolutionary biology studies even further by linking geographic patterns of ecological and genetical variation in evolutionary processes (Cushman *et al.*, 2006; Knouft *et al.*, 2006).

In the present study, we have combined geostatistics and GIS to examine morphological variability in a viper of the genus *Vipera* living in the Western Mediterranean basin. European vipers offer an interesting model that can be used to study biogeographic patterns of morphological variability. They differentiated from other Eurasian vipers in the early Miocene, constitute a monophyletic group, and partially evolved under the Quaternary dynamic scenario (Lenk *et al.*, 2001; Garrigues *et al.*, 2005). Among European vipers, *Vipera latastei* Boscá, 1878, probably shows the most complex evolutionary scenario: the land-bridges between the Iberian Peninsula and the Maghreb contributed to the expansion of this viper through north-western Africa to the Krômimir Mountains of Tunisia (Saint-Girons, 1980); subsequently, the opening of the Strait of Gibraltar separated Iberian from African populations; and the Pleistocene glaciations likely favoured the isolation of small populations in refugia. Evolutionary allopatric processes possibly gave rise to geographic differentiation (subspecies) and favoured differentiation of the High Atlas populations, which were initially described as subspecies (Saint-Girons, 1953), but later recognized as full species: *Vipera monticola* Saint-Girons, 1954 (Beerli, Billing & Schätti, 1986). In the rest of the area occupied by *V. latastei*, Saint-



**Figure 1.** Study area and location of  $10 \times 10$  km UTM cells with specimens of *Vipera latastei* and *Vipera monticola* used in the analysis (WGS84 projection). The analysis mask delimits the area for which continuous morphological trait surfaces were derived. Thick black lines delimit the traditional species and subspecies separation proposed by Saint-Girons (1977): 1 – *Vipera latastei latastei*; 2 – *Vipera latastei gaditana*; 3 – *Vipera monticola*. Thin black lines delimit regions mentioned in the text.

Girons (1977, 1978) described two subspecies based on morphological traits: *Vipera latastei latastei* in most of the Iberian Peninsula, and *Vipera latastei gaditana* in south-western Iberian Peninsula and Africa, except the High Atlas (Fig. 1). Nevertheless, Saint-Girons (1977) found a complex pattern of morphological variation across the Strait of Gibraltar with wide variability within the *gadicana* subspecies, suggesting that the Algerian populations might represent an undescribed taxon.

In a recent study, we used classical tools such as principal components and discriminant function analyses, for re-analysing variation in morphological traits across the range of *V. latastei* (Brito *et al.*,

2006). A clinal morphological variation was detected in a similar pattern than that observed by Saint-Girons (1977) and the morphological variability among vipers between the two sides of the Strait of Gibraltar was reconfirmed. However, classical tools were unable to confirm or invalidate geographic trends in morphology and the distribution area of Iberian subspecies remained unclear (Brito *et al.*, 2006). The present morphological study aimed to infer evolutionary scenarios for *V. latastei* and *V. monticola* across their distribution area using geostatistics and GIS-based niche models, with the intention to solve several biogeographical questions that remained unclear after the application of classical morphologi-

**Table 1.** Environmental factors used to test relationships with spatial variation in morphological variability of *Vipera latastei* and *Vipera monticola*

Type	Variable	Range and units	Code
Topographical	Altitude	0–3958 m a.s.l.	ALTIT
	Slope	0–71%	SLOPE
Climatic	Temperature seasonality	2.09–7.53 °C	TSEAS
	Maximum temperature of warmest month	10.2–39.6 °C	TMAXWM
	Minimum temperature of coldest month	–15.0 to 10.4 °C	TMINCM
	Temperature annual range	12.1–39.6 °C	TEMPAR
	Precipitation seasonality	12–88 mm	PSEAS
	Precipitation of wettest month	25–272 mm	PRECWM
	Precipitation of driest month	0–107 mm	PRECDM

cal analytical tools. The specific questions addressed are: (1) are there any geographic trends in the distribution of morphological traits; (2) how is morphological variability spatially distributed; and (3) is the distribution of morphological variability related to environmental factors? The linking of geostatistics, ecological niche modelling, and GIS is expected to allow the identification of morphological coherent groups, environmental sources of adaptation for such groups, and areas of secondary contact and probable hybridization among groups.

## MATERIAL AND METHODS

### THE DATA

#### *Specimens*

The study area includes all the distribution range of the species: the Iberian Peninsula and north-western Africa, from Morocco to Algeria, along the Rif, Middle Atlas, High Atlas, and Tell Atlas mountain chains (Fig. 1). A total of 606 specimens (598 *V. latastei* and eight *V. monticola*) with clear geographic assignment were examined from the collections of several institutions (Brito *et al.*, 2006). Additional data on 24 specimens was collected from two sources: (1) published data for four *V. latastei* and 18 *V. monticola* were recorded from the literature (Dolfus & Beau-rioux, 1928; Wettstein, 1933; Saint-Girons, 1977; Beerli *et al.*, 1986; Pillet, 1994); and (2) data from two live *V. latastei* observed in field expeditions to the Rif and Middle Atlas Mountains (Fahd *et al.*, 2005, 2007).

The geographic location of the specimens examined in museum collections and literature records was determined on the Universal Transverse Mercator (UTM) Projection. UTM coordinates were gathered manually from four map series (1 : 250 000 from Instituto Geográfico do Exército for Portugal; 1 : 250 000 from Mapa Militar Digital de España, version 1.5, for Spain; 1 : 250 000 and 1 : 200 000 from Institut Géographique National for Morocco and

for Algeria, respectively). The locations of the 630 specimens analysed (604 *V. latastei* and 26 *V. monticola*) were inscribed in a georeferenced database and assigned to the corresponding 10 × 10 km UTM grid cell. The ambiguity and imprecision in location description in some museum collections precluded the use of finer scales of analysis (5 × 5 or 1 × 1 km grid cell). A total of 224 UTM 10 × 10 km grid cells with the location of specimens were displayed in the GIS ArcMap 9.0 (ESRI, 2004) on the WGS84 datum (Fig. 1).

#### *Morphological characters*

For each specimen, a total of ten meristic characters were recorded: number of ventral scales, excluding preventrals and anal, *sensu* Saint-Girons (1978) (NVENT); subcaudal scales in males and females (SUBCAM, SUBCAF); dorsal scale rows at mid-body (DMBOD); apical scales (APICA); supralabial scales (SUPRR); infralabial scales (INFRR); pericocular scales (PERIR); loreal scales (LORER); intercantal plus intersupraocular scales (INTER); and dorsal marks (DMARK). When bilateral, characters were considered on the right side of the head. These characters were reported to present geographic variation in previous morphological analyses (Saint-Girons, 1977; Beerli *et al.*, 1986; Brito *et al.*, 2006). As there is no significant sexual dimorphism in *V. latastei-monticola*, except for the number of sub-caudal scales (Saint-Girons, 1977; Brito *et al.*, 2006), analyses combined male and female data except for the above trait.

#### *Environmental factors*

A set of nine uncorrelated ( $r < 0.800$ ) environmental factors or ecogeographical variables (EGV) known to affect the distribution of viperid snakes were selected for the analyses (Brito & Crespo, 2002; Santos *et al.*, 2006; Martínez-Freiria *et al.*, 2008). Two types of EGV were considered (Table 1): (1) topographical: a 30 arc second (approximately 1 km) digital elevation model



**Table 2.** Statistical measures to assess the performance of the Kriging interpolation of morphological traits from specimens of *Vipera latastei* and *Vipera monticola*

Trait	Mean error	Root-mean-square error	Average SE	Root-mean-square SE	<i>N</i> UTM cells	<i>N</i> specimens
NVENT	-0.043	3.773	3.922	0.961	203	547
SUBCAM	-0.047	2.687	2.261	1.182	146	286
SUBCAF	-0.012	2.683	2.570	1.042	107	207
DMBOD	0.001	0.446	0.324	1.327	220	608
APICA	0.007	0.918	0.774	1.161	216	584
SUPRR	-0.008	0.574	0.569	1.009	195	485
INFRR	0.015	0.759	0.670	1.127	195	464
PERIR	0.018	0.772	0.777	0.993	203	534
LORER	0.017	1.231	1.173	1.043	196	521
INTER	-0.032	5.874	5.001	1.163	202	535
DMARK	0.057	4.143	4.585	0.903	165	400

*N* UTM cells refer to the number of 10 × 10 km UTM cells for which data was available for a given trait.

NVENT, number of ventral scales, excluding preventrals and anal; SUBCAM, SUBCAF, subcaudal scales in males and females; DMBOD, dorsal scale rows at mid-body; APICA, apical scales; SUPRR, supralabial scales; INFRR, infralabial scales; PERIR, periocular scales; LORER, loreal scales; INTER, intercantal plus intersupraocular scales; DMARK, dorsal marks.

was obtained from the United States Geological Survey (USGS, 2006). Slope was subsequently derived using 'Slope' function of ArcMap GIS; (2) climatic: a set of seven climate layers (climate grids) with a spatial resolution of 1 km<sup>2</sup> were downloaded in digital format from Worldclim, version 1.4 (Hijmans *et al.*, 2005). These climatic EGV represent seasonal and extreme trends of temperature and precipitation (Table 1). Although the size of the grid cells (pixel) of the EGV was approximately 1 km<sup>2</sup>, the location of specimens was available at a 10 × 10 km resolution (see above). Therefore, to combine both morphological and environmental data, the EGV were resampled to a coarser resolution (10 × 10 km) using the 'Aggregate' function of ArcMap 9.0. In the new EGV, each output grid cell contains the mean value of the input cells that are encompassed by the extent of the output cell. All EGV were quantitative. Coastline, borders and major rivers were extracted from ArcAtlas – Our Earth (ESRI, 1996). All variables were projected in the WGS84 datum.

