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Predictions based on phylogeography and climatic niche modelling depict an uncertain future scenario for giant blister beetles (*Berberomeloe*, Meloidae) facing intensive greenhouse expansion and global warming

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Abstract

1. Large-scale agricultural and tourism development are the main threats to biodiversity in south-eastern Spain. Species with low dispersal abilities, such as some endemic insects from this region, are particularly vulnerable to fragmentation and loss of genetic and morphological diversity. Here, we studied the current and future threat of climate and land-use change on the intraspecific diversity of one such group of endemics, the giant blister beetles of the *Berberomeloe insignis* species group.
2. Using a phylogeographic approach and morphometrics, we identified intraspecific variation within the *B. insignis* species group. These data were coupled with ecological niche modelling (ENM) to determine the effect of agricultural and climate change on the connectivity across phylogroups.
3. We identified a marked geographic structure within *B. insignis*, with the time to the most recent common ancestor (TMRCA) of the two main mtDNA clades dating back to 2.1 Ma. Cyto-nuclear discordances found across parapatric populations suggest past events of genetic introgression. *B. insignis* presents a head pattern that is geographically structured and mostly congruent with the four mitochondrial lineages. In contrast, diversification within *Berberomeloe tenebrosus* is more recent, dating back to less than 1 Ma. Future ENMs highlight the role of mountains as potential refugia under a climate warming scenario but predict extinctions in the lowlands.
4. Based on our analyses, two lineages of *B. insignis* are threatened and should be urgently considered as independent conservation units, and their current geographic distribution areas protected to conserve, at the very least, a portion of the species' evolutionary heritage.

KEYWORDS
Berberomeloe insignis, *Berberomeloe tenebrosus*, climatic niche modelling, conservation, diversification, phylogeography

INTRODUCTION

We are currently witnessing an accelerated rate of biodiversity loss resulting from human activity during the Anthropocene (Waters et al., 2016). Consequences of this global-scale footprint have plunged the Earth into the sixth wave of mass extinction, affecting not only organisms but the entire biodiversity continuum, from ecosystems to genes and their evolutionary processes (Dirzo et al., 2014; Latta, 2008; Moritz, 1999, 2002). The south-eastern coast of Spain, due to habitat loss and fragmentation, is one of the most disturbed regions of the western Mediterranean Basin (Barragán & Borja, 2011; de Andrés et al., 2017). Although human settlement and agricultural exploitation are known to have occurred in this region since Neolithic times (Castro et al., 2000), only in the last 60 years have many of its natural landscapes been massively transformed by deep changes in land use (Mota et al., 1996). Recurrent drivers of biodiversity loss and habitat destruction throughout south-eastern Iberia include industrial, tourism and urban development, and the large-scale expansion of horticultural greenhouses (Aznar-Sánchez et al., 2011; de Andrés et al., 2017; Martín García, 2007; Viciana Martínez-Lage, 2007). Currently, greenhouses occupy a vast region in south-eastern Spain, especially across the coastal plains—in the province of Almería alone, greenhouses extend over 30,000 ha, making this region one of the world's largest areas of concentrated greenhouses (Díez-Garretas et al., 2019). These constructions, which also occupy natural habitats of European interest (Benito et al., 2009), have led to the extinction of several local populations of vulnerable taxa. For example, almost 92% of the range regressions of the regionally endangered scrub *Maytenus senegalensis europaea* (Boiss.) Rivas Mart. ex Güemes and Crespo (Celastraceae) were registered from 1957 to 1994, a consequence of habitat destruction directly associated with the expansion of greenhouses in Campo de Dalías and Llanos del Ejido (Almería) (Mendoza-Fernández et al., 2015; Mota et al., 1996). Similar patterns of range regression have been reported among populations of narrow endemic animal taxa across south-eastern Spain (Diogo et al., 1999; García-París & Ruiz, 2011). However, the extent of intraspecific diversity loss within these taxa is unknown.

A noteworthy case of range regression in south-eastern Spain caused by habitat destruction has been detected in giant flightless blister beetles of the genus *Berberomeloe* Bologna, 1989 (García-París & Ruiz, 2008, 2011). To complicate matters, recent phylogenetic investigations have revealed the existence of marked intra- and inter-specific diversity within *Berberomeloe*, especially in the *Berberomeloe majalis* species group, one of the two main clades comprising the genus (Sánchez-Vialas et al., 2020). The other clade, the *Berberomeloe insignis* species group, which is restricted to high mountainous and semiarid areas in south-eastern Spain, has not been studied in detail and no phylogeographic studies have been carried out across its entire distribution. The *B. insignis* species group includes two species: *B. insignis* (Chapentier, 1818), a lowland, xerophilous species distributed across the coastal provinces of Murcia, Almería and Granada, where it is considered threatened at the regional and national levels (García-París & Ruiz, 2008, 2011), and *Berberomeloe tenebrosus* Sánchez-Vialas et al., 2020, a montane species limited to high elevations across the south-eastern mountain chains of Sierra Nevada and Sierra de Los Filabres (Sánchez-Vialas et al., 2020). Although *B. tenebrosus* is a morphologically conserved species (Sánchez-Vialas et al., 2020), *B. insignis* presents notable variability in its head phenotype, particularly in the extension and shape of the red colouration on the temples and, in some cases, the presence of an additional red mark on the frons (García-París, 1998; Sánchez-Vialas et al., 2020). There is a well-defined geographic correspondence among populations exhibiting differentiated morphological traits (e.g., pronotum shape and sculpture, shape of the coloured bands, male genitalia) and phylogeographic units (based on mtDNA) in the *B. majalis* clade. This correspondence led to the description of a set of new species hypothesised to be independent evolutionary units (Sánchez-Vialas et al., 2020). In addition, specific mate selection was previously reported in syntopic encounters between specimens representing different evolutionary units of *Berberomeloe* in the Almería region (García-París et al., 1999); however, this selection is probably not directly related with specific differences in morphological traits. Despite these observations, it remains uncertain if the morphological diversity observed in *B. insignis* arose from an allopatric pattern of diversification, similar to the situation documented for the *B. majalis* species group (Sánchez-Vialas et al., 2020) or, was it a response to local environmental pressures. With regard to the possibility of the first case, putative evolutionary units within *B. insignis* would likely have become extinct even before conservation measures could be established. The natural histories of *B. insignis* and *B. tenebrosus* are poorly known (García-París & Ruiz, 2008, 2011; Ruiz & García-París, 2013). Species of *Berberomeloe* present limited dispersal abilities at larval and adult stages (Bravo et al., 2017; Percino-Daniel et al., 2013). They also depend on the presence of large solitary bee populations (Apoidea: Anthophoridae, Andrenidae and perhaps Megachilidae), which their larvae parasitise (Bologna, 1989, 1991; Bologna & Pinto, 2002; López-Estrada et al., 2022). Owing to this dependence, these endemic beetle species are highly vulnerable to any degradation or habitat loss that induces changes in bee communities (García-París et al., 2006; García-París & Ruiz, 2008, 2011; Sánchez-Vialas et al., 2020).

The limited dispersal ability and distribution area of these endemic species offer an exceptional model to study the vulnerability of evolutionary lineages facing (1) habitat destruction (e.g., greenhouse expansion) that could eliminate entire populations or lineages, thereby affecting gene flow across the species' range, and (2) a future climate change scenario in south-eastern Spain, one of the most arid regions of Europe, that could affect their long-term survival. Moreover, the threatened status and narrow distribution range of *B. insignis*

TABLE 1 Species identity, collection locality, voucher number and corresponding GenBank accession numbers of the samples of *Berberomeloe insignis* and *Berberomeloe tenebrosus* analysed in this study.

Species	Locality	Voucher number	GenBank#CoxI	GenBank#16S	GenBank#ITS2
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB181	KC853087	KC853066	
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB184	KC853088	KC853067	
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB193	MN252816	MN252646	
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB194	MN252817	MN252647	
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB195	MN252818	MN252648	
Berberomeloe insignis	Spain: Almería: El Puntal	BiMAB196	MN252819	MN252649	
Berberomeloe insignis	Spain: Almería: Peñas Negras-Los Perales	BiMAB192	MN252815	MN252645	
Berberomeloe insignis	Spain: Almería: 4.5 Km southern of Zurgena	BiMAB182	KC853086	KC853065	
Berberomeloe insignis	Spain: Almería: Tabernas	ASV18010	OQ151517	OQ151611	OQ159079
Berberomeloe insignis	Spain: Almería: Tabernas	ASV19009	OQ151521	OQ151615	OQ159081
Berberomeloe insignis	Spain: Almería: Tabernas	ASV19010	OQ151522	OQ151616	OQ159078
Berberomeloe insignis	Spain: Almería: El Sabinar	ASV18011	OQ151518	OQ151612	OQ159074
Berberomeloe insignis	Spain: Almería: Las Marinas	ASV18012	OQ151519	OQ151613	OQ159075
Berberomeloe insignis	Spain: Granada: La Garnatilla	ASV18041	OQ151523	OQ151617	OQ159073
Berberomeloe insignis	Spain: Granada: La Garnatilla	ASV18042	OQ151524	OQ151618	
Berberomeloe insignis	Spain: Granada: La Garnatilla	ASV18043	OQ151525	OQ151619	OQ159072
Berberomeloe insignis	Spain: Granada: Polopos	ASV18009	OQ151516	OQ151610	OQ159071
Berberomeloe insignis	Spain: Murcia: Águilas	ASV18014	OQ151526		OQ159076
Berberomeloe insignis	Spain: Murcia: Las Palas	ASV18023		OQ151620	OQ159082
Berberomeloe insignis	Spain: Murcia: Mazarrón	ASV18040	OQ151527	OQ151621	OQ159077
Berberomeloe insignis	Spain: Murcia: Morata	ASV18013	OQ151520	OQ151614	OQ159080
Berberomeloe tenebrosus	Spain: Almería: Puerto La Ragua	APP17005	MN252870	MN252751	OQ159083
Berberomeloe tenebrosus	Spain: Almería: Puerto La Ragua	APP17006	MN252871		OQ159084
Berberomeloe tenebrosus	Spain: Almería: Calar Alto	ASV16028	MN252867	MN252748	OQ159088
Berberomeloe tenebrosus	Spain: Almería: Calar Alto	ASV16036	MN252868	MN252749	OQ159087
Berberomeloe tenebrosus	Spain: Almería: Puerto de Escúllar	ASV16080	MN252864	MN252745	
Berberomeloe tenebrosus	Spain: Granada: Pradollano	ASV16075	MN252849	MN252732	OQ159085
Berberomeloe tenebrosus	Spain: Granada: El Veleta	ASV16071	MN252869	MN252750	OQ159086

make it an important species for conservation. However, at present, the lack of sufficient knowledge on the geographic distribution of the genetic diversity of this narrow Iberian endemic precludes accurate conservation strategies, including the definition of evolutionary significant units (ESUs) for this species.

In this study, we determine whether the populations of the south-eastern endemics *B. insignis* and *B. tenebrosus* are genetically structured and, using this data, define conservation units for their protection and the preservation of their genetic heritage, evolutionary potential and morphological diversity. We also identify the vulnerability of these species under different climate change scenarios, considering the ESUs defined. Specifically, we analysed (1) the intraspecific genetic structure within *B. insignis* and *B. tenebrosus* through phylogeographic and phylogenetic analyses; (2) the geographic structure of these lineages and, in the case of *B. insignis*, correlations between this structure and morphological variation (including colour pattern) through morphometric and quantitative analyses; and (3) species vulnerability based on climatic niche modelling and lineage-specific threats.

MATERIALS AND METHODS

DNA extraction and sequencing

A total of 28 specimens of *B. insignis* (21 samples from 12 populations) and *B. tenebrosus* (seven samples from five populations) were collected across their known geographic range and used for molecular analyses (Table 1).

Tissue samples were collected from the coxae or femora of ethanol-preserved specimens that had been stored at −20°C. Genomic DNA was extracted using the Qiagen DNeasy kit. Polymerase chain reaction (PCR) was used to amplify the nuclear internal transcriber spacer-2 (ITS2) region using primers CAS28Sb1d/CAS5p8sFt

(Ji et al., 2003) and two mitochondrial regions: cytochrome oxidase I (*Cox1*) using primers LCO 1490/ COI-H (Folmer et al., 1994; Machordom et al., 2003) and 16S ribosomal RNA (16S) using primers 16Sar/16Sbr (Palumbi et al., 1991). PCR conditions used to amplify *Cox1* and 16S were as described by Sánchez-Vialas et al. (2020) and were the same for ITS2, except that an annealing temperature of 48°C was used. PCR purification and Sanger sequencing were performed by Macrogen Spain Inc. (Madrid, Spain).

Sequence alignment and phylogenetic and time estimation analyses

The data set consisted of 18, 27 and 26 sequences of ITS2, *Cox1* and 16S, respectively, for *B. insignis* and *B. tenebrosus* (Table 1). Sequences were revised using Sequencher v4.9. and aligned with MAFFT (Katoh & Toh, 2008). Alignments were visually checked in Mesquite v3.04 (Maddison & Maddison, 2016).

Coalescent Bayesian phylogenetic analysis and time divergence estimates for the mitochondrial DNA (mtDNA) were performed in BEAST v1.8.3 (Drummond et al., 2012). Analyses were run for 100×10^6 generations, sampling every 10,000, under a Yule Process tree prior and a lognormal relaxed molecular clock with the following substitution models calculated in JModelTest v.2 (Darriba et al., 2012): HKI + G + I for *Cox1* and TN93 + G + I for 16S. As no fossils are available to calibrate a molecular clock for this group, we used substitution rates suggested by Papadopoulou et al. (2010) to estimate time to the most recent common ancestor (TMRCA). We used lognormal distributions in real space with the following values for the ucld.mean prior: initial value = 0.0178 substitutions/site/my, Log (Mean) = 0.0178, Log (SD) = 0.2 for *Cox1* and initial value = 0.0049 substitutions/site/my, Log (Mean) = 0.0049, Log (SD) = 0.2 for 16S. Trace plots and effective sample size (ESS) were examined in TRACER v1.5 to evaluate the convergence of runs. The results were summarised and annotated on a maximum clade credibility tree, with a 25% burn-in, using TreeAnnotator v1.7.5 (Drummond et al., 2012).

Networks for the observed nuclear ITS2 alleles were built with PopArt v1.7 (Leigh & Bryant, 2015) using a minimum spanning network for *B. insignis* and *B. tenebrosus* independently. Haplotype diversity was calculated in DnaSP 6 (Rozas et al., 2017).

Morphometric analyses and qualitative morphological traits of *B. insignis*

We examined quantitative morphological variation across 94 ethanol-preserved specimens belonging to the same populations as the genotyped individuals. We assessed the following traits: head width; pronotum distal width (between the posterior apexes) and proximal width (between the fore apexes); pronotum length along the sagittal plane and length of the metafemur, metatibia and metatarsus (Table S1). These traits have proven to be diagnostic between two related species of *Berberomeloe* (Sánchez-Vialas et al., 2020). All measurements were made from images taken on a Leica MZ16A stereomicroscope fitted with a Leica DFC550 camera and processed with the software LAS v4.3. Data were analysed using 'R' software v1.5.001 (RStudio Team, 2019). Trait measurements were size corrected by taking the residuals from a regression against head width. Head width in *Berberomeloe* was selected as a better proxy of specimen size than total body length, which is highly variable and depends on the extent of hypertrophy of the abdomen (it is very short in recently emerged adults, but expands while feeding, and becomes shorter under starvation conditions or after ovoposition). To visualise the relationship among the data, a principal component analysis (PCA) was performed and plotted with the packages 'psych' (Revelle, 2019) and 'factoextra' (Kassambara, 2017). Linear discriminant analysis (LDA), using the packages 'MASS' (Venables & Ripley, 2002) and 'ggplot2' (Wickham, 2009), was also performed to verify whether priori-defined groups (i.e., main phenotypically distinguishable mtDNA clades) could be discriminated.

The head colour pattern of 135 specimens (ethanol- or dry-preserved) of *B. insignis*, all held in the entomological collection of the Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain, was examined and characterised as a qualitative morphological trait. This pattern can be unambiguously characterised regardless of specimen age or preservation method (ethanol or dried). Four distinct phenotypes can be classified based on the shape and extension of the red- to orange-coloured blotches on the temples and, in some instances, on the frons. Three of these phenotypes are illustrated in García-París (1998).

Climatic niche modelling

Presence localities of *B. insignis* ($n = 55$) and *B. tenebrosus* ($n = 21$) were extracted from the literature and unpublished records (Table S2). Species distribution models (SDMs) based on climatic niche modelling were used to evaluate the effect of climate change predicted for the years 2080–2100 on the habitat suitability for each species. For the climatic niche modelling, we downloaded the 19 bioclimatic variables from WorldClim v2.1 (www.worldclim.org) at a resolution of 2.5 min (Fick & Hijmans, 2017). We then performed a correlation analysis on these variables (pairs with a value ≥80% were considered correlated) using the package 'corrgram' (Wright & Wright, 2018), implemented in R v1.5.001. After considering the observed correlations among different bioclimatic variables and their biological relevance, the following four were selected for the SDMs: annual mean temperature (Bio1), temperature seasonality (Bio4), annual precipitation (Bio12) and precipitation of driest quarter (Bio17). The SDMs were built with MaxEnt v3.4.0 (Phillips et al., 2004), which estimates the interaction between environmental variables and species presence over a geographic area to create suitability models. We ran MaxEnt with 10 bootstrap replicates and 10% random values; the model was evaluated by using the AUC criterion (Peterson et al., 2011).

Projections of future distributions of the species (for 2080–2100) were also produced with MaxEnt v3.4.0 using the most recent data

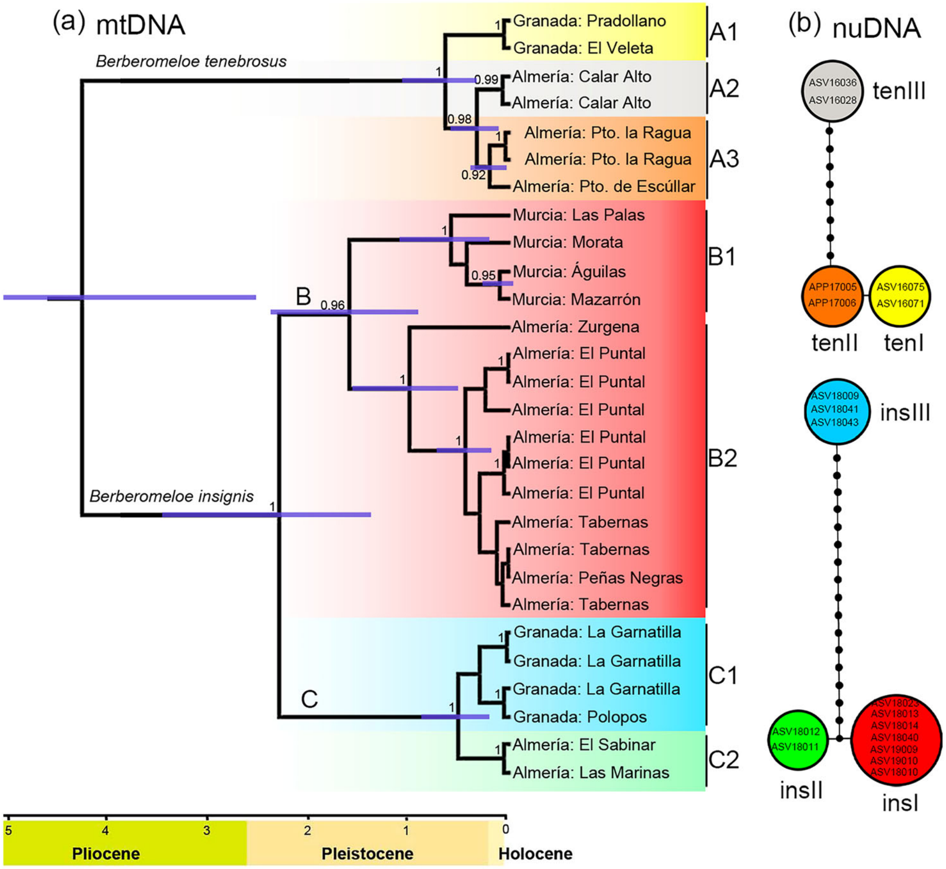

FIGURE 1 (a) Bayesian chronogram based on mtDNA of the sister species pair *Berberomeloe insignis* and *Berberomeloe tenebrosus*. Number on each node represents the posterior probability. Relevant lineages discussed in this work are labelled. (b) Minimum spanning network of nuclear alleles. Each studied specimen is shown within its corresponding allele. The colour of the allele group corresponds with the colour of its respective mtDNA clade in A.

from the Climate Modelling Intercomparison Project, Phase 6 (CMIP6) (Eyring et al., 2016) under future climate scenarios based on two Representative Concentration Pathways (RCPs): RCP4.5 and RCP8.5, representing moderate and high greenhouse gas emission levels, respectively. All MaxEnt outputs were edited with QGIS v3.8.3 (www.qgis.org) to generate the projected distribution maps.

RESULTS

Phylogeographic patterns and divergence time estimations

The mtDNA alignment is 1167 bp in length (*Cox1*: 657 bp, 16S: 510 bp), and the ITS2 alignment, which did not contain any ambiguous nucleotide signal, is 342 bp in length. Based on the mtDNA data, the TMRCA of *B. insignis* and *B. tenebrosus* dates back to 4.33 Ma (95% HPD: 2.4–6.4 Ma) (Figure 1a).

The mitochondrial phylogeography of *B. tenebrosus* depicts three late Pleistocene lineages: lineage A1 from the western Sierra Nevada (675,000 years; 95% HPD: 0.3–1.1 Ma), representing the sister group to the clade formed by lineage A2 from the central region of Sierra de los Filabres and lineage A3 from the eastern Sierra Nevada and western Sierra de los Filabres (375,000 years, 95% HPD: 0.15–0.6 Ma) (Figure 1a). Six and three haplotypes were recovered for *Cox1* (Haplotype diversity [Hd]: 0.95) and 16S (Hd: 0.6), respectively.

Two main well supported and geographically structured mitochondrial clades were recovered within *B. insignis* (Figure 1a): Clade B from Murcia (lineage B1) and eastern Almería (lineage B2) and Clade C from south-eastern Granada (Sierra de la Contraviesa and surroundings of Motril, lineage C1) to southern Almería (Campo de Dalías, lineage C2) (Figure 1a). According to the divergence time estimates, the TMRCA of the main lineages B and C dates back to 2.13 Ma (95% HPD: 1.3–3.2 Ma). The TMRCA of lineages B1 and B2 is around 1.5 Ma (95% HPD: 0.81–2.2 Ma), and lineages C1 and C2, 565,000 years (95% HPD: 0.22–0.91 Ma). Fourteen and nine haplotypes were recovered for *Cox1* (Hd: 0.95) and 16S (Hd: 0.87), respectively.

As the nuclear ITS2 networks for *B. insignis* and *B. tenebrosus* differ markedly in the number of mutations, they are represented independently for easier visualisation (Figure 1b). Populations of *B. insignis* present three different nuclear alleles: the first (insI), which is congruent with lineage B (from Murcia and eastern Almería), differs by two mutational steps from the second allele (insII), which is congruent with lineage C2 from southern Almería (Figure 1b); the third

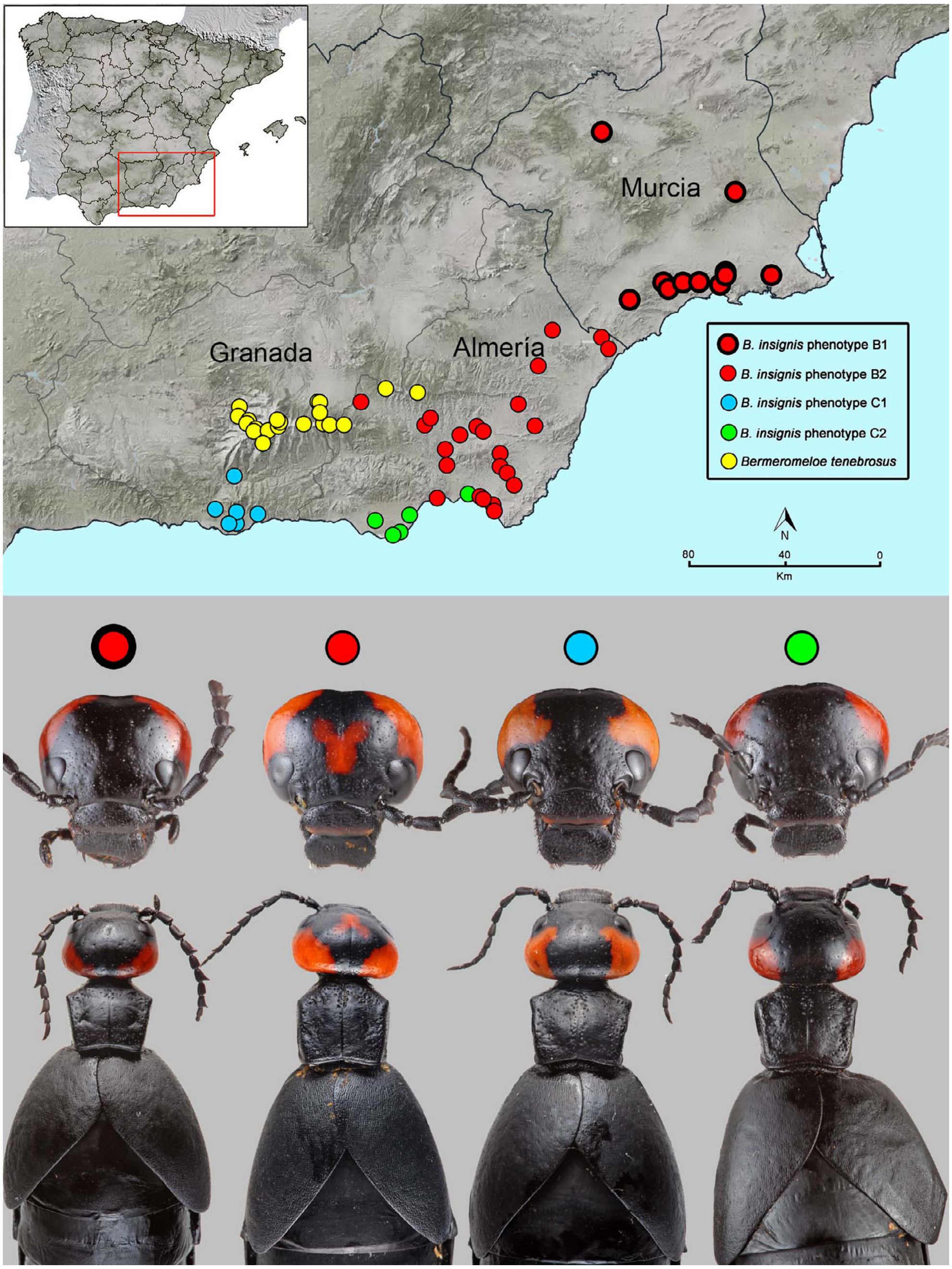

FIGURE 2 Geographic map of the current known populations of *Berberomeloe insignis* and *Berberomeloe tenebrosus* in the south-eastern Iberian Peninsula. Colour of each locality indicates the head phenotype found at that locality (see legend), which is congruent with the main mitochondrial lineages found in *B. insignis*.

(insIII), congruent with lineage C1 from Granada, is the most differentiated allele (18 mutational steps from the other two alleles).

The studied populations of *B. tenebrosus* also present three ITS2 alleles: tenI, tenII and tenIII. TenI is found in populations located in the western Sierra Nevada, concordant with lineage A1. It differs by only one mutation from tenII, which is present in populations from the eastern Sierra Nevada, represented by lineage A3. TenIII is present in the central region of Sierra de los Filabres, concordant with lineage A2, and differs from TenI and TenII by 10 and nine mutations, respectively (Figure 1b). This pattern does not match the mitochondrial phylogeographic structure, which shows Filabres and eastern Sierra Nevada populations as more closely related.

Morphological diversification in *Berberomeloe insignis*

Our analyses of head colour patterns in *B. insignis* reveals four unambiguously diagnosable phenotypes that represent a geographically structured distribution (Figure 2). Populations from Sierra de la Contraviesa and the surroundings of Motril (Granada) show two

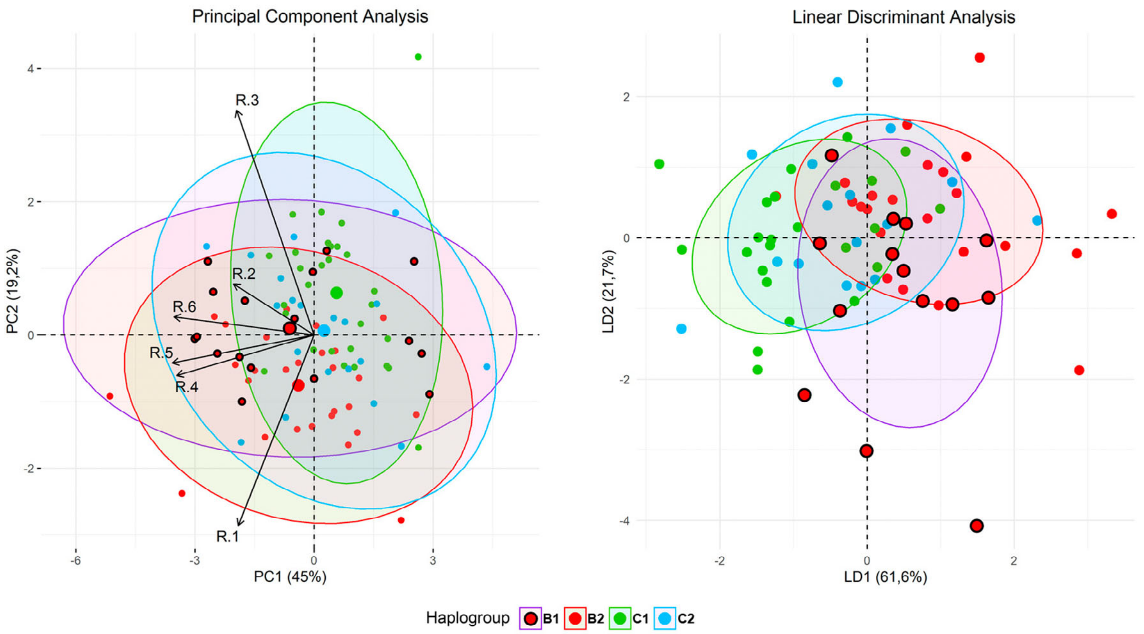

FIGURE 3 Representation of the first two axes of the principal component (PC1 and PC2) and linear discriminant (LD1 and LD2) analyses based on six size-corrected morphometric variables of *Berberomeloe insignis*. The different colours represent the four main mitochondrial lineages. Ellipses show the 80% confidence intervals for each lineage.

symmetric red- to orange-coloured blotches on the temples, of which the inner lower margin, after bordering the eye, extends slightly into the frons (but not reaching the frons midline) (Figure 2: phenotype C1; genotyped specimens of lineage C1/allele insIII present this phenotype). Populations from eastern Almería and Pasillo de Fiñana Valley (between Sierra Nevada and Sierra de los Filabres) have two symmetric red- to orange-coloured blotches over the temples that do not border the upper eye area and a single, isolated smaller blotch on the frons that is generally 'V' or 'Y' shaped (Figure 2: phenotype B2; genotyped specimens of lineage B2/allele insI show this phenotype). The third and fourth phenotypes resemble that of B2 but lack the frontal blotch on the frons. Populations presenting these phenotypes are allopatrically distributed in Murcia (Figure 2: phenotype B1; genotyped specimens of lineage B1/allele insI show this phenotype) and southern Almería at Campo de Dalías, and in isolation in El Alquián (not sequenced) (Figure 2: phenotype B2; genotyped specimens of lineage C2/allele insII show this phenotype). Phenotype B1 differs from B2 by the narrower and elongated shape of its blotches.

Our morphometric results from the PCA and LDA analyses show extensive overlap among the functions PC1 (45%) and PC2 (19.2%), and LD1 (61.6%) and LD2 (21.7%). None of the studied traits show morphometric differentiation; therefore, no correlation is observed between any of these traits and genetic and head phenotypic diversity (Figure 3).

Climatic niche modelling

The models of current and future suitable climatic areas show bioclimatic variables Bio12 (annual precipitation) and Bio4 (temperature seasonality) contributing most to the models of *B. insignis* and Bio1 (annual mean temperature) and Bio4, to the models of *B. tenebrosus* (AUC range: 0.93–0.99) (Appendix A).

The predictions for the period 2080–2100 indicate a decrease in habitat suitability for both species under the two RCPs scenarios (Figure 4). A greater reduction in habitat suitability is observed under the RCP8.5 than RCP4.5. Under RCP8.5, the current range of *B. insignis* would no longer be suitable for this species, especially in the coastal areas. Instead, the most suitable areas would likely be steep slopes in the low- and mid-elevation areas in the Sierra Nevada, Sierra de los Filabres and Sierra de Gádor mountain systems. Under both future climatic scenarios, habitat suitability for *B. tenebrosus* is predicted to remain stable in the upper parts of Sierra Nevada and Sierra de los Filabres, where the species currently occurs (Figure 4). Sierra de Gádor, located in southern Almería, about 15 km south of Sierra Nevada, also represents a currently suitable area for *B. tenebrosus*; however, to date, the species has not been found in this mountain range.

DISCUSSION

Phylogeographic and morphological patterns in *B. insignis* and *Berberomeloe tenebrosus*: A history of geographic isolation

Diversification within *B. insignis* and *B. tenebrosus* started about 2.5 Ma and 675,000 years ago, respectively. A pattern of cyto-nuclear discordance was observed in both species, specifically between the parapatric lineages B2 (eastern Almería) and C2 (western Almería) of *B. insignis*, and between A1 (western Sierra Nevada) and A3 (eastern Sierra Nevada) of *B. tenebrosus*.

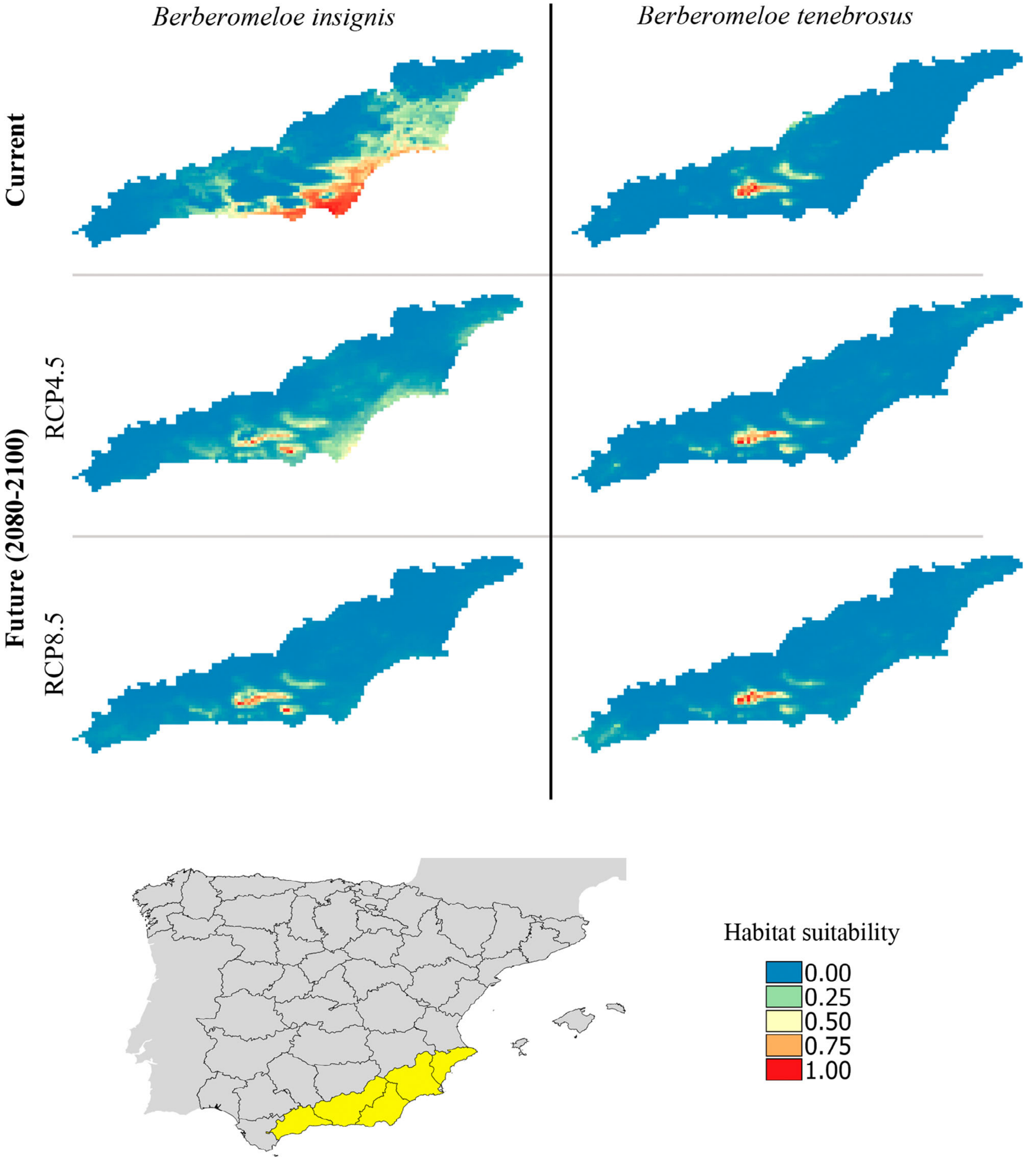

FIGURE 4 Climatic niche modelling for *Berberomeloe insignis* and *Berberomeloe tenebrosus* under current and future scenarios. Habitat suitability for the years 2080–2100 is predicted under two greenhouse gas emission scenarios, RCP4.5 and RCP8.5.

Intraspecific cyto-nuclear discordances are often found in species inhabiting regions with a complex geographic and climatic history (Freitas et al., 2020; McGuire et al., 2007; Velo-Antón et al., 2018). These discordances are usually driven by genetic introgression between lineages or taxa that have undergone rapid range expansions during climatically favourable periods (García-París et al., 2003; Pereira et al., 2016). Incomplete lineage sorting (Freitas et al., 2020; Neigel & Avise, 1993), sex-biased dispersal (Bonnet et al., 2017; Toews & Brelsford, 2012) and processes derived from inherited symbionts, such as *Wolbachia* in invertebrates (Hurst & Jiggins, 2005), have also been invoked to explain cyto-nuclear discordances. Under an incomplete lineage sorting scenario, a geographically unstructured

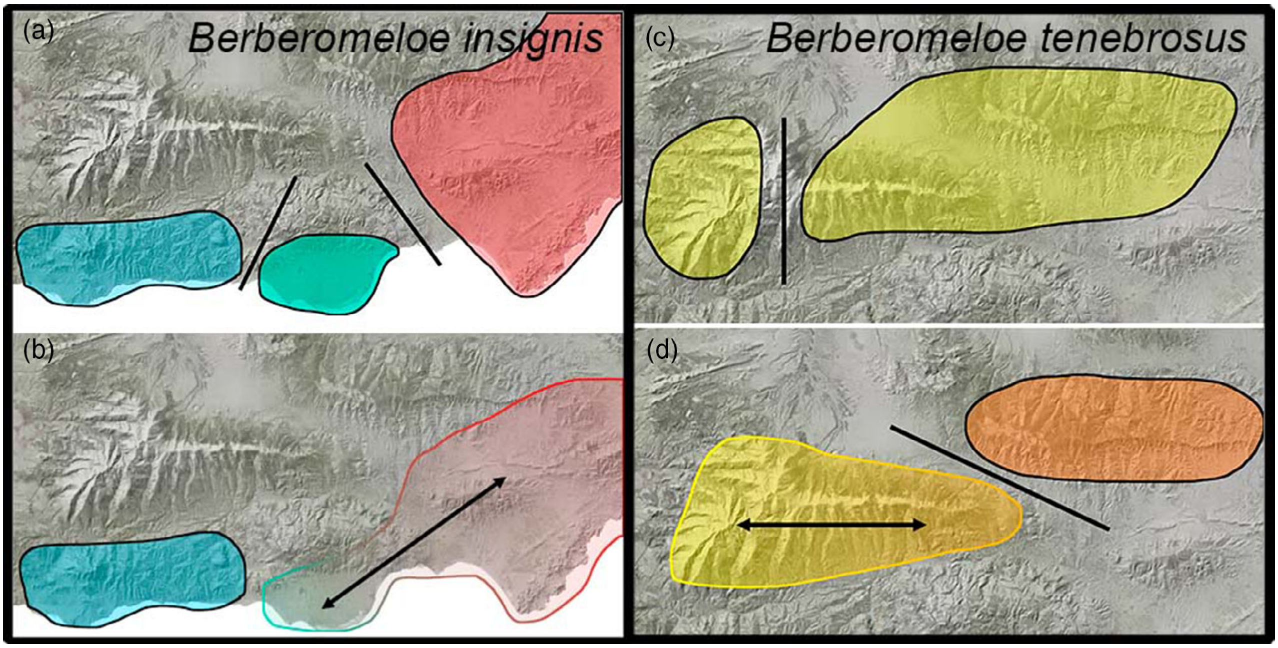

FIGURE 5 Hypothetical reconstruction of the major historical cladogenetic and gene flow events uncovered among some lineages of *Berberomeloe insignis* and *Berberomeloe tenebrosus* at different times. In the scenario of *B. insignis*, three major allopatric events occurred in its southern range (a) the oldest one is depicted by the eastern Almería lineage (red), followed by the split between the lineage from Sierra de Contraviesa (blue) and Campo de Dalías (green); after the allopatric differentiation, (b) an event of genetic introgression could have occurred across the lowlands that would have allowed contact between the eastern and southern Almería lineages. In the scenario of *B. tenebrosus*, (c) an allopatric split first occurred between the western Sierra Nevada and eastern Sierra Nevada/Sierra de los Filabres, followed by (d) an event of genetic introgression across Sierra Nevada and the isolation of the Sierra de los Filabres populations.

distribution pattern of genetic diversity would be expected (Albert et al., 2007); however, in our case, the mtDNA and nDNA lineages are geographically structured. Because *Wolbachia* is a genus of maternally inherited bacteria that spread by cytoplasmic transmission, it usually promotes the selection of particular mtDNA haplotypes (Hurst & Jiggins, 2005; Turelli et al., 1992). However, the haplotypic and phenotypic patterns found in *Berberomeloe* are not congruent with the effects of *Wolbachia*. The sex-biased dispersal hypothesis cannot be discarded because we lack data for estimating differential migration rates (López-Uribe et al., 2014). However, direct field observations show that both sexes seem to be equally poor dispersers (pers. obs., authors), suggesting that the sex-biased dispersal hypothesis likely does not explain the observed discordance in the beetle species. Because the mitochondrial and nuclear gene trees (*Cox1* plus 16S, and ITS2) show well-structured topologies that are mostly consistent with one another, except for the relationship between some parapatric lineages, we hypothesise that the observed cyto-nuclear discordances are likely a consequence of gene introgression following secondary contact between some of those parapatric lineages (García-París et al., 2003; Ivanov et al., 2018; Mas-Peinado et al., 2022). The substitution rates of our markers are too slow to differentiate between contemporaneous or historic introgression processes. In this sense, additional data such as single nucleotide polymorphism (SNP) could be used to investigate patterns of gene flow among lineages.

We suggest the discordant patterns in *B. insignis* could be the result of early allopatric or parapatric splits that are currently reflected in the mtDNA data set (Figure 5a). Following these splits, migratory events may have favoured contact and gene flow across secondary contact zones between lineages C2 (south-east of Almería) and B2 (south of Almería) (Figure 5b). A subsequent and more recent isolation event would explain the parapatric distribution of the nuclear alleles and head phenotypes, with no evidence of intermixing. Due to their phenotypic morphological singularity, lineage C2 (southern Almería) remains clearly distinguishable from the parapatric lineages (C1 from Granada and B2 from south-eastern Almería). Thus, populations from southern Almería seem to form a cohesive reproductive group that is geographically restricted and evolutionarily independent.

The existence of secondary contact zones between some parapatric lineages is likely influenced by the topography of the region, as has been reported for other organisms and areas (MacCallum et al., 1998; Velo-Antón et al., 2021). This is likely the case for lineages C2 and B2 of *B. insignis*, which occur mostly in lowlands with no marked structural landforms and a relatively homogeneous habitat structure. The coastline where many populations currently occur repeatedly experienced sea level fluctuations during the Pleistocene climatic changes (Alcántara-Carrió et al., 2013), which could have resulted in range expansions favouring secondary contact zones, as has been observed in other taxa inhabiting coastal regions (Senczuk et al., 2019). In contrast, the environmental heterogeneity and complex topology of the mountains where lineage C1 occurs might have favoured the geographic isolation of these populations. Similar results have been observed for other groups occurring in arid mountainous landscapes in which elevation can impact population-level biodiversity (Garrick, 2011; Pepper et al., 2011). Topography is also an important factor related to diversification (Baselga et al., 2011; García-París et al., 2000; Habel et al., 2012; Wiens et al., 2007). Mountain regions provide adaptive and non-adaptive opportunities for diversification, as montane populations can become easily isolated over time from conspecific ones, interrupting gene flow among them (Habel et al., 2012). This could be the case for lineage C1, and future research should explore the factors influencing its evolutionary trends.

Within *B. tenebrosus*, the mtDNA data show a pattern of geographic isolation from west to east (Figure 5c); however, the nuclear evidence suggests contemporaneous population intermixing between the eastern and western Sierra Nevada (Figure 5d). Climatic fluctuations during the Pleistocene could have resulted in recurrent range shifts in these lineages, leading to the formation and disappearance of secondary contact zones and thus the contrasting cyto-nuclear patterns (Pereira et al., 2016). The isolation of populations from Sierra de los Filabres with respect to Sierra Nevada could be typical of warm interglacial periods, when the lowlands between these mountain systems would have been unsuitable for *B. tenebrosus*, thereby restricting them to high elevation areas (Sánchez-Vialas et al., 2020). Another possible explanation could be related to the competence exerted by congeneric species present across areas of low to medium altitude between Sierra Nevada and Sierra de los Filabres, such as *Berberomeloe indalo* (Sánchez-Vialas et al., 2020).

Although general morphological (body proportions) differences have not been found across the geographic range of *B. insignis* (see Figure 3), phenotypic diversity is observed in the pattern of head colouration. Head phenotypes in *B. insignis* are geographically structured, and each of the recovered mitochondrial clades (see Figures 1 and 2) consistently coincides with one of these phenotypes, and in general, are potentially useful for lineage identification. However, not every morphologically revised population has been genotyped. This noteworthy pattern in which the most similar phenotypes are geographically separated by another distinctive phenotype, with no mixed phenotypes close to their contact zones, represents a challenge to investigate. Gene flow among phenotypically differentiated species or lineages has driven colour variation in many taxa (Medina et al., 2013). Additional sampling across the eastern region of Sierra de la Contraviesa, where lineage C1 is expected, and western Almería, including low to medium altitudes of Sierra de Gádor, where lineage C2 is expected, is needed to delimit the distribution boundaries of these lineages and to test for the existence of gene flow between them. The mechanisms for retaining two very distinctive phenotypes within clade B (B1 from Murcia and B2 from south-eastern Almería) remain unknown. The boundaries between lineages B1 and B2 are expected to span a narrow region corresponding to the political border between the provinces of Murcia and Almería (see Figure 2). Future studies of these lineages should focus on improving the sampling effort and including additional nuclear markers while increasing the numbers of individuals used in genetic analyses, in order to obtain fine-scale results across the different contact zones. This will allow for a proper discussion of the processes involved in phenotype delimitation.

Conservation of intraspecific diversity in a changing scenario

In this study, our phylogenetic analyses recovered four old, independent evolutionary lineages within the limited geographic range of *B. insignis*. According to Moritz (1999, 2002), historical, isolated intraspecific lineages deserve special conservation protection as they represent independently evolving sets of populations. As our results indicate the existence of ongoing processes of diversification in *B. insignis*, we suggest that these four lineages should be considered independent ESUs for conservation purposes.

Preservation of the areas where the 4 units occur will preserve the history of contact among phylogroups (in south and eastern Almería where lineages C2 and B2 occur in parapatry) or of the areas in which a different process is currently operating to maintain distribution boundaries between phenotypes (i.e., along the boundary between Murcia and Almería, where lineages B1 and B2 occur, respectively). As interpreted by Hewitt (1988), hybrid zones are 'natural laboratories for evolutionary studies' as they provide experimental areas in which to study factors shaping gene flow between divergent lineages (Andújar et al., 2014; Dinis et al., 2019; Dufresnes et al., 2020) and the nature of genetic barriers. In this sense, the narrow region of Campo de Dalías, where lineage C2 occurs, merits special conservation status for preserving both historical and current evolutionary processes (Cozzolino et al., 2006; Evans et al., 2012).

The current trend in which entire populations are being lost is diminishing the resilience of lineages to respond to global changes (e.g., climate change), therefore increasing the risk of species extinction. Future climate change models predict that suitable areas for *B. insignis* will largely shift from low to mid-elevation mountain systems. Under changing climatic scenarios (particularly RCP8.5), most, if not all, lowland populations would become extinct unless they disperse altitudinally, potentially surviving on the slopes of the mountains currently inhabited by the species. The south-eastern Iberian mountain systems may preserve suitable ecological conditions, acting as refugia for some lineages, such as those in south-eastern Almería (B2) and in Granada (C1). The most vulnerable lineage, especially under the most extreme scenario (RCP8.5), is lineage B1 from Murcia, where there are no high mountain systems within the species' range.

The habitat of *B. insignis* is disappearing across a large part of its range at an alarming rate (García-París & Ruiz, 2008, 2011). Unless specific conservation plans are developed, some lineages will soon become highly threatened, and lineage C2 from southern Almería will become extinct. Coastal plains across Spain are being transformed into agricultural fields and greenhouses (Caparros, 2008; Castro et al., 2019; Viciana Martínez-Lage, 2007), especially in the natural region of Campo de Dalías, where the world's largest concentration of intensive agriculture is located (Aznar-Sánchez et al., 2011) (Figure 6). Restoration of connectivity among anthropogenically isolated populations is important for the long-term viability of metapopulations across fragmented systems (Moritz, 1999), particularly considering future ecological suitability trends. This lineage, only found across the 1960 ha of Paraje Natural Punta Entinas-Sabinar (Sánchez-Vialas

FIGURE 6 Greenhouse expansion in Almería threatens lineage C2 of *Berberomeloe insignis*. (a) Aerial view of the vast concentration of greenhouses in Campo de Dalías, Almería. The red asterisk indicates an area in which greenhouse expansion resulted in the recent destruction (in 2020) of the remaining natural steppes in the region. (b) The same region with a view from the southern slope of Sierra de Gádor showing the 'plastic sea' (photo taken in 2020).

et al., 2020), is bordered by the greenhouses of Campo de Dalías to the north, urban developments to the east and west and the Mediterranean Sea to the south, making it the most threatened phylogroup of *B. insignis* (Figure 6).

It seems particularly necessary to protect the Sierra de la Contraviesa and Sierra de Lújar mountain ranges to conserve the unique and geographically narrow evolutionary lineage C1. As an example, while conducting field observations in Sierra de Lújar in March 2020, 11 road-killed specimens were detected over a 7 km road section between Gualchos and Lújar (A. Sánchez-Vialas pers. obs.). This is particularly worrying since excessive road traffic was not observed during the sampling period, suggesting that even moderate levels of fast road traffic could represent a considerable problem for the lineage. The possible attraction of males to a dead female's volatile compounds on the road could exacerbate the situation (Figure 7).

In the case of *B. tenebrosus*, conservation efforts should focus on the two main nuclear lineages from Sierra Nevada and Sierra de los Filabres. Future climate scenarios of global warming suggest that the range suitability of *B. tenebrosus* will remain relatively stable and limited to the highlands of Sierra Nevada (maximum altitude: 3479 m), Sierra de los Filabres (2168 m) and Sierra de Baza (2269 m). The conservation status of *B. tenebrosus* is more favourable than that of *B. insignis* as the ranges of the detected lineages are mostly within protected natural regions: Sierra Nevada for lineages A1 and A3, and Sierra de Baza (which is connected to Sierra de los Filabres) for lineage A2 (Sánchez-Vialas et al., 2020). Despite this, the distribution range of *B. tenebrosus* is small and will become even smaller if climate change predictions come true. Given its narrow ecological niche and its large size and conspicuous appearance, *B. tenebrosus* is an ideal biological indicator for global change across the high elevation areas of Sierra Nevada and Filabres.

FIGURE 7 Two specimens, female and male, of *Berberomeloe insignis* from Lújar, lineage C1, dead on the road. Some males were found inspecting female carcasses on the same road.

This study is only a token of the massive diversity loss faced by one of the most endemic arthropod-rich areas in Europe (Sanchez Piñero, 2006). The superfamily Tenebrionoidea alone harbours more than 100 species endemic to the provinces of Granada, Murcia and Almería (Cobos, 1988; Escalera, 1905; Piñero et al., 2011; Sanchez Piñero, 2006; Viñolas & Cartagena, 2005) that have very reduced geographic ranges and are facing immense pressure from agricultural and tourism development. We believe it is time that the European Union and the Spanish and Andalusian governments take direct steps to conserve some of their most important natural heritage, including these local endemic taxa (and their evolutionary potential). This is especially relevant for the *B. insignis* species group, which has been isolated in a small corner of south-western Europe for more than 5 Ma and includes some of the largest beetles found in the Western Palaearctic.

AUTHOR CONTRIBUTIONS

Alberto Sánchez-Vialas: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Arnau Calatayud-Mascarell:** Data curation (equal); formal analysis (equal); methodology (equal); writing – original draft (equal). **Ernesto Recuero:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); supervision (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **José Luis Ruiz:** Conceptualization (equal); investigation (equal); resources (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **Mario García-París:** Conceptualization (equal); data curation (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (lead); supervision (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at (1) the entomological collection of the National Museum of Natural Sciences and (2) in the web server Genbank, that will become available once the manuscript is accepted.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Morphometric measurements (in mm) of the specimens of *Berberomeloe insignis* used in this study.

Table S2. Currently known populations of *Berberomeloe insignis* and *Berberomeloe tenebrosus*. The geographic coordinates, locality and original reference of each record are provided.

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APPENDIX A: PERCENT CONTRIBUTIONS AND PERMUTATION IMPORTANCