Environmental niche divergence between genetically distant lineages of an endangered water beetle

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Historically, there has been considerable disagreement between researchers about the criteria used to discriminate among species. Decisions based on traditional morphological and genetic data alone can be potentially problematic, especially if the hypotheses are contradictory. Today, taxonomy is integrating new methods from different disciplines that study species' limits and evolution; this diverse range of evidence aids researchers in the recognition of species. Differences in niche characteristics could become a new and useful criterion in helping to decide the status of conflicting taxonomical entities. *Ochthebius glaber* (family Hydraenidae) is an endangered water beetle typical from southeast Iberian hypersaline streams that shows three clear discrete genetic units within its distribution range. However, there is no evidence to date that these lineages of *O. glaber* exhibit any adaptive morphological or ecological divergence. Using a modelling approach directed to generate niche representation from distributional data, we found a significant environmental niche divergence for allopatric lineages of *O. glaber* that followed an aridity gradient. Although we can not conclude firmly at present that the separate populations of *O. glaber* studied represent separate, reproductively isolated species, the present study complements and supports previous phylogeographic analyses through the inclusion of measures of another form of evolutionary change; in this case, ecological diversification. Despite the existence of some methodological limitations, also discussed in the present study, we emphasize the importance of recent conceptual advances that allow taxonomy to improve species delimitation practices through the integration of theory and methods from disciplines that study the origin and evolution of species. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **103**, 891–903.

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INTRODUCTION

Traditionally, there has been considerable disagreement between researchers about the criteria used to recognize species. Because lineage separation and divergence constitutes a temporally continuous process that may render populations monophyletic for haplotype variation, reproductively isolated, ecologically divergent, and/or morphologically distinctive

(De Queiroz, 1998; Leaché *et al*., 2009), the different criteria for species discrimination encouraged a historical debate about the merits of alternative species concepts (De Queiroz, 1998, 2007). A practical yet conservative strategy adopted by many researchers has been to apply a diverse range of evidence to support the recognition of species (e.g. fixed or non-overlapping differences in morphological, behavioural or ecological characteristics, molecular divergence thresholds, additional quantitative methods, or geographic isolation), thereby meeting the requirements of several species criteria (De Queiroz, 1998, 2007;

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Raxworthy *et al*., 2007; Hemp *et al*., 2010; Padial *et al*., 2010; Tobias *et al*., 2010). In this sense, decisions based on traditional morphological and genetic data alone can be problematic, especially if the hypotheses are contradictory; for examples of conflicts between morphology and phylogenetic history in water beetles, see Ribera, Bilton & Vogler (2003) and Hawlitschek *et al*. (2011). Thus, potential differences in ecological niche characteristics could become a new and useful criterion in helping to decide the status of conflicting taxonomical entities (Wiens & Graham, 2005; Raxworthy *et al*., 2007; Martínez-Gordillo, Rojas-Soto & de los Monteros, 2010).

A relevant question in evolutionary biology is whether ecology generally drives diversification, as proposed in ecological speciation models in which divergent natural selection leads to reproductive isolation (Schluter, 2009; McCormack, Zellmer & Knowles, 2010). Although the maintenance of ancestral ecological adaptations along an evolutionary lineage (niche conservatism) promotes allopatric divergence in fragmented habitats by limiting adaptation to new environments, the occurrence of new ecological adaptations or niche divergence within a lineage may facilitate the sympatric formation of species when populations adapt to new environments (Graham *et al*., 2004; Wiens & Graham, 2005). The mechanism by which this happens is well defined in terms of the fragmentation of a continuous habitat, which promotes geographical isolation, or dispersal into a new habitat, after which natural selection subsequently promotes ecological differentiation (Coyne & Orr, 2004; Pryon & Burbrink, 2009). Although there are several definitions of niche, Grinnell's niche concept was followed in this study (i.e. a niche is the set of environmental conditions within which each species can survive and maintain its populations without migration (Grinnell, 1917). The recent development of environmental niche models (ENMs), which are generated by combining species occurrence data with environmental geographical information system managed data layers (Guisan & Zimmermann, 2000), makes it possible to partially quantify niches at an unprecedented scale (Kozak, Graham & Wiens, 2008; Jiménez-Valverde *et al*., 2011). Such data can provide a starting point for quantitative adaptive differentiation analyses (Nakazato, Bogonovich & Moyle, 2008) or be used to assess likely ecological and evolutionary forces acting to shape species' geographical distributions and habitat preferences (Graham *et al*., 2004).

The comparison of ENMs between species or populations, as is necessary when assessing niche divergence, poses new conceptual and statistical challenges (Kozak *et al*., 2008; McCormack *et al*., 2010). For example, it is assumed that ecology plays an important role in speciation when ENMs or niche representations show little overlap between closely related taxa (Rice, Martínez-Meyer & Peterson, 2003; Ruegg, Hijmans & Moritz, 2006; Rissler & Apodaca, 2007). If a set of populations of uncertain taxonomic status show niche divergence (i.e. populations occur under different climatic conditions) or even if those populations are geographically separated by areas that are outside of their climatic niche envelope, gene flow between populations is unlikely (Wiens & Graham, 2005). This pattern would support the hypothesis that the different populations could represent distinct species, or at least that divergent speciation processes are occurring. These similarities and differences in niche characteristics can be visualized and analyzed statistically within the framework of ecological niche modelling and niche conservatism or divergence (Wiens & Graham, 2005; Raxworthy *et al*., 2007; Warren, Glor & Turelli, 2008).

The present study focuses on *Ochthebius glaber* Montes & Soler (family Hidraenidae), a small (< 3 mm), highly threatened (Sánchez-Fernández *et al*., 2008a) water beetle endemic to the south of the Iberian Peninsula. This is one of the most characteristic species of the macroinvertebrate community in Iberian hypersaline streams (Abellán *et al*., 2007). The species shows high habitat specificity, occurring only in running hypersaline waters (generally associated with marl soils) with salinity values from 40 g/L to over 250 g/L (Abellán *et al*., 2007). Although it may be abundant locally, the species is limited to only a few saline streams grouped in three geographical areas: the basins of the rivers Guadalquivir, Segura, and Júcar (the latter in just two known sites; Fig. 1).

By contrast to expectations based on the taxonomy and biology of the species, molecular studies have revealed that these three populations groups belong to distinct lineages that probably evolved as a consequence of isolation generated by long-term barriers to gene flow between the different river basins (Abellán *et al*., 2007; Abellán, Millán & Ribera, 2009). Thus, the discontinuous and patchy distribution of aquatic saline environments, determined by local geological and climatic conditions, appears to have promoted high levels of genetic divergence amongst isolated populations (e.g. the average pairwise genetic distance among populations is 3.6%; Abellán *et al*., 2009), which is even higher than those observed among other widely distributed and morphologically well-characterized Iberian endemic water beetles species (Ribera & Vogler, 2004; Abellán *et al*., 2009). The existence of these three clear genetic units within the distribution range of *O. glaber*, which appear to have remained genetically isolated from each other for significant periods of time (approximately 1–2 Myr; Abellán *et al*., 2009) allowed Abellán *et al*.
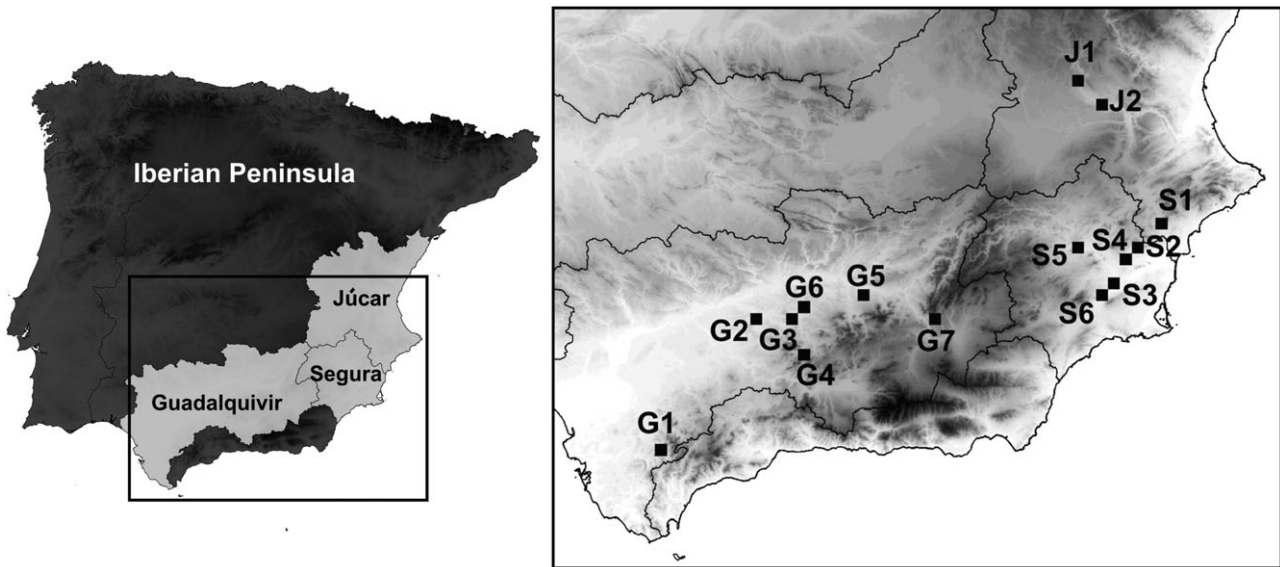
Figure 1. Localities of all known permanent populations of *Ochthebius glaber* (for locality codes, see Table 1). The three river basins (Júcar; Segura, and Guadalquivir) are indicated in the map on the left.

(2007) to consider each lineage as an independent evolutionary significant unit (ESU). To date, there is no evidence that these three groups of *O. glaber* populations exhibit any adaptive morphological or ecological divergence. Nevertheless, the sort of deep genetic structuring found in *O. glaber* could have promoted local adaptations, even though these may not be readily distinguishable by obvious, external 'adaptive' differences (Fraser & Bernatchez, 2001).

In the present study, an ENM approach was applied using distributional data from the two phylogenetic groups of *Ochthebius glaber* populations with enough information to estimate if the obtained niche estimations could provide useful evidence of ecological niche divergence. Using large-scale climatic and geological data, the specific aims of this study were to: (1) estimate the potential distribution of *O. glaber* as well as its main lineages; (2) characterize the basic niches of these lineages; (3) assess whether niche divergence accompanied the geographic isolation of *O. glaber* populations; and (4) investigate the role of current climate and topographic–geological factors in the maintenance of the genetic isolation between these two populations.

MATERIAL AND METHODS

SPECIES OCCURRENCES

Records for this species came from an exhaustive database (ESACIB 'EScarabajos ACuáticos IBéricos') that included all the available taxonomic and distributional data for Iberian water beetles (Sánchez-Fernández *et al.*, 2008b). This species is distributed

across three Iberian geographical areas belonging to the basins of the rivers Guadalquivir, Segura, and Júcar (Fig. 1). After rejecting three records with non-precise information and/or individual vagrants collected in clearly unsuitable habitats, we used data from 15 localities or populations. Seven of these populations are located in the Guadalquivir basin, six in the Segura basin, and just two sites in the Júcar basin (Fig. 1, Table 1). The low number of occurrences found in the Júcar basin hinders the generation of potential distribution simulations based only on the data of this lineage.

ENVIRONMENTAL VARIABLES

Climatic data were obtained from WORLDCLIM, version 1.3 (http://www.worldclim.org) (Hijmans *et al.*, 2005). WORLDCLIM contains climate data (i.e., monthly precipitation and monthly mean, minimum and maximum temperatures) obtained by interpolation of climate station records from 1950–2000. In the present study, 17 climate variables were used as predictors: annual mean temperature, isothermality, temperature seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, annual temperature range, mean temperature of the wettest quarter, mean temperature of the warmest quarter, mean temperature of the coldest quarter, annual precipitation, precipitation of the wettest month, precipitation of the driest month, precipitation seasonality, precipitation of the wettest quarter, precipitation of the driest quarter, precipitation of the warmest quarter, and precipitation of the

Table 1. Locality data of all known permanent populations of *Ochthebius glaber*, showing the Spanish provinces and the main basin to which they belong (Fig. 1)

Code	Locality	Province	Basin	Longitude	Latitude
G 1	Salinas de Hortales	C	Guadalquivir	−5.54	36.74
G 2	Ayo. Salinas de Duernas	CO	Guadalquivir	−4.60	37.70
G 3	Salinas de la Maturra	CO	Guadalquivir	−4.36	37.68
G 4	Río salado de Priego	CO	Guadalquivir	−4.18	37.41
G 5	Ayo. Salinas de Brujuelo	J	Guadalquivir	−3.67	37.89
G 6	Arroyo de las Salinas de Porcuna	J	Guadalquivir	−4.22	37.79
G 7	Salinas de Chíllar, Hinojares	J	Guadalquivir	−3.00	37.71
J 1	Rambla de Minglanilla	CU	Júcar	−1.57	39.56
J 2	Ayo. hipersalino en R. Cabriel	V	Júcar	−1.30	39.34
S 1	Estrecho de la Salineta	A	Segura	−0.78	38.43
S 2	Rambla de Albatera	A	Segura	−0.95	38.24
S 3	Rambla de Sangonera	M	Segura	−1.29	37.95
S 4	Rambla Salada de Fortuna	M	Segura	−1.12	38.13
S 5	Ayo. Salinas de la Ramona	M	Segura	−1.62	38.21
S 6	Rambla de Librilla	M	Segura	−1.37	37.91

C, Cuenca; CO, Córdoba; J, Jaén; CU, Cuenca; V, Valencia; A, Alicante; M, Murcia.

coldest quarter. Five geological variables were also used: percentage of land with gypsum, basic rocks, acidic rocks, basic deposits, and acidic deposits. These data were obtained from the Geological Survey of Spain (Instituto Geológico y Minero de España; IGME). These geological variables were added because the nature of the substratum could highly influence the conductivity and pH of the water, and thus the presence and abundance of a species adapted to saline water bodies. We extracted the data from all of these considered environmental variables at a spatial resolution of 100 km² UTM cells.

Niche metrics

We estimated the niche discrepancy between the two lineages by using two different procedures to gauge the species' distributions: a simple, multidimensional envelope procedure (MDE) to represent the potential species distribution (a distributional hypothesis able to reflect all of the environmentally suitable places in which a species can occur according to the values of a group of environmental variables in the observed localities; Chefaoui & Lobo, 2008; Jiménez-Valverde *et al.*, 2011), and a widely-used modelling method (MaxEnt; Phillips, Anderson & Schapire, 2006), which probably generates distribution ranges closer to the realized species distribution (Jiménez-Valverde, Lobo & Hortal, 2008). Using both methods, we obtained a complete distribution representation based on all occurrence information, as well as specific distributions based on data from the Guadalquivir and Segura basins separately.

MDE aims to provide a geographical representation of regions with favourable environmental conditions (both colonized and uncolonized) based on climatic and geological information from observed occurrences (Jiménez-Valverde *et al.*, 2008). First, the prevailing environmental conditions were estimated at observed occurrence localities for the species (or population). Then, the extreme yet reliable values for these variables were calculated to derive a binary distributional hypothesis about the areas with environmentally suitable conditions (potential distribution). This potential distribution can be considered to be the geographical projection of niche characteristics derived from recorded occurrences. Because distributional simulations obtained by MDE are highly dependent on the number of selected predictors (Beaumont, Hughes & Poulsen, 2005), the minimum set of explanatory variables relevant to explain the occurrence was estimated using an ecological niche factor analysis (ENFA) procedure based on Iberian data (Hirzel *et al.*, 2002; Calenge & Basille, 2008). ENFA compares the climatic data of presence localities against climatic conditions in the entire study area, thereby computing uncorrelated factors that can explain both species or lineage marginality (i.e. the distance between the species' optimum and average conditions in the study area) and specialization (i.e. the ratio of ecological variance in the study area to that associated with the focal taxon). The number of retained factors was based on their eigenvalues relative to a 'broken-stick' distribution (Hirzel *et al.*, 2002). The predictor-selected variables were those showing the highest correlation with the retained

ENFA factors (factor loadings ≥ 0.30). Thus, we obtained three potential distribution representations: (1) using all the distributional information; (2) using just the data from the Guadalquivir basin (PD_G); and (3) using only the distributional records from the Segura basin (PD_S).

Finally, we compared the values of all the considered predictor variable features between the favourable and unfavourable cells of each potential distribution as well as between the favourable cells of both lineages by using nonparametric Mann–Whitney U-tests with Bonferroni corrections.

MAXENT is a machine-learning method that estimates organisms distribution by finding the probability distribution of maximum entropy (i.e. the most uniform), given the constraint that the expected value of each environmental predictor under this estimated distribution matches the empirical average of sample locations (Phillips *et al.*, 2006). MAXENT simulations of realized distributions produce continuous suitability scores for each cell (from 0 to 1), and we calculated Schoener's D metric (Schoener, 1968) using ENMtools (Warren *et al.*, 2008) to assess niche similarity among populations, where $D\ (pX,\ pY) = 1 - \frac{1}{2}\Sigma\ (pXi - pYi)$. This metric assumes probability distributions defined over geographic space, in which pXi (or pYi) denotes the probability assigned by the modelling method to species X (or Y) in the i cell ranging from 0 (niches do not overlap) to 1 (niches completely overlap). Because niche differences may be simply be a result of the spatial autocorrelation of the used explanatory environmental variables (background environmental divergence (Warren *et al.*, 2008), strong evidence for niche divergence requires two conditions: (1) that niche characteristics differ between the two considered populations and (2) that these differences are greater than the background environmental divergence (McCormack *et al.*, 2010). Niche conservatism, on the other hand, would be supported if niche differences are smaller than the obtained background environmental divergence. Thus, comparison of environmental characteristics from these two classes of data should allow discrimination between differences as a result of simple spatial autocorrelation caused by geographic distance and strong niche divergence that occurs because two species occupy different environmental conditions. To test the null hypothesis that niches are similarly divergent in comparison to background environments, we used the 'background similarity test' procedure implemented in ENMtools, estimating 100 niche overlap values generated by comparing model suitability values of one population to those generated from random cells drawn from the geographic range of the other population (Warren, Glor & Turelli, 2009). One conceptual issue that arises when this test is used is the definition and

justification of what is considered to be 'background'. Little is known regarding the dispersal capability of *O. glaber*, although it is known that it is able to fly and that its small size could probably facilitate passive wind dispersal. Other species within the genus *Ochthebius* in similar habitats show massive swarming flights under certain environmental conditions (A. Millán, unpubl. observ.), although this has never been observed in *O. glaber*. Because this consideration could have altered the results, the analyses were repeated considering four different background regions: the Iberian Peninsula, the Iberian Peninsula excluding those cells with more than 80% of acidic soils, the potential distribution of *O. glaber* using all observed cells, and the basin limits of each lineage.

GENETIC DISTANCES AND ISOLATION

To estimate the role played by current climatic and topographic–geological factors in the maintenance of the genetic isolation among the *O. glaber* populations, the relation between genetic distances and both climatic and topographic–geological resistances was studied. Low resistance values were assigned to the most permeable to movement conditions and high resistances assigned to poor dispersal conditions or movement barriers (McRae, 2006). The genetic distances between populations were obtained from Abellán *et al.* (2009) from mitochondrial DNA sequences. This measure of genetic distance is based on the phylogenetic distances of haplotypes in one locality to haplotypes in the other. We calculated the mean pairwise distance (Webb, Ackerly & Kembel, 2008; Vamosi *et al.*, 2009), as implemented in PHYLOCOM software (Webb *et al.*, 2008). Although originally intended to measure interspecific phylogenetic diversity (Graham & Fine, 2008; Vamosi *et al.*, 2009), it can also be used to calculate these metrics at an intraspecific level by considering individual haplotypes as terminal taxa.

We calculated climatic resistances as inverse suitability values generated by the MAXENT distribution model, with 0 as the value for those cells with higher climatic favourability or low resistance, and 100 for those cells with lower favourability or high resistance values. Topographic–geological resistances were calculated using the cell values of the variables: altitude, slope, and percentage of acidic and basic soils (both deposits and rocks). Because *O. glaber* occurs in lowlands with basic soils, each one of the variables were rescaled from 0 to 100 so that cells with a value of 100 were those which harboured higher altitudes, slopes, and higher percentages of acidic soils (higher resistance). To calculate the final topographic–geological resistance, the individual resistance values of the four considered variables were averaged.
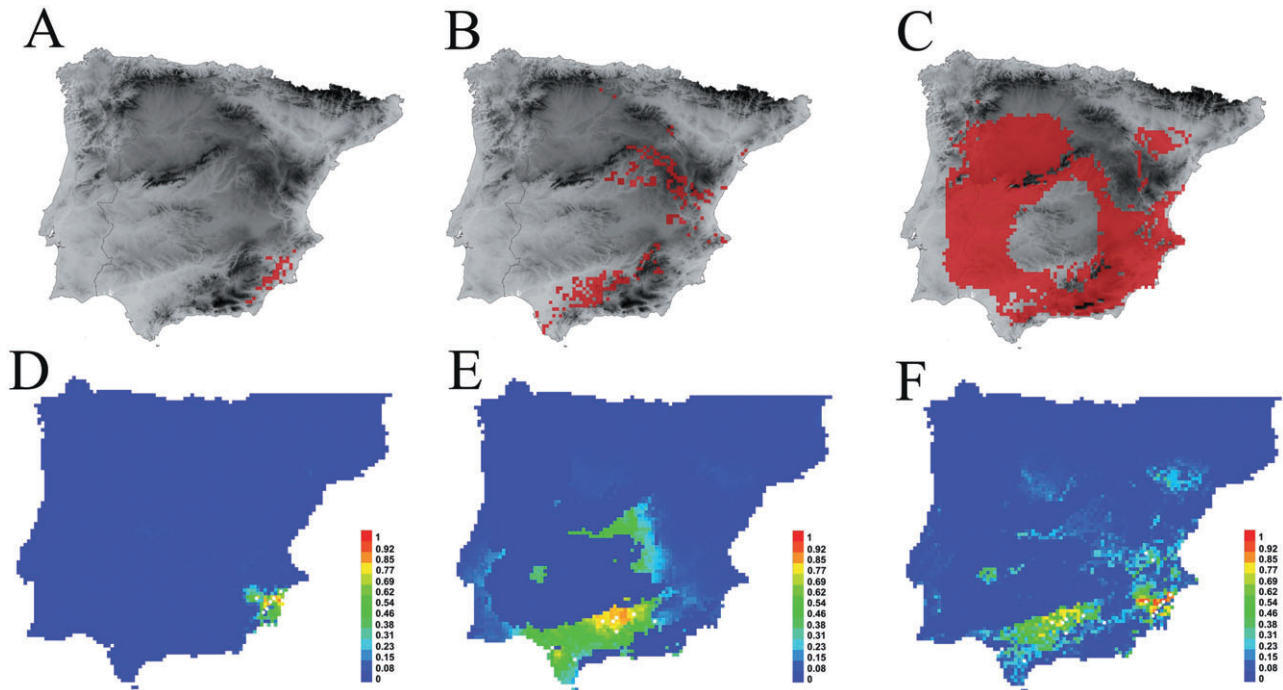
Figure 2. Potential distribution (red surface) using a multidimensional enveloping procedure (see Material and methods) for the Segura lineage (PD$_G$) (A); the Guadalquivir lineage (PD$_S$) (B); and the whole species (PDw) (C). Environmental niche models (ENMs) using MAXENT from the Segura populations (D), the Guadalquivir populations (E), and all known populations (F). These continuous measures of habitat suitability were used in ENM-based tests of niche divergence and conservatism.

We used the software CIRCUITSCAPE, version 2.2 (McRae, 2006; McRae *et al*., 2008), based on circuit theory, to calculate the connectivity between the observed presence cells according to the two previously mentioned resistance maps. We selected the pairwise option so that connectivity, or current flow, was calculated between all pairs of observed presence cells, and then, these pairwise current maps were overlapped (averaged) to obtain a cumulative current (or connectivity) map. We calculated two connectivity maps, using climatic and topographic-geological resistance maps separately. Finally, the values of these two cumulative connectivity maps were correlated (Pearson's correlation coefficient) with genetic distances using a Mantel test computed with 1000 randomizations to estimate whether more genetically distant populations were also more isolated by climatic or by topographic–geological limitations.

RESULTS

POTENTIAL DISTRIBUTION SIMULATION

The ENFA procedure showed that the three groups of populations occurred in very restricted environmental conditions far from the average climatic conditions present in the Iberian Peninsula. More specifically, marginality values showed that the optimum conditions for *O. glaber* were relatively far from the mean available conditions in the study area (1.92) and that the Segura population occurred under more distant environmental conditions (2.49) than the Guadalquivir population (2.25) with respect to average Iberian conditions. The high specialization values obtained in all cases (> 1000) indicated that both species and each of the two lineages inhabit a narrow interval of environmental conditions more than one thousand times smaller than the whole range of available variations. Percentage of gypsum, precipitation of the driest quarter, and temperature seasonality were the most relevant predictors in explaining the distribution range of *O. glaber*. Percentage of gypsum, annual precipitation, and isothermality were the most important predictors for the Guadalquivir populations, whereas annual precipitation, temperature seasonality, and minimum temperature of the coldest month were the most relevant factors for the Segura lineage. The MDE-derived potential distributions (Fig. 2) indicated that the Segura lineage (PD$_S$) showed a very narrow potential range, confined to 37 100 km^2 UTM cells close to the number of occurrence points (Fig. 2A). The potential distribution of the Guadalquivir lineage (PD$_G$) displayed a narrow

distribution (207 cells) including the Guadalquivir basin, as well as part of the South plateau (Fig. 2B). The two potential distributions did not overlap. The potential distribution generated using all known populations (Fig. 2C) showed a very wide representation (2263 cells) in the Iberian Peninsula that occupied extensive areas around the North and South plateaus.

The Mann–Whitney U-test showed that the environmental conditions in PD_G and PD_S cells were clearly different from those of the remaining Iberian cells (Table 2), as well as the environmental conditions between the potential distributions of both lineages. In general, the Guadalquivir population was characterized by higher precipitation, lower temperatures and higher percentages of land with gypsum than those of the Segura population (Table 2). Although the scarcity of data did not allow high significant values to be obtained, observed presences supported the same pattern of environmental differences between both populations (Table 2).

REALIZED DISTRIBUTION SIMULATION AND NICHE OVERLAP

Projected maps using MAXENT provided a similar distributional representation of the Segura and Guadalquivir lineages (Fig. 2D, E) but a more restricted one for the species as a whole (Fig. 2F). A value of Schoener's $D = 0.136$ of niche overlap between ENMs from the Segura and Guadalquivir populations was obtained. The observed overlap (Schoener's D-value) between the two lineages (Fig. 3, black arrow) was lower than expected under the null hypothesis, indicating that the two lineages were more divergent than expected by chance (Warren $et\ al.$, 2008). Thus, it was observed: (1) that niche characteristics differed between the two considered lineages; (2) that these differences were greater than the background environmental divergence, thus supporting niche divergence; and (3) that these results were independent of the considered background region (Fig. 3A, B, C, D).

GENETIC DISTANCES AND ISOLATION

Connectivity maps (Fig. 4) using both climatic and topographic–geological resistances showed that the three populations, or phylogenetic lineages, appeared to be more isolated when topographical and geological variables were considered. However, Mantel tests indicated that statistically significant correlation values appeared between both genetic distances and topographic–geological connectivity values ($r = 0.582$; $P < 0.001$) and between genetic distances and climate connectivity values ($r = 0.314$; $P = 0.013$).

DISCUSSION

Potential distribution of the Segura River basin lineage showed a very narrow representation confined to the occurrence points, meaning that individuals from these six localities live under practically the same environmental conditions. On the other hand, the potential distribution of the Guadalquivir lineage showed a wider distribution range that was divided into two areas (Fig. 2B); a main area following the Guadalquivir basin, and another suitable northern area that belongs to other basins, yet unoccupied, probably as a result of limited dispersal abilities. Potential distribution of the Júcar lineage was not estimated in the present study as a result of very limited data (only two populations). However, neither of these two potential distributions (Guadalquivir and Segura lineages) properly predicted the Júcar populations, although their environmental conditions appear to be close to the Guadalquivir lineage. Because both lineages are spatially separated, a pattern of niche divergence could result either from actual niche differences between the populations or simply as a result of spatial autocorrelation in environmental variables (Costa $et\ al.$, 2008) between the regions in which both lineages are distributed. Thus, the null model approach adopted in the present study provides strong evidence for niche divergence because niche differences between the two lineages were greater than background environmental divergence (Broennimann $et\ al.$, 2008; Warren $et\ al.$, 2008), and this pattern was consistent across the different background environmental scenarios; for an example of lack of niche divergence among closely-related allopatric taxa, see McCormack $et\ al.$, (2010).

The Guadalquivir and Segura lineages of $O.\ glaber$ currently do not overlap geographically but, according to the results obtained in the present study, they apparently do not overlap environmentally either, so the geographical projections of the estimated niche conditions were unconnected, at least at the resolution used in the present study. The spatial pattern found for $O.\ glaber$, with both lineages displaying different climatic niches and the environmental conditions in the area between them outside of their climatic tolerances (see below), was consistent with the hypothesis that divergence in their climatic niches was involved in the maintenance of their current range limits. Hence, the present study complements and supports previous phylogeographic analyses (Abellán $et\ al.$, 2007) through the inclusion of measures of another form of evolutionary change (i.e. ecological diversification). These results suggest that niche divergence processes have acted in separating the genetically structured lineages from the Segura and Guadalquivir basins. Taxonomic entities

Table 2. Comparison of environmental variables (climatic and geological) between the suitable cells according to the multidimensional envelope procedure potential distribution carried out considering the Guadalquivir basin data (PD_G), the Segura basin data (PD_S), and the remaining (REM) Iberian cells (N $PD_G = 207$, N $PD_S = 27$, N REM $= 5893$)

	PD_G versus REM			PD_S versus REM			PD_G versus PD_S			D_G versus D_S		
	U	P		U	P		U	P		U	P	
Annual precipitation	555367.5	0.0285	−	2062.0	**<0.001**	−	0.0	**<0.001**	+	0.0	0.003	+
Annual mean temperature	575125	0.1623	=	18527.5	**<0.001**	+	1289.0	**<0.001**	−	15.0	0.391	=
Isothermality	490967	**<0.001**	−	39967.0	**<0.001**	+	381.5	**<0.001**	−	0.0	0.003	−
Precipitation of coldest quarter	534178	0.0024	−	10889.5	**<0.001**	−	205.5	**<0.001**	+	0.0	0.003	+
Precipitation of driest month	498472	**<0.001**	−	34563.5	**<0.001**	−	2247.5	**<0.001**	+	16.5	0.520	=
Precipitation of driest quarter	460294.5	**<0.001**	+	25543.5	**<0.001**	−	2796.5	0.009	+	9.5	0.100	−
Precipitation seasonality	503987.5	**<0.001**	−	77091.0	**0.002**	+	3604.5	0.569	=	6.0	0.032	+
Precipitation of warmest quarter	462436.5	**<0.001**	−	50753.5	**<0.001**	−	3403.5	0.281	=	5.5	0.027	−
Precipitation of wettest month	603185	0.7867	=	10094.5	**<0.001**	−	20.0	**<0.001**	+	0.0	0.003	+
Precipitation of wettest quarter	587636.5	**0.3708**	=	3189.5	**<0.001**	−	0.0	**<0.001**	+	0.0	0.003	+
Maximum temperature of warmest month	493497.5	**<0.001**	+	49172.5	**<0.001**	+	2761.5	0.007	−	12.0	0.199	=
Minimum temperature of coldest month	556948.5	0.0334	−	50888.5	**<0.001**	+	2019.5	**<0.001**	−	16.5	0.520	=
Mean temperature of coldest quarter	592462	0.4832	=	27643.5	**<0.001**	+	774.0	**<0.001**	−	5.5	0.027	−
Mean temperature of warmest quarter	504668	**<0.001**	+	23832.0	**<0.001**	+	1714.0	**<0.001**	−	14.5	0.353	=
Mean temperature of wettest quarter	523455.5	**0.0005**	+	29581.5	**<0.001**	+	577.0	**<0.001**	−	0.0	0.003	−
Temperature seasonality	394213	**<0.001**	+	87296.0	0.036	−	1109.5	**<0.001**	+	6.0	0.032	+
Temperature annual range	428534	**<0.001**	+	102779.0	0.548	=	2035.5	**<0.001**	+	6.0	0.032	+
% Land with gypsum	33799	**<0.001**	+	85728.0	0.025	+	816.0	**<0.001**	+	12.5	0.225	=
% Land with basic rocks	259914.5	**<0.001**	+	98654.5	0.318	=	947.0	**<0.001**	+	21.0	1.000	=
% Land with acidic rocks	355459	**<0.001**	−	106548.5	0.812	=	1568.0	**<0.001**	−	17.5	0.617	=
% Land with basic deposits	400761	**<0.001**	+	76395.0	**0.002**	+	3570.5	0.512	=	10.0	0.116	+
% Land with acidic deposits	543645	0.0078	−	44805.0	**<0.001**	+	828.5	**<0.001**	−	3.0	0.010	−

The final column shows the comparison between the same environmental variables in the localities of the Guadalquivir basin (D_G), and the Segura basin (D_S) (N $D_G = 7$, N $D_S = 6$). Variables with statistically significant differences ($P < 0.05$) are highlighted (Mann–Whitney U-test). Signs indicate whether median values for the term on the left are higher (+), lower (−) or equal (=) than those of the term on the right. Bold values are those statistically significant after a Bonferroni corrected P-value ($0.05/22 = 0.002$).

can be recognized not only by examining genetic differences (Cracraft, 1987), but also by discriminating ecological discrepancies (Templeton, 2001) because they can contribute to the reproductive isolation of populations (Nosil, Crespi & Sandoval, 2003). Therefore, the evolutionary outcome of niche differentiation among lineages could be a shift in environmental preferences.

However, results such as these should be always considered with caution. Although the use of current distribution data suggests significant environmental niche divergence for allopatric lineages, it is always possible that observed differences in the macroclimatic conditions detected in the distribution areas of both lineages are irrelevant because the niche of the populations may be mainly determined by the probable similar microscale environmental conditions at which these beetles live. Are niche characteristics determined by macro- or micro-environmental conditions? Furthermore, it is also possible that the niche of this species is broad enough to allow it to cope with the conditions in the separate basins, so that the causes of the allopatric occurrence of these two populations are unrelated with the existence of local adaptation processes. Additional physiological information may help in determining the answer to this question. These caveats are ultimately related with one of the main shortcomings of these modelling techniques: that the environmental conditions in the occupied localities by a species can only provide partial representations of their fundamental niche (Colwell & Rangel, 2009; Jiménez-Valverde et al., 2011). The geographic projection of the niche conditions derived from the occupied area will be biased not only by the role played by restrictive forces such as dispersal limitations and biotic interactions, but also because environmental conditions in present-day occurrence localities may represent only a portion of the full range of conditions inhabitable by the species.

Taking into account all of these considerations, we can conclude that molecular discrimination of the two studied populations of O. glaber was supported by niche divergence estimations based on present-day distribution data, although supplementary and additional evidence intending to detect physiological, reproductive or trophic local adaptations is necessary to clearly recognize these populations as separate species. Because these lineages are monophyletic groups that do not share an environmental niche (assuming the uncertainties exposed above), it is hypothesized that they could experience a speciation model in which reproductive isolation built up in allopatry with significant contributions from ecologically mediated divergent natural selection (i.e. the different lineages of O. glaber may have been caused by two vicariant processes; Abellán et al., 2007, 2009),

and the adaptation to different climatic regimes could have arisen after they split into separate lineages. Interdisciplinary research can be very useful in species delimitation, especially for cryptic species and groups displaying fine-scaled patterns (Leaché et al., 2009; Ross et al., 2009; Martínez-Gordillo et al., 2010; Nakazato, Warren & Moyle, 2010). We emphasize the importance of recent conceptual advances as niche modelling methods that allow taxonomy to improve species delimitation practices through the integration of theory and methods from different disciplines (Alström et al., 2008; Padial et al., 2010). However, in the case of the so-called niche models, we consider that these procedures are not capable of firmly concluding niche differences among populations or species.

Furthermore, our results also suggest that current climatic and, especially topographic–geological factors, play (and probably have played in the past) a pivotal role in the maintenance of the genetic isolation between these two populations. The discontinuous and patchy distribution of aquatic saline environments appears to have promoted allopatric speciation and high levels of divergence amongst isolated populations in their invertebrate biota, with habitat discontinuities operating as effective barriers to gene exchange and emerging as critical factors shaping phylogeographic structure (Gómez, Carvalho & Lunt, 2000; Gómez et al., 2002; Abellán et al., 2009). We assume that speciation processes based in niche divergences could have happened in the same region in which these populations currently occur. Of course, the climatic conditions of the studied area have changed over the last hundred thousand years, even since the Holocene (Martín-Puertas et al., 2010), and we do not have fossil data demonstrating the past occurrence of this species in the area. Recent evidence suggests that this region has experienced a general arid climate ever since the middle Miocene (Carrión et al., 2010), and also that the discontinuous distribution of the saline habitats in the south eastern corner of the Iberian Peninsula is likely to have been the norm through the Pleistocene (Gómez et al., 2000; Muñoz et al., 2008). Thus, we suggest that climatic changes during the Pleistocene could have promoted the occurrence of evolutionary processes that lead to niche divergence as a result of the isolation of populations inhabiting patchy saline habitats located within a region submitted to arid conditions during thousands of years.

From a conservation point of view, the Guadalquivir and Segura lineages, previously considered as independent ESUs by Abellán et al. (2007), should be treated as different entities. Efficient conservation plans should recognize these conservation units and manage them separately. Furthermore, it is
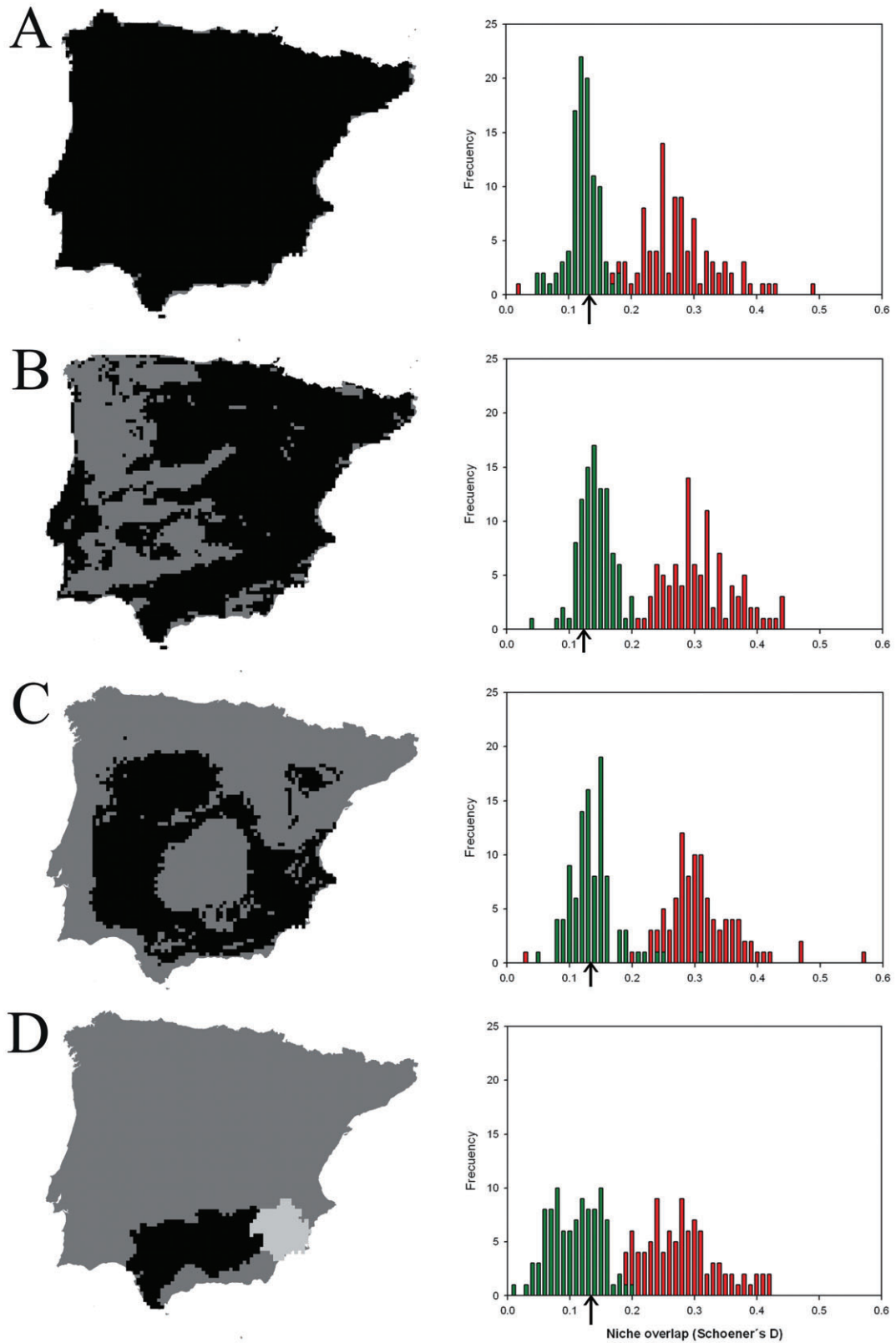
Figure 3. Tests of niche divergence and conservatism from environmental niche models (ENMs). Observed niche-overlap values (arrows) compared wirh a null distribution using four different background regions. Each comparison produces two reciprocal analyses: one in which the niche model for the Segura lineage is compared with a niche model generated from random points from each background area of the Guadalquivir lineage, and vice versa (Guadalquivir, red; Segura, green). The analyses were repeated considering four different background regions: the Iberian Peninsula (A); the Iberian Peninsula excluding those cells with more than 80% of acidic soils (B); the potential distribution of *Ochthebius glaber* using all observed cells (C); and the basin limits of each lineage (D).
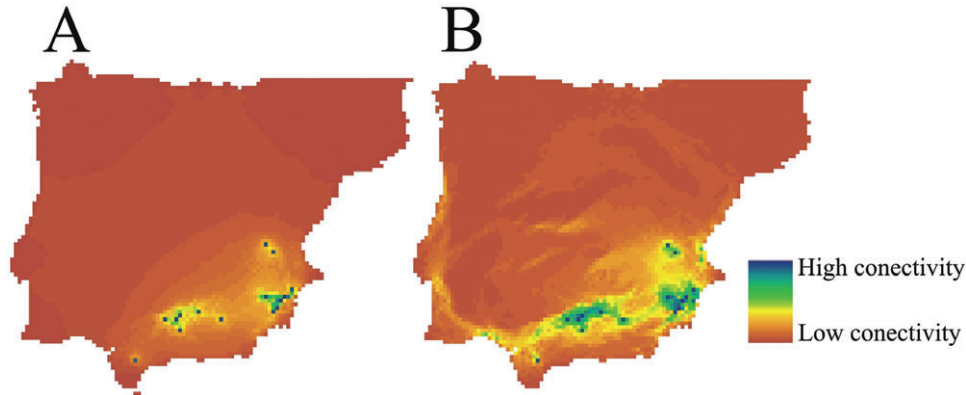
Figure 4. Maps of current flow (connectivity) between the fifteen observed occurrence localities of *Ochthebius glaber* (focal patches): climatic connectivity map (A) and topographic-geological connectivity map (B). Each map highlights portions of the territory critical for movement between the focal patches.

important to note that other independent evolutionary lineages with environmentally divergent niches can also be found in other saline water taxa (indistinguishable by external differences), as in the case of *O. glaber*. If the consequences of the present study are applied to other saline water beetle species, they may have deep conservation findings. Saline systems, despite their high conservation interest and narrow distribution in a global context (Sánchez-Fernández *et al.*, 2008a; Millán *et al.*, 2011), are adversely affected by a range of anthropogenic activities, especially those that are a result of agricultural practices (Velasco *et al.*, 2006). The main impacts on saline streams are modifications of channel bed geomorphology as well as a decrease in water flow and quality. A number of studies show that increases in freshwater inputs have a dramatic effect on of hypersaline water communities (Velasco *et al.*, 2006; Gutiérrez-Cánovas, Velasco & Millán, 2009), by increasing diversity (through an influx of generalist taxa), yet with a reduction in abundance or the elimination of hypersaline specialists (Velasco *et al.*, 2006). Thus, it is important to highlight that anthropogenic habitat transformations are not equally dramatic in the three areas where the populations of *O. glaber* are found. Land-use changes have been particularly intense in the southeast of Spain, especially in the Segura basin, where agricultural irrigation has increased rapidly, resulting in the disappearance of saline streams.

Therefore, although all conservation units should be considered as highly threatened, the population in the Segura basin appears to be critically endangered.

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