#### MODELLING PROCEDURES

##### *Spatial patterns in morphological character variation*

The specimens examined did not cover all UTM grid cells of the study area (Fig. 1). Therefore, it was necessary to interpolate trait values at unsampled locations (Kidd & Ritchie, 2000). For each trait, a continuous surface was created with ArcMap 9.0, using the Geostatistical Analyst extension (Johnston *et al.*, 2001). A Kriging function was used as interpo-

lation method using weights from a semi-variogram estimated through the spatial structure of the data (Oliver, 1990). Some grid cells included more than one specimen; thus, the mean for the trait in each grid cell was used. A number of statistical measures can be used to assess the performance of the Kriging interpolation (Table 2). For a model that provides accurate predictions, the mean error should be close to 0, the root-mean-square error and average standard error should be as small as possible, and the root-mean-square standardized error should be close to 1 (Johnston *et al.*, 2001).

Each standard trait map was reclassified into equal intervals between their respective maximum and minimum values for the trait. The reclassified surfaces were overlaid with a Boolean sea/land mask to remove areas outside the distribution area of *V. latastei-monticola* in the Iberian Peninsula, Africa and sea areas (Fig. 1). Noticeable spatial variation in traits was determined by visual inspection of surface maps and such traits were exported to Idrisi Kilimanjaro 14.01 (Clark Labs, 2003) using an equal grid cell size, resulting in maps with identical number of rows and columns (1426 × 2116).

##### *Spatial patterns in morphological variability*

To identify areas with multivariate clines and high morphological variability, a spatial principal components analysis (SPCA) was undertaken using the continuous morphological trait surfaces derived in the previous step. PCA is a procedure for finding structural relationships among specimens without a priori

subdivision into discrete groups (e.g. Brito *et al.*, 2006; Tomović, 2006). The SPCA maps were created using PCA function from Idrisi Kilimanjaro 14.01, which produces a new set of uncorrelated images (PC maps), which explain progressively less of the variance found in the original set of surface trait maps. PC maps were derived using only traits presenting spatial variation (see above). Trait surfaces were unstandardized and used variance/covariance matrixes, implying that the surface trait maps with a greater morphological variance had greater factor loadings in the SPCA (Kidd & Ritchie, 2000). For each PC map, the factor loadings were used as a measure of the association between surface trait maps and the total variance accounting for each eigenvalue was used to evaluate the analysis explanation level.

SPCA maps were used for aiding the identification of spatial patterns. The study area was partitioned into geographical areas (groups) according to the clines observed in the SPCA map that explained the highest percentage of morphological trait variability. These groups were tested for significance with discriminant function analysis (DFA). The discriminant functions computed by the DFA are the linear combinations of the original variables that maximized differences between given groups. DFA was performed to clarify the relative importance of such traits as discriminators between a priori groups and the relative positions of the centroids of those groups (Brito *et al.*, 2006). Only the traits with the highest factor loadings in the SPCA were included in the multivariate DFA. Classification into potential populations was tested using only specimens with all traits sampled ( $N = 215$ ); specimens with missing values for any trait in any population were excluded from the analysis. Males ( $N = 137$ ) and females ( $N = 78$ ) were analysed separately due to the significant sexual dimorphism in the number of the subcaudal scales (Brito *et al.*, 2006). A stepwise classification procedure was used to evaluate population membership in SPSS, version 13.0 (LeadTools, 2004). Reallocation with cross-validation was used to assess the distinctness of the specimen grouping by classifying each specimen through the functions derived from all specimens other than that specimen. The percentage of correct assignment of specimens to each population was taken as a measure of model robustness.

#### *Biogeographic patterns in morphological variability distribution*

The examined specimens were assigned into geographic areas in the ArcMap 9.0, following the same groups tested with DFA (see above) that were hypothesized from the SPCA maps. The accurate determination of the absence of *V. latastei-monticola* in a given region is complicated because the home range

size is very small, the active season is short, and individuals remain mostly inside bushes throughout the year (Saint-Girons, 1953; Brito, 2003a, b). Therefore, to detect biogeographic patterns in the distribution of morphological variability, the Maximum Entropy was used. This general-purpose machine learning method uses presence-only occurrence data and is particularly well suited to noisy or sparse information, as is typical of species occurrence data (Phillips, Dudík & Schapire, 2004; Phillips, Anderson & Schapire, 2006). Comparative analyses of model performance revealed that Maximum Entropy models consistently outperform more established methods, especially when samples sizes are low (Elith *et al.*, 2006; Hernandez *et al.*, 2006). A matrix with specimens-localities for each group and the set of EGVs were imported into Maxent 3.0.4 beta software (<http://www.cs.princeton.edu/~schapire/maxent>). From the available squares with presence records for each group, 80% were randomly selected as training data and 20% reserved for model testing.

Maxent estimates the range of groups by finding the distribution of maximum entropy (i.e. the most uniform distribution across the study area), with the constraint that the expected value of each EGV (or its transform and/or interactions) should match its empirical average (i.e. the average value for a set of sample points taken from the group-target distribution) (Phillips *et al.*, 2004, 2006). The program starts with a uniform probability distribution (gain = 0) and iteratively alters one weight at a time to maximize the likelihood of the occurrence dataset. The gain is a measure of the likelihood of the samples. The algorithm converges to the optimum probable distribution; thus, the gain can be interpreted as representing how much better the distribution fits the sample points than the uniform distribution does (Phillips *et al.*, 2004, 2006).

Maximum Entropy models were tested with receiver operated characteristics (ROC) plots. Absence data was selected uniformly at random by Maxent from the background squares (Phillips *et al.*, 2006). The area under the curve (AUC) of the ROC plot was taken as a measure of the overall fit of the model. The importance of each EGV for explaining the distribution of vipers was determined by jackknife analysis of the average gain with training and test data and also with AUC and average percent contribution of each EGV for the models (Phillips *et al.*, 2006). The relationship between occurrence of vipers and each EGV was determined by the examination of the profiles of response curves plots from univariate models.

To identify potential contact zones between groups in the Iberian Peninsula, the Maxent models were imported into ArcMap 9.0. The Maximum Entropy model classifies squares with a continuous value of

probability of occurrence between 0 and 100; thus, it was necessary to determine the threshold above which it is considered that the species is present. The tenth percentile training presence given by Maxent was chosen because 'true' absence data was not available, and models were reclassified to display the areas of probable absence and of probable presence for each group. To identify areas of probable contact zones between groups, these maps were overlaid in the GIS and the squares of probable presence common to both geographic groups represented probable sympatry.

## RESULTS

### SPATIAL PATTERNS IN MORPHOLOGICAL CHARACTER VARIATION

The continuous surface for the number of supralabial scales presented no spatial variation; therefore, this trait was excluded from further analysis. Several basic patterns of spatial variation in morphological traits could be observed. Some surfaces only exhibited characteristics of one of the basic patterns; others appear to be combinations of more than one type. There were four basic spatial patterns (Fig. 2) as outlined below.

#### (1) North-east/south-west cline in the Iberian Peninsula

Several traits exhibited apparent clines with a variety of locations, steepness and directions. All clines roughly separate south-western Iberia from the remaining area of the peninsula. Specimens from south-western Iberia presented low number of dorsal marks and of ventral scales (Fig. 2B, D), and high number of loreal scales (Fig. 2E). Also, there was a very sharp transition between western and eastern Iberia in the number of infralabial scales (Fig. 2C). Other relatively marked transitions were observed in the Iberian Central System axis (Malcata-Gata mountains) and the Spanish Guadalquivir River valley (for toponimies, see Fig. 1).

#### (2) Private character trends for the Moroccan High Atlas and for Eastern Algeria

Despite the low number of sampled localities, there was a trend for specimens from the High Atlas exhibiting exclusive trait states, such as the lowest number of rows of scales at midbody and the highest number of dorsal marks (Fig. 2A, B) and for specimens from Eastern Algeria to exhibit the lowest number of ventral scales and the highest number of loreal scales (Fig. 2D, E).

#### (3) Local maxima and minima for north-eastern and south-eastern Iberian Peninsula, respectively

Local maxima in the north-eastern were observed for several traits, including number of dorsal marks and

of subcaudal scales (Fig. 2B, F), and local minima in the south-eastern was observed in the number of infralabial and ventral scales (Fig. 2C, D).

#### (4) Congruent variation across the Strait of Gibraltar

Concordant transition across several characters was observed in specimens from both sides of the Strait of Gibraltar. However, variation was related either with Western or Eastern Iberian Peninsula. For example, similar patterns between Morocco and Western Iberia were observed for the number of loreal and ventral scales (Fig. 2D, E), whereas similar variation between Morocco and Eastern Iberia was observed for the number of dorsal marks and of infralabial scales (Fig. 2B, C).

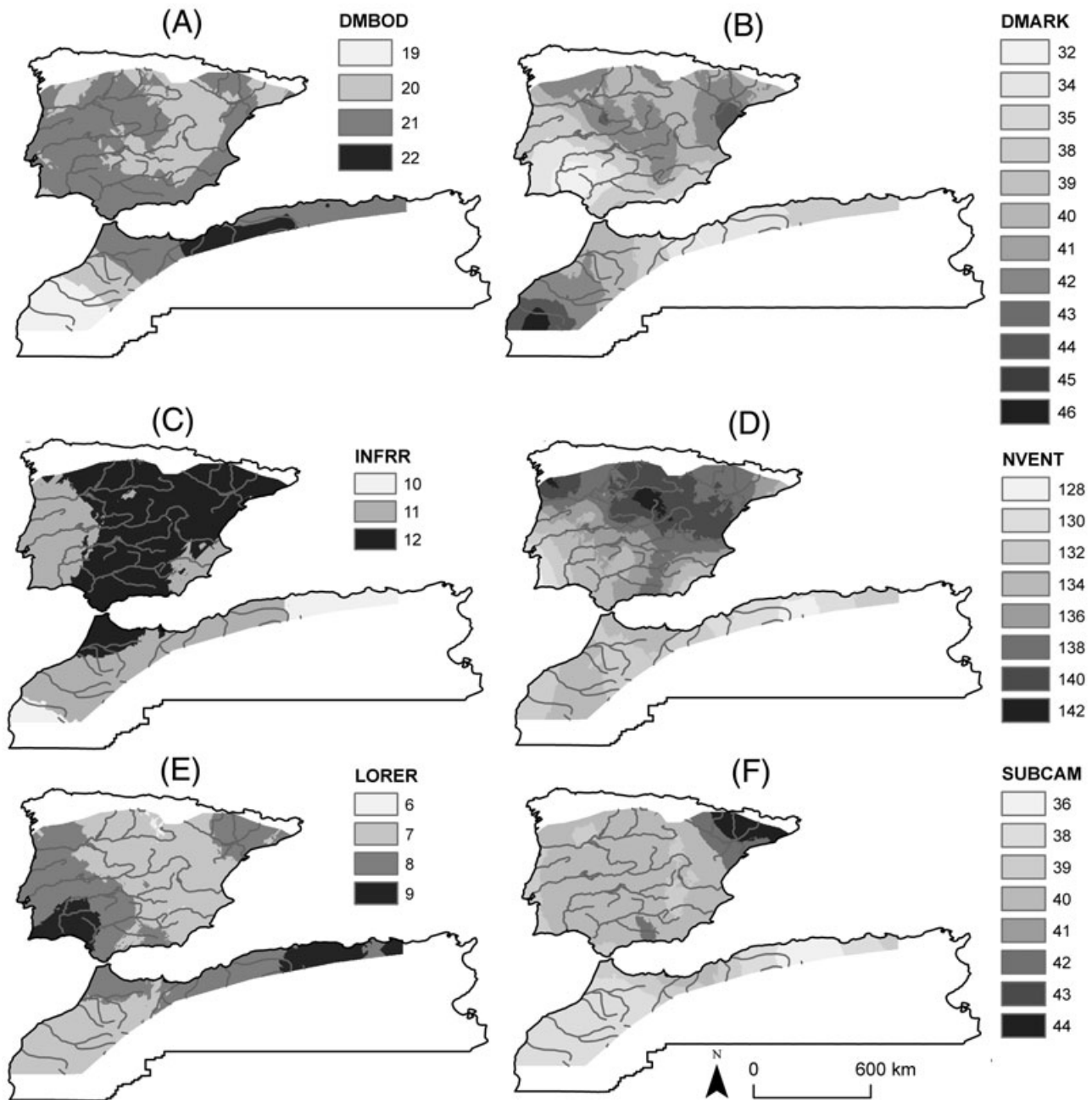
### SPATIAL PATTERNS IN MORPHOLOGICAL VARIABILITY

The variance accounted by the two most explicative dimensions, SPC1 and SPC2, on the SPCA were 87.7% and 3.4%, respectively. All traits explained a significant amount of variation of the SPC1 but, for the SPC2, only the number of ventral scales explained a marginal portion of the variation (Table 3). Several basic patterns of variation were observed with the SPC1: low values in western and south-eastern Iberia, eastern Algeria and High Atlas, high values in north-eastern Iberia, and intermediate values in the remaining areas (Fig. 3A). The SPC1 illustrates also steep clines along the High Atlas, across the Ebro

**Table 3.** Loading scores on the first two principal components extracted according to the spatial principal components analysis of individual surfaces of variation of morphological traits from *Vipera latastei* and *Vipera monticola*

Loading	SPC1	SPC2
APICA	0.796	-0.101
DMARK	0.961	-0.274
DMBOD	0.954	0.221
INFRR	0.964	-0.012
INTER	0.934	-0.017
LORER	0.954	0.272
PERIR	0.942	0.037
SUBCAF	0.943	0.037
SUBCAM	0.944	0.037
NVENT	0.901	-0.351

APICA, apical scales; DMARK, dorsal marks; DMBOD, dorsal scale rows at mid-body; INFRR, infralabial scales; INTER, intercantal plus intersupraocular scales; LORER, loreal scales; PERIR, periocular scales; SUBCAM, SUBCAF, subcaudal scales in males and females; NVENT, number of ventral scales, excluding preventrals and anal.

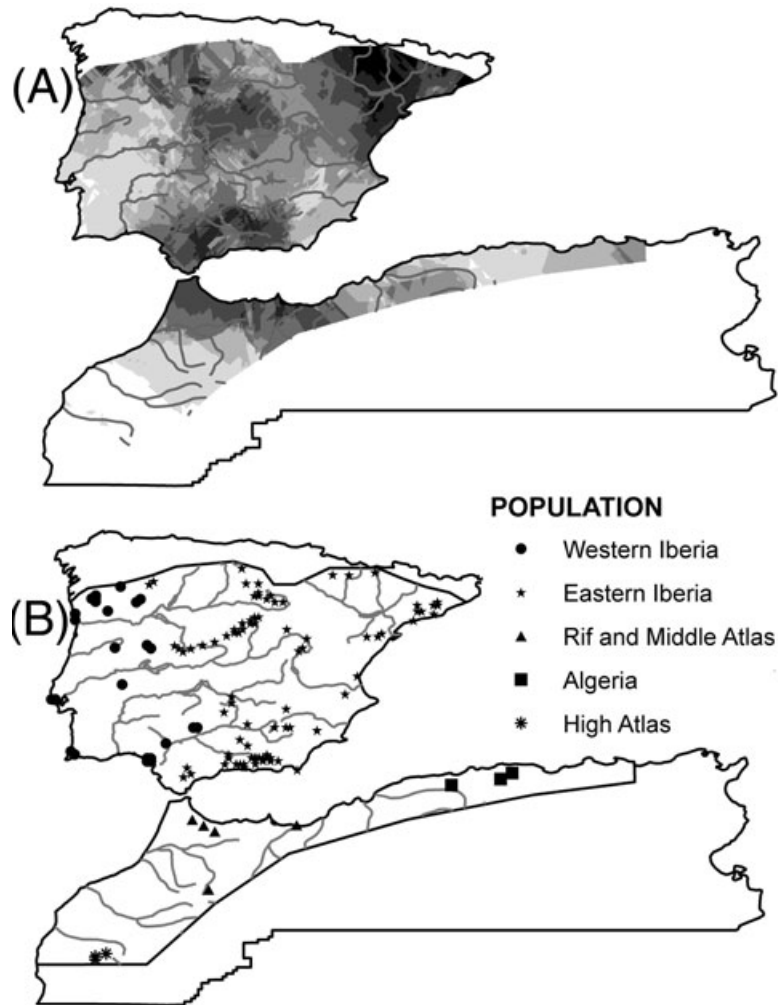


**Figure 2.** Spatial variation patterns of the morphological traits from *Vipera latastei* and *Vipera monticola* with higher loading scores in the spatial principal components analysis (see Table 3) (WGS84 projection). Continuous surfaces were derived using Kriging interpolation algorithm (see methods for details). A, number of scale rows at mid-body (DMBOD). B, number of dorsal marks (DMARK). C, number of infralabial scales (INFRR). D, number of ventral scales (NVENT). E, number of loreal scales (LORER). F, number of subcaudal scales in males (SUBCAM).

river valley, and also across a north/south axis, including the Trás os Montes-Sanabria axis, the Central System axis (Malcata–Gata) and Guadalquivir river valley, separating specimens from western Iberia of those from central and eastern Iberia. The SPC2 (not shown) presented a similar variation pattern with SPC1.

The high explained variance of SPC1 suggested that the greatest subdivisions within the study area concerned the SPC1 clines. To assess the validity of subdivisions, DFA was carried out on five hypothesized groups: Western Iberia, Eastern Iberia, Rif plus Middle Atlas, Algeria, and High Atlas (Fig. 3B). Two additional groups were also tested, south-eastern





**Figure 3.** Spatial principal component map (SPC1) for morphological variability of *Vipera latastei* and *Vipera monticola* (A) and population subdivision tested with discriminant function analysis (B). Each symbol in map (B) represents a UTM  $10 \times 10$  km cell for which specimens with complete morphological data were available (WGS84 projection).

Iberia and the Pyrenees, as suggested by the putative clines observed in SPC1 (Fig. 3A). However, correct classification rates for the specimens from these groups were low (less than 50%) and suggested their inclusion in the Eastern Iberia group.

The total correct classification of specimens into the five geographic groups was relatively high: 85% and 78% for males and females, respectively (Table 4). The cross-validation procedure yielded slightly lower correct classification rates: 80% and 64% for males and females, respectively. The best supported groups were the Rif and Middle Atlas, Algeria (females only) and High Atlas, with correct classification rates of 100%, although low sample size for these groups could have affected classification rates. For the Iberian Peninsula, the correct classification of the two groups ranged from 76% in Western Iberia to 82% in Eastern Iberia (sexes combined). However, there were

15% of misclassifications of specimens (sexes combined) from Western Iberia as Eastern Iberia, and 16% vice versa (Table 4). Trait variation among groups is presented in Appendix Table S1 in the Supporting information.

The first two discriminant axes explained 88.6% and 84.8% of the variability for males and females, respectively (Appendix Fig. S2). Although the sample size of African groups was low, the relative position of individual specimens and group centroids from the Rif and Middle Atlas, Algeria, and High Atlas males and females did not overlap with the remaining samples. The two Iberian groups were less clearly discriminated in both sexes. Although there was an evident separation in the relative position of group centroids, several specimens from Western Iberia fell inside the distribution cloud of specimens from Eastern Iberia, and vice versa.

**Table 4.** Number and percentage of *Vipera latastei* and *Vipera monticola* specimens classified in each group according to the first four canonical discriminant functions of morphological traits

Population	Sex	Western Iberia	Eastern Iberia	Rif and Middle Atlas	Algeria	High Atlas	<i>N</i>
Western Iberia	M	27 (87%)	4 (13%)	0	0	0	31
	F	11 (65%)	3 (18%)	3 (18%)	0	0	17
Eastern Iberia	M	14 (14%)	83 (84%)	2 (2%)	0	0	99
	F	8 (15%)	41 (79%)	3 (6%)	0	0	52
Rif and Middle Atlas	M	0	0	1 (100%)	0	0	1
	F	0	0	4 (100%)	0	0	4
Algeria	M	1 (33%)	0	0	2 (67%)	0	3
	F	0	0	0	3 (100%)	0	3
High Atlas	M	0	0	0	0	3 (100%)	3
	F	0	0	0	0	2 (100%)	2

Analyses were conducted for males and females separately due to significant sexual dimorphism in the number of subcaudal scales.

**Table 5.** Percent contribution and gain for each environmental factor with training data, Area Under the Curve (AUC) and number of presence records (*N*) for the Maximum Entropy models for each group of *Vipera latastei* and *Vipera monticola*

	Western Iberia	Eastern Iberia	Rif/Middle Atlas	Algeria	High Atlas
ALTIT	0.4–0.058	11.2–0.392	1.6–0.009	0.0–0.020	10.9–0.454
SLOPE	1.7–0.159	30.0–0.398 (+)	45.7–0.286 (+)	7.0–0.289 (+)	0.0–0.223
TSEAS	5.8–0.347	1.1–0.089	0.0–0.002	0.1–0.000	21.5–0.550 (–)
TMAXWM	9.3–0.443 (–)	0.8–0.257	0.0–0.017	0.0–0.014	0.0–0.118
TMINCM	1.9–0.142	1.8–0.353	0.0–0.000	0.0–0.139	5.4–0.282
TEMPAR	0.8–0.461	10.1–0.056	0.0–0.000	0.0–0.000	31.0–0.995 (+)
PSEAS	12.9–0.436 (+)	7.6–0.303	40.9–0.307 (+)	0.0–0.513	0.0–0.204
PRECWWM	66.7–1.084 (+)	12.6–0.257 (–)	0.0–0.026	50.8–0.838 (+)	1.064–0.047
PRECDM	0.4–0.074	24.9–0.375 (–)	11.8–0.236 (–)	42.1–0.714 (–)	30.2–0.660 (–)
Training AUC	0.952	0.902	0.860	0.973	0.997
Test AUC	0.885	0.847	0.812	0.960	0.959
<i>N</i> Training	43	117	8	3	4
<i>N</i> Test	9	29	1	1	1

For the three most explaining variables (for variable acronyms, see Table 1) for each group, the relationship between group occurrence and environmental variables is presented: +, positive; –, negative.

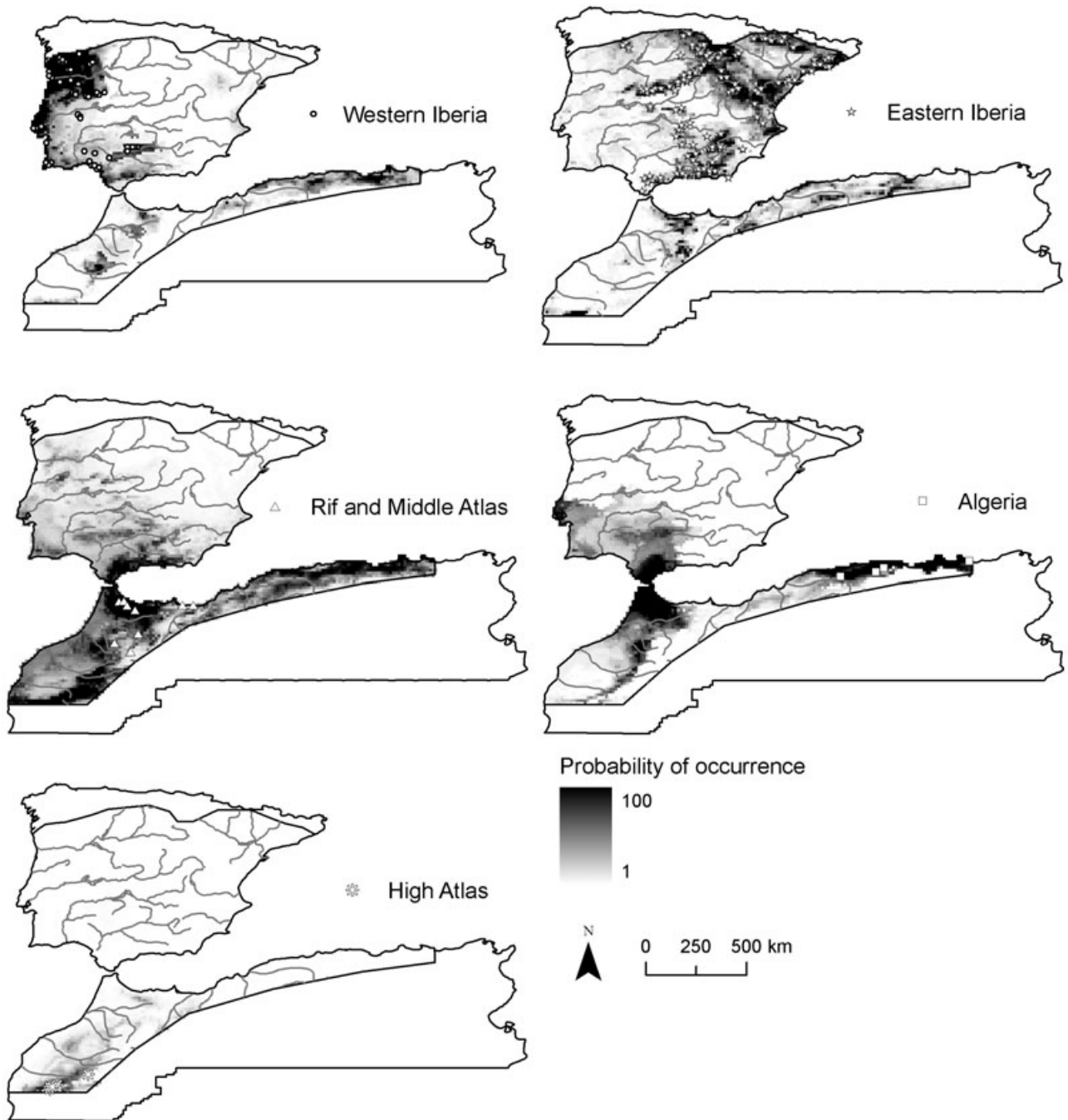
#### BIOGEOGRAPHIC PATTERNS IN MORPHOLOGICAL VARIABILITY DISTRIBUTION

The Maximum Entropy models identified a set of topographical and climatic EGVs explaining the distribution of groups (Table 5). The distribution of some groups was influenced by common EGVs, such as slope for Eastern Iberia, Algeria, and Rif and Middle Atlas, precipitation seasonality for Western Iberian and Rif and Middle Atlas, precipitation in the wettest month for Western and Eastern Iberia and Algeria, and precipitation in the driest month for all groups except Western Iberia (Table 5).

The analysis of EGV profiles by pairs of groups reveals that: (1) populations from Eastern Iberia, Algeria, and Rif and Middle Atlas occur in areas of high slope; (2) populations from Western Iberian and

Rif and Middle Atlas occur in areas of high precipitation seasonality; (3) populations from Western Iberia and Algeria occur in areas with high precipitation in the wettest month whereas populations from Eastern Iberia exhibit an opposite trend; (4) all African groups and Eastern Iberia occur in areas with low precipitation in the driest month (Table 5). The AUC for both training and test data was relatively high for all groups, suggesting a reasonable fit for all models (Table 5).

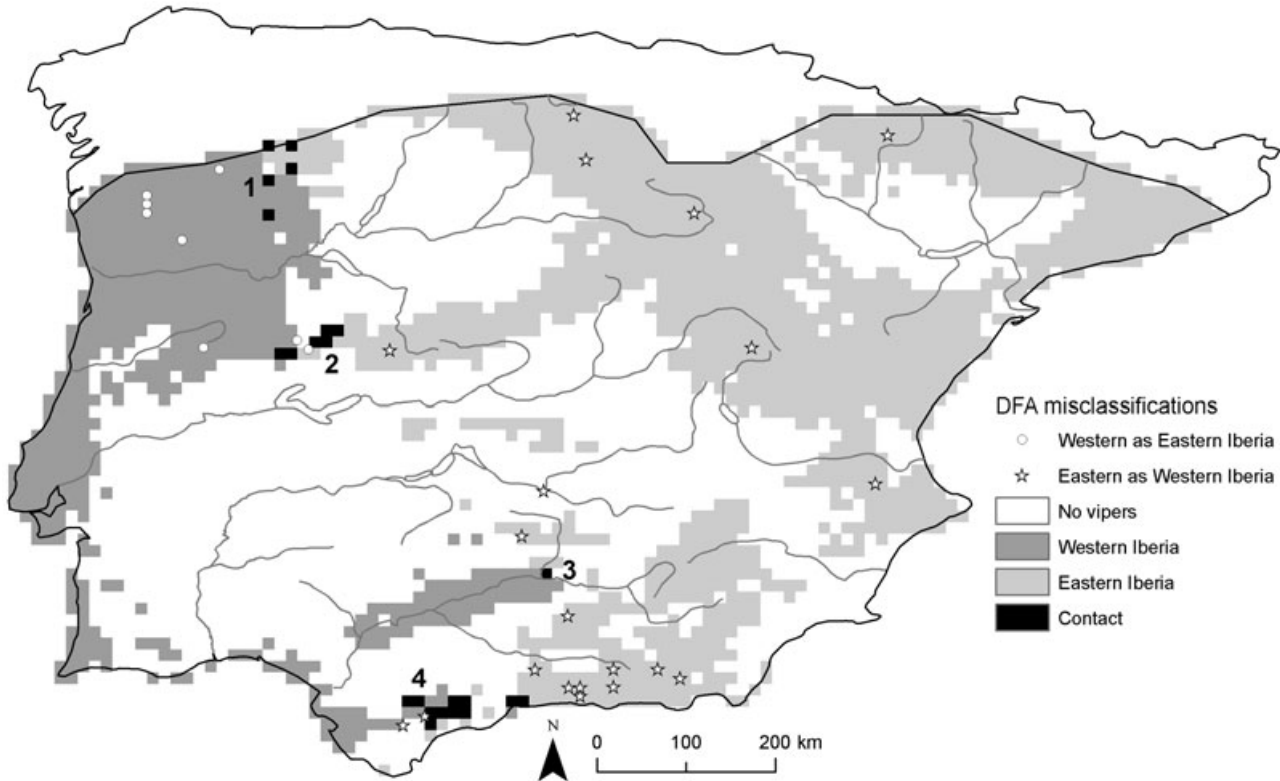
Areas of potential occurrence of geographic groups (Fig. 4) were identified for: (1) Western Iberia in continuous areas along western Iberian Peninsula, scattered patches corresponding to Moroccan mountain areas and in northern Algeria; (2) Eastern Iberia along the mountain ranges of eastern Iberian Penin-



**Figure 4.** Probability of occurrence for each group of *Vipera latastei* and *Vipera monticola* suggested by SPCA (Figs 2, 3A), derived by Maximum Entropy at a  $10 \times 10$  km scale (WGS84 projection).

sula and northern Africa; (3) Rif and Middle Atlas in continuous areas from Algeria to the High Atlas but also across the Strait of Gibraltar to south-western Iberian Peninsula and Almeria region; (4) Algeria, in scattered mountain areas of northern Africa and also across the Strait of Gibraltar to south-western Iberian Peninsula; and (5) High Atlas exclusively in high altitude peaks of the High Atlas (Fig. 4).

Areas of probable sympatry between Iberian groups were identified for: (1) Trás os Montes–Sanabria axis, including the mountains of Bornes, Nogueira, Montesinho, Culebra, and Sanabria; (2) Central System axis, including the mountains of Malcata and Gata; (3) Guadalquivir–Jándula river valleys; and (4) Cadiz–Malaga axis, including the mountains of Aljibe, Grazalema, Bermeja, and



**Figure 5.** Location of potential contact zones between Western and Eastern Iberia groups of *Vipera latastei* at a  $10 \times 10$  km scale (WGS84 projection) according to Maximum Entropy and location of Iberian misclassified specimens by the discriminant function analysis. 1 – Trás os Montes–Sanabria axis; 2 – Malcata–Gata Mountains; 3 – Guadalquivir–Jándula river valleys; 4 – Cadiz–Malaga axis.

Ronda (Fig. 5). Approximately 61% of the Iberian specimens misclassified by the DFA (Table 4) are located within areas of probable sympatry or in the vicinities of these areas (Fig. 5).

## DISCUSSION

### SPATIAL VARIATION PATTERNS IN MORPHOLOGICAL VARIABILITY

The present study demonstrates that geostatistics and GIS are powerful tools for studying evolutionary patterns across complex biogeographical scenarios including interrupted ranges and asymmetrical sample sizes. This innovative approach should be applied to other complex biogeographical scenarios because it can identify: (1) spatial variation patterns in morphological characters, such as clines or regions with private character trends; (2) spatial patterns of morphological variability, such as regions with multivariate clines and high morphological differentiation, and abrupt clines between stable regions; and (3) biogeographic patterns of morphological variability distribution, such as environmental factors limiting the distribution of differentiated groups, areas for

the potential occurrence of each group and for contact among groups. However, spatially biased samples affected predictions and artefacts should be taken into account when interpreting patterns of morphological variation. These artefacts were a consequence of both the uneven distribution of sample locations and the interpolation algorithm (Fig. 2). For example, artificial clinal variation patterns were observed along the Moroccan Atlas Mountains and trends for private trait states in western Algeria, as a consequence of the lacking of sampling points in these regions (compare with Fig. 1). Artefact patterns were also observed in the SPC map (Fig. 3A) and resulted from the accumulation of artefacts in the individual surfaces of morphological variation.

The individual surfaces and SPCA revealed four general patterns of morphological variation.

#### *Pattern 1*

A multivariate cline, including number of dorsal marks and of ventral, loreal and infralabial scales, divided the Iberian Peninsula along a north-east/south-west cline (Figs 2, 3A). In some locations, the cline was abrupt and corresponded to natural



barriers, such as the Sanabria-Trás os Montes axis of mountains, the Malcata–Gata Mountains in the Iberian Central System axis and the valley of Guadalquivir–Jándula rivers in Andalusia. DFA gave a reasonable support for Iberian subdivision and ecological niche modelling evidenced that groups have coherent probable occurrence areas. Probable contact zones between Western and Eastern Iberian groups spatially matched the areas of morphological sharp transition and were located in the Trás os Montes–Sanabria axis, Malcata–Gata Mountains, Guadalquivir–Jándula river valleys and Cadiz–Malaga axis. Different environmental factors affect the range of Iberian groups, maximum temperature of the warmest month and precipitation seasonality for Western Iberia and slope and precipitation of the driest month for Eastern Iberia, suggesting distinct environmental sources of adaptation. Moreover, precipitation of the wettest month is related with the distribution of both groups but exhibiting opposite relationships: positive with Western and negative with Eastern Iberia. However, the present range of *V. latastei* in the Iberian Peninsula has been strongly modified by human activities and the species is now fragmented and restricted mostly to mountainous areas (Santos *et al.*, 2006). Thus, separating environmental adaptation from human-related fragmentation is a difficult task and caution should be taken when interpreting evolutionary scenarios for this species.

#### Pattern 2

Private character trends for the Moroccan High Atlas and Eastern Algeria isolated these groups along putative multivariate clines. For the High Atlas, a cline was suggested for the region of the el Abid river valley and included the lowest number of scale rows at midbody and the highest number of dorsal marks. DFA and ecological niche modelling further suggested strong morphological differentiation and habitat adaptation for these groups. Temperature seasonality, temperature annual range, and precipitation of the driest month restricted the probable range of this group to the highest points of the Atlas, south of el Abid river valley. Unfortunately, the rarity of these vipers along the Atlas Mountains excludes the use of ecological-niche modelling for detecting contact zones between High and Rif-Middle Atlas populations. Individual surfaces and SPCA map also suggested high differentiation for Eastern Algeria, with specimens from this area presenting the lowest number of ventral and highest number of loreal scales. Although the artefact patterns observed difficult inferences at this stage, Saint-Girons (1977) and Brito *et al.* (2006) suggested that Algerian populations represented an

undescribed taxon due to the high morphological differentiation of these populations.

#### Pattern 3

Local maxima or minima in north-eastern Iberian Peninsula roughly separated this group from the remaining ones along a putative cline. Specimens from this area exhibited the highest number of ventral and lowest number of loreal scales, a trend approaching these populations from the sister-taxon *Vipera aspis* (Linnaeus, 1758). In north-eastern Iberia, where the ranges of *V. aspis* and *V. latastei* meet, specimens with intermediate morphological characteristics (similar scale counts and colour pattern) are found in syntopic populations (Martínez-Freiría *et al.*, 2008). The putative cline for this *V. latastei* population was not supported by DFA; but, probable introgression with *V. aspis* should be investigated with genetic markers.

#### Pattern 4

Congruent variation patterns across the Strait of Gibraltar were observed for the number of loreal, infralabial and ventral scales, and number of dorsal marks. This would be the reason for frequent DFA misclassification of specimens from both Iberian groups into African groups. Ecological niche modelling further showed that similar environmental factors affected the potential range of *V. latastei* groups across the Strait of Gibraltar (Fig. 4).

#### BIOGEOGRAPHY OF *V. LATASTEI* AND *V. MONTICOLA*

The opening of the Strait of Gibraltar (approximately 5.33 Mya) separated Iberian and African populations. This event caused considerable genetic differentiation in several taxa (Busack, 1986; Carranza & Arnold, 2004; Albert, Zardoya & García-París, 2007). Nevertheless, populations of *V. latastei* across the Strait of Gibraltar presented similar morphological variation patterns. At least two hypotheses can be addressed to explain this scenario: (1) populations on both sides of the Strait might have evolved under similar environmental conditions, which lead to similar morphology as an adaptation to local conditions. Indeed, ecological niche modelling showed that similar environmental factors affected the range of populations across the Strait and that areas favourable for the occurrence of Iberian populations spanned over African mountain areas, and vice versa; (2) gene flow among populations across the Strait might have occurred until recently, as observed in other trans-Mediterranean snakes (Carranza *et al.*, 2006a). During the Quaternary climatic oscillations, the sea level descended and the maximum gap between Iberia and Africa was approximately 3.5 km (Zazo *et al.*, 2000). Never-

theless, *V. latastei* has very small home range size and limited dispersal capacities (Brito, 2003a), which might hamper latter colonization events by rafting. Most likely, *V. latastei* colonized North Africa during the Miocene period through a land bridge across the site of the Strait of Gibraltar, as suggested by the presence of *aspis*-like fossils (*V. maghrebiana*) from the Moroccan middle Miocene (approximately 7–8 Mya; Szyndlar & Rage, 2002), and remained in contact until the opening of the Strait.

Population refugia during the Quaternary climate shifts followed by post-glacial expansion and secondary contact are the most likely causes for the multivariate clines observed within the Iberian Peninsula. During the cold periods, multiple refugia occurred probably in the south-western Atlantic coast and in the south-eastern mountains, with environmental adaptation and probably genetic drift. This pattern of 'refugia within refugia' is consistent with other Iberian flora and fauna (Gómez & Lunt, 2006; Albert *et al.*, 2007; Santos *et al.*, 2008).

When conditions ameliorated, probably there was range expansion and secondary contact occurred along intermediate regions, such as the Sanabria-Trás os Montes axis of mountains, the Malcata-Gata Mountains and Guadalquivir-Jándula rivers valleys. The relatively constant DFA misclassification (around 15%) of specimens from the two Iberian groups with each other could be related with gene flow and morphological convergence in contact zones, as suggested also for *V. aspis* in Western Europe (Zuffi, 2002) and *Vipera ammodytes* (Linnaeus, 1758) in the Balkans (Tomović, 2006). In fact, probable contact zones between Western and Eastern Iberian populations roughly matched the areas of morphological sharp transition. Patterns of introgression across hybrid zones have been detected for these regions in other vertebrates, including lizards (*Lacerta schreiberi*; Godinho *et al.*, 2006) and rabbits (*Oryctolagus cuniculus*; Gerales, Ferrand & Nachman, 2006).

The small sample size for African groups precludes extensive biogeographic inferences. Nevertheless, two patterns could be suggested: (1) populations from the High Atlas might have been isolated during the Quaternary and probably suffered extreme environmental adaptations. Differentiation under isolation should be also responsible for the occurrence of other mountain endemics in the Atlas, including geckos (*Quedenfeldtia trachyblepharus*) and lizards (*Atlantolacerta andreanszkyi*); (2) populations from northern Morocco might have been isolated from Algerian populations as a consequence of Pliocene sub-Riffain marine transgression (Duggen *et al.*, 2003) or more recent Plio-Pleistocene climatic oscillations (Claussen *et al.*, 2003). Fluctuations between humid and hyper-arid

phases appear to have affected cladogenesis processes in several taxa, including false-smooth snakes (*Macroprotodon*; Carranza *et al.*, 2004) and white-toothed shrews (*Crocidura*; Cosson *et al.*, 2005) and could explain the broad multivariate cline observed in the region of Oran, between western and eastern regions of the Moulouya River.

#### SYSTEMATIC INFERENCES

Previous taxonomic works reported the existence of two subspecies of *V. latastei* in the Iberian Peninsula, *latastei* and *gaditana* (Saint-Girons, 1977; Brito *et al.*, 2006). Current analyses show that Pattern 1 of morphological variation broadly matches the suggested distributions for the *latastei* and *gaditana* subspecies, but the range of *gaditana* is larger than previously reported, crossing north of Mondego river valley and reaching the Peneda-Gerês Mountains of northern Portugal. Beerli *et al.* (1986) proposed that populations of the High Atlas were highly differentiated and ascribed them to *V. monticola*. Current analysis support environmental adaptation for these populations and Pattern 2 matches the proposed distribution for *V. monticola*. It has been suggested that Algerian populations might belong to an undescribed taxa (Saint-Girons, 1977; Brito *et al.*, 2006). The putative clines observed in the present analysis also supported this hypothesis, but low sample size precludes further inferences.

The innovative techniques presented in the present study are a new step in our interest to perform an integrative approach to solve the systematics and taxonomy of these vipers. The future combination of morphological and molecular data in a geographical context should provide clearer and stronger evolutionary insights, as has been demonstrated previously for several taxonomic groups (Wüster *et al.*, 1995; Giribet, Edgecombe & Wheeler, 2001).

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## REFERENCES

- Albert EM, Zardoya R, García-París M. 2007.** Phylogeographical and speciation patterns in subterranean worm lizards of the genus *Blanus* (Amphisbaenia: Blanidae). *Molecular Ecology* **16**: 1519–1531.
- Arif S, Adams DC, Wicknick JA. 2007.** Bioclimatic modeling, morphology, and behaviour reveal alternative mechanisms regulating the distributions of two parapatric salamander species. *Evolutionary Ecology Research* **9**: 843–854.
- Beerli P, Billing H, Schätti B. 1986.** Taxonomischer status von *Vipera latasti monticola* Saint Girons, 1953 (Serpentes: Viperidae). *Salamandra* **22**: 101–104.
- Brito JC. 2003a.** Seasonal variation in movements, home range and habitat use by male *Vipera latastei* in northern Portugal. *Journal of Herpetology* **37**: 155–160.
- Brito JC. 2003b.** Seasonal and daily activity patterns of *Vipera latastei* in northern Portugal. *Amphibia-Reptilia* **24**: 497–508.
- Brito JC, Crespo EG. 2002.** Distributional analysis of two vipers (*Vipera latastei* and *V. seoanei*) in a potential area of sympatry in the Northwestern Iberian Peninsula. In: Schuett GW, Höggren M, Douglas ME, Greene HW, eds. *Biology of the vipers*. Eagle Mountain, UT: Eagle Mountain Publishing, 129–138.
- Brito JC, Santos X, Pleguezuelos JM, Fahd S, Llorente GA, Parellada X. 2006.** Morphological variability of the Lataste's viper (*Vipera latastei*) and the Atlas dwarf viper (*Vipera monticola*): patterns of biogeographical distribution and taxonomy. *Amphibia-Reptilia* **27**: 219–240.
- Brown RP, Thorpe RS. 1991.** Description of within-island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus*, with testing of causal hypothesis. *Biological Journal of the Linnean Society* **44**: 47–64.
- Busack SD. 1986.** Biochemical and morphological differentiation on Spanish and Moroccan populations of *Discoglossus* with the description of a new species from Southern Spain (Amphibia, Anura, Discoglossidae). *Annals of Carnegie Museum* **55**: 41–61.
- Carranza S, Arnold EN. 2004.** History of West Mediterranean newts, *Pleurodeles* (Amphibia: Salamandridae), inferred from old and recent DNA sequences. *Systematics and Biodiversity* **1**: 327–337.
- Carranza S, Arnold EN, Pleguezuelos JM. 2006a.** Phylogeny, biogeography, and evolution of two Mediterranean snakes, *Malpolon monspessulanus* and *Hemorrhois hippocrepis* (Squamata, Colubridae), using mtDNA sequences. *Molecular Phylogenetics and Evolution* **40**: 532–546.
- Carranza S, Arnold EN, Wade E, Fahd S. 2004.** Phylogeography of the false smooth snakes, *Macroprotodon* (Serpentes, Colubridae): mitochondrial DNA sequences show European populations arrived recently from Northwest Africa. *Molecular Phylogenetics and Evolution* **33**: 523–532.
- Carranza S, Harris DJ, Arnold EN, Batista V, González de la Vega JP. 2006b.** Phylogeography of the lacertid lizard, *Psammodromus algirus*, in Iberia and across the Strait of Gibraltar. *Journal of Biogeography* **33**: 1279–1288.
- Clark Labs. 2003.** *Idrisi Kilimanjaro* Version 14.01. Worcester, MA: Clark Labs, Clark University.
- Claussen M, Brovkin V, Ganopolski A, Kubatzki C, Petoukhov V. 2003.** Climate change in northern Africa: the past is not the future. *Climatic Change* **57**: 99–118.
- Cosson J-F, Hutterer R, Libois R, Sarà M, Taberlet P, Vogel P. 2005.** Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic fluctuations in the western Mediterranean: a case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). *Molecular Ecology* **14**: 1151–1162.
- Cushman SA, McKelvey KS, Hayden J, Schwartz MK. 2006.** Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *American Naturalist* **168**: 486–499.
- Dawson AG. 1996.** *Ice age earth: late quaternary geology and climate*. New York, NY: Routledge.
- De Jong H. 1998.** In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. *Biological Journal of the Linnean Society* **65**: 99–164.
- Dolfus RP, Beaurieux C. 1928.** Tableau pour la détermination facile des serpents du Maroc. *Variétés Scientifiques de la Société des Sciences Naturelles et Physiques du Maroc* **1**: 1–29.
- Duggen S, Hoernle K, van den Bogaard P, Rupke L, Morgan JP. 2003.** Deep roots of the Messinian salinity crisis. *Nature* **422**: 602–606.
- ESRI. 1996.** *ArcAtlas: our earth*. Redlands, CA: Environmental Systems Research Institute, Inc and DATA+.
- ESRI. 2004.** *ArcMap 9.0*. Redlands, CA: Environmental Systems Research Institute, Inc.
- Elith J, Graham CH, Anderson RP, Dudyk M, Freer S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, McOverton J, Peterson AT, Phillips S, Wisz MS, Zimmermann NE. 2006.** Novel methods improve prediction of species' distributions from occurrence data. *Ecography* **29**: 129–151.
- Fahd S, Barata M, Benítez M, Brito JC, Caro J, Carvalho S, Chiroso M, Feriche M, Herrera T, Márquez-Ferrando R, Nesbitt D, Pleguezuelos JM, Reques R, Rodríguez MP, Santos X, Sicilia M, Vasconcelos R. 2007.** Presencia de la víbora hocicuda *Vipera latastei* en el Atlas Medio (Marruecos) y otras citas herpetológicas para la región. *Boletín de la Asociación Herpetológica Española* **18**: 26–34.
- Fahd S, Benítez M, Brito JC, Caro J, Chiroso M, Feriche M, Fernández-Cardenete JR, Martínez-Freira F, Márquez-Ferrando R, Nesbitt D, Pleguezuelos JM, Reques R, Paz Rodríguez M, Santos X, Sicilia M. 2005.** Distribución de *Vipera latastei* en el Rif y otras citas her-



- petológicas para el norte de Marruecos. *Boletín de la Asociación Herpetológica Española* **16**: 19–25.
- García-París M, Alcobendas M, Buckley D, Wake DB. 2003.** Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution* **57**: 129–143.
- Garrigues T, Dauga C, Ferquel E, Choumet V, Failloux A-B. 2005.** Molecular phylogeny of *Vipera* Laurenti, 1768 and the related genera *Macrovipera* (Reuss, 1927) and *Daboia* (Gray, 1842), with comments about neurotoxic *Vipera aspis aspis* populations. *Molecular Phylogenetics and Evolution* **35**: 35–47.
- Gaubert P, Fernandes CA, Bruford MW, Veron G. 2004.** Genets (Carnivora, Viverridae) in Africa: an evolutionary synthesis based on cytochrome b sequences and morphological characters. *Biological Journal of the Linnean Society* **81**: 589–610.
- Geraldes A, Ferrand N, Nachman MW. 2006.** Contrasting patterns of introgression at X-linked loci across the hybrid zone between subspecies of the European Rabbit (*Oryctolagus cuniculus*). *Genetics* **173**: 919–933.
- Giribet G, Edgecombe GD, Wheeler WC. 2001.** Arthropod phylogeny based on eight molecular loci and morphology. *Nature* **413**: 157–161.
- Godinho R, Mendonça B, Crespo EG, Ferrand N. 2006.** Genealogy of the nuclear *b-fibrinogen* locus in a highly structured lizard species: comparison with mtDNA and evidence for intragenic recombination in the hybrid zone. *Heredity* **96**: 454–463.
- Guillaumet A, Pons JM, Godelle B, Crochet PA. 2006.** History of the Crested Lark in the Mediterranean region as revealed by mtDNA sequences and morphology. *Molecular Phylogenetics and Evolution* **39**: 645–656.
- Gómez A, Lunt DH. 2006.** Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: Weiss S, Ferrand N, eds. *Phylogeography of Southern European Refugia*. Dordrecht: Springer, 155–188.
- Hernandez PA, Graham CH, Master LL, Albert DL. 2006.** The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* **29**: 773–785.
- Hewitt GM. 1996.** Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247–276.
- Hewitt GM. 1999.** Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* **68**: 87–112.
- Hewitt GM. 2000.** The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005.** Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.
- Hoffmann MH, Glab AS, Tomiuk J, Schmuths H, Fritsch RM, Bachmann K. 2003.** Analysis of molecular data of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) with Geographical Information Systems (GIS). *Molecular Ecology* **12**: 1007–1019.
- Johnston K, Ver Hoef JM, Krivoruchko K, Lucas N. 2001.** *Using ArcGIS geostatistical analyst*. Redlands, CA: Environmental Systems Research Institute, Inc.
- Kidd DM, Ritchie MG. 2000.** Inferring the patterns and causes of geographic variation in *Ephippiger ephippiger* (Orthoptera, Tettigoniidae) using Geographical Information Systems (GIS). *Biological Journal of the Linnean Society* **71**: 269–295.
- Kidd DM, Ritchie MG. 2001.** A Geographical Information Science (GIS) approach to exploring variation in the bush-cricket *Ephippiger ephippiger*. In: Millington AC, Walsh SJ, Osborne PE, eds. *GIS and remote sensing applications in biogeography and ecology*. Dordrecht: Kluwer, 193–211.
- Kidd DM, Ritchie MG. 2006.** Phylogeographic information systems: putting the geography into phylogeography. *Journal of Biogeography* **33**: 1851–1865.
- Knouft JH, Losos JB, Glor RE, Kolbe JJ. 2006.** Phylogenetic analysis of the evolution of the niche in lizards of the *Anolis sagrei* group. *Ecology* **87**: S29–S38 (supplement).
- LeadTools. 2004.** *SPSS 13.0*. Chicago: Lead Technologies, Inc.
- Lenk P, Kalyabina S, Wink M, Joger U. 2001.** Evolutionary relationships among the True Vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **19**: 94–104.
- Martínez-Freiría F, Sillero N, Lizana M, Brito JC. 2008.** GIS-based niche models identify environmental correlates sustaining a contact zone between three species of European vipers. *Diversity and Distributions* **14**: 452–461.
- Martínez-Solano I, Teixeira J, Buckley D, García-París M. 2006.** Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): evidence for old, multiple refugia in an Iberian endemic. *Molecular Ecology* **15**: 3375–3388.
- Mateo JA, Pleguezuelos JM, Fahd S, Geniez P, Martínez-Medina FJ. 2003.** *Los anfibios, los reptiles y el Estrecho de Gibraltar. Un ensayo sobre la herpetofauna de Ceuta y su entorno*. Ceuta: Instituto de Estudios Ceutíes.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000.** Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.
- Oliver MA. 1990.** Kriging: a method of interpolation for geographical information systems. *International Journal of Geographic Information Systems* **4**: 313–332.
- Perera A, Vasconcelos R, Harris DJ, Brown RP, Carretero MA, Pérez Mellado V. 2007.** Complex patterns of morphological and mtDNA variation in *Lacerta perspicillata* (Reptilia; Lacertidae). *Biological Journal of the Linnean Society* **90**: 479–490.
- Phillips SJ, Anderson RP, Schapire RE. 2006.** Maximum Entropy modeling of species geographic distributions. *Ecological Modelling* **190**: 231–259.
- Phillips SJ, Dudík M, Schapire RE. 2004.** A maximum entropy approach to species distribution modeling. In: *Proceedings of the 21st International Conference on Machine Learning*. New York, NY: ACM Press, 655–662.
- Pillet JM. 1994.** Nouvelles données sur la répartition et l'écologie de la Vipère naine du Haut Atlas *Vipera monticola*



- Saint Girons, 1954 (Reptilia, Viperidae). *Revue suisse de Zoologie* **101**: 645–653.
- Saint-Girons H. 1953.** Une vipère naine: *Vipera latastei montana*. *Bulletin de la Société Zoologique de France* **78**: 24–28.
- Saint-Girons H. 1977.** Systématique de *Vipera latastei latastei* Bosca, 1878 et description de *Vipera latastei gaditana*, subsp. n. (Reptilia, Viperidae). *Revue suisse de Zoologie* **84**: 599–607.
- Saint-Girons H. 1978.** Morphologie externe comparée et systématique des vipères d'Europe (Reptilia, Viperidae). *Revue suisse de Zoologie* **85**: 565–595.
- Saint-Girons H. 1980.** Biogéographie et évolution des vipères européennes. *Comptes Rendus de la Société de Biogéographie* **496**: 146–172.
- Santos X, Brito JC, Sillero N, Pleguezuelos JM, Llorente GA, Fahd S, Parellada X. 2006.** Inferring habitat-suitability areas with ecological modelling techniques and GIS: a contribution to assess the conservation status of *Vipera latastei*. *Biological Conservation* **130**: 416–425.
- Santos X, Roca J, Pleguezuelos JM, Donaire D, Carranza S. 2008.** Biogeography and evolution of the smooth snake *Coronella austriaca* (Serpentes: Colubridae) in the Iberian Peninsula: evidence for Messinian refuges and Pleistocene range expansions. *Amphibia-Reptilia* **29**: 35–47.
- Spear SF, Peterson CR, Matocq MD, Storfer A. 2005.** Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology* **14**: 2553–2564.
- Swenson NG. 2006.** GIS-based niche models reveal unifying climatic mechanisms that maintain the location of avian hybrid zones in a North American suture zone. *Journal of Evolutionary Biology* **19**: 717–725.
- Szyndlar Z, Rage J. 2002.** Fossil record of the True vipers. In: Schuett GW, Höggren M, Douglas ME, Greene HW, eds. *Biology of the vipers*. Eagle Mountain, UT: Eagle Mountain Publishing, 419–444.
- Thorpe RS. 1987a.** Clines: character number and the multivariate analysis of simple patterns of geographic variation. *Biological Journal of the Linnean Society* **26**: 201–214.
- Thorpe RS. 1987b.** Complex clines: the predictivity of complicated patterns of geographic variation portrayed by multivariate analysis. *Biological Journal of the Linnean Society* **31**: 75–88.
- Thorpe RS. 1996.** The use of DNA divergence to help determine the correlates of evolution of morphological characters. *Evolution* **50**: 524–531.
- Thorpe RS, Brown RP, Malhotra A, Wüster W. 1991.** Geographic variation and population systematics: distinguishing between ecogenetics and phylogenetics. *Bolletino di Zoologia* **58**: 329–335.
- Thorpe RS, Báez M. 1987.** Geographic variation within an island: univariate and multivariate contouring of scalation, size and shape of the lizard, *Gallotia galloti*. *Evolution* **41**: 256–268.
- Tomović L. 2006.** Systematics of the Nose-horned viper (*Vipera ammodytes* Linnaeus, 1758). *Herpetological Journal* **16**: 191–201.
- USGS. 2006.** GTOPO30 – global digital elevation model. Available at <http://edc.usgs.gov/products/elevation/gtopo30/gtopo30.html>
- Weiss S, Ferrand N, eds. 2007.** *Phylogeography of southern European refugia*. Dordrecht: Springer.
- Wettstein O. 1933.** Bemerkungen zur reptilienfauna SW-Marokkos. *Zoologischer Anzeiger* **105**: 62–63.
- Wüster W, Thorpe RS, Cox MJ, Jintakune P, Nabhitabhata J. 1995.** Population systematics of the snake genus *Naja* (Reptilia: Serpentes: Elapidae) in Indochina: multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology* **8**: 493–510.
- Zazo C, Goy JL, Hillaire-Marcel J, Lario J, Dabrio J, Hoyos CJ, Bardaji M, Silva PG, Somoza L. 2000.** The record of highstand sea-level during the last interglacials. In: Finlayson C, Finslayson G, Fa D, eds. *Gibraltar during the Quaternary: the southernmost part of Europe in the last two million years*. *Gibraltar Government Heritage Publications Monographs n°1*: 87–92.
- Zuffi MAL. 2002.** A critique of the systematic position of the asp viper subspecies *Vipera aspis aspis* (Linnaeus, 1758), *Vipera aspis atra* Meisner, 1820, *Vipera aspis francisciredi* Laurenti, 1768, *Vipera aspis hugyi* Schinz, 1833 and *Vipera aspis zinnikeri* Kramer, 1958. *Amphibia-Reptilia* **23**: 191–213.

## SUPPORTING INFORMATION

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

**Figure S1.** Relative position of individual male and female *Vipera latastei* and *Vipera monticola* belonging to five populations (1 – Western Iberia; 2 – Eastern Iberia; 3 – Rif and Middle Atlas; 4 – Algeria; 5 – High Atlas) in the projection of the first two canonical variates of a Discriminant Function Analysis of morphological traits.

**Table S1.** Descriptive statistics of meristic traits of *Vipera latastei* and *Vipera monticola*.

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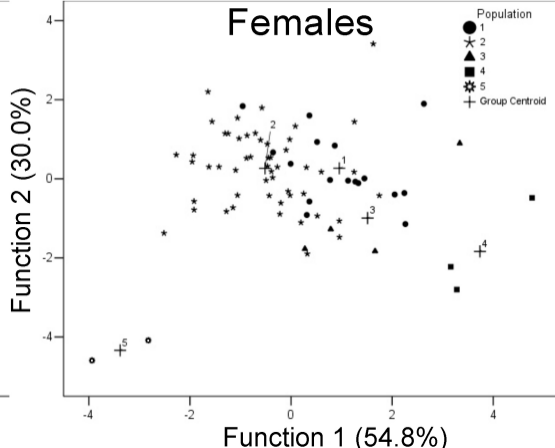
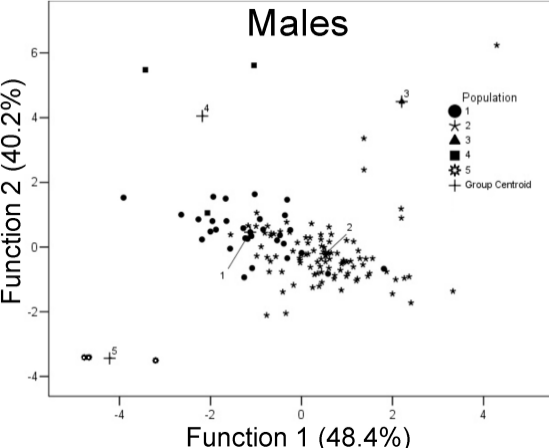


Table S1

Trait	Population	N	Mean	SD	Minimum	Maximum	Kruskal Wallis, H	df	p
APICA	Western Iberia	189	4.7	1.0	3	8	24.146	4	<0.001
	Eastern Iberia	363	4.9	1.1	2	9			
	Rif and Middle Atlas	10	6.9	2.1	4	11			
	Algeria	13	5.1	1.7	3	8			
	High Atlas	9	4.2	0.8	3	5			
DMARK	Western Iberia	95	37.2	6.5	26	52	45.614	4	<0.001
	Eastern Iberia	285	41.5	5.8	27	59			
	Rif and Middle Atlas	5	39.8	4.1	33	44			
	Algeria	7	34.0	5.3	27	41			
	High Atlas	7	50.0	6.1	39	59			
DMBOD	Western Iberia	185	21.0	0.5	19	23	215.482	4	<<0.001
	Eastern Iberia	374	21.0	0.5	19	25			
	Rif and Middle Atlas	10	21.4	0.8	21	23			
	Algeria	13	21.6	1.0	21	23			
	High Atlas	26	19.0	0.0	19	19			
INFRR	Western Iberia	118	11.9	0.9	10	14	45.592	4	<0.001
	Eastern Iberia	318	12.3	0.9	10	16			
	Rif and Middle Atlas	10	12.3	0.9	11	14			
	Algeria	10	10.9	1.1	9	12			
	High Atlas	8	10.4	0.5	10	11			
INTER	Western Iberia	176	35.4	7.1	13	57	14.441	4	<0.001
	Eastern Iberia	327	35.3	6.4	19	63			
	Rif and Middle Atlas	11	39.1	8.7	25	59			
	Algeria	12	42.4	6.4	33	50			
	High Atlas	8	34.5	2.5	32	39			
LORER	Western Iberia	149	8.3	1.4	5	12	57.392	4	<0.001
	Eastern Iberia	328	7.6	1.5	3	13			
	Rif and Middle Atlas	11	8.2	1.7	6	12			
	Algeria	13	9.4	1.8	7	13			
	High Atlas	20	6.3	1.0	5	8			
PERIR	Western Iberia	159	9.9	0.9	7	12	22.076	4	<0.001
	Eastern Iberia	343	9.7	0.9	7	12			
	Rif and Middle Atlas	11	9.8	1.4	6	11			
	Algeria	13	10.6	0.9	9	12			
	High Atlas	8	9.1	0.6	8	10			
SUBCAF	Western Iberia	70	33.8	3.0	24	39	11.187	4	0.025
	Eastern Iberia	117	34.8	2.8	28	41			
	Rif and Middle Atlas	4	35.3	3.3	31	39			
	Algeria	5	32.2	2.3	30	35			
	High Atlas	11	32.7	2.3	29	37			
SUBCAM	Western Iberia	97	41.1	3.1	34	47	32.240	4	<0.001
	Eastern Iberia	168	41.9	2.6	36	50			
	Rif and Middle Atlas	4	39.0	2.9	35	42			
	Algeria	5	36.2	3.0	33	41			
	High Atlas	12	37.8	2.2	35	41			
NVENT	Western Iberia	168	137.9	5.7	123	147	67.651	4	<<0.001
	Eastern Iberia	332	138.7	4.4	127	153			
	Rif and Middle Atlas	8	132.8	1.8	130	135			
	Algeria	13	128.0	5.0	123	138			
	High Atlas	26	133.4	2.5	129	138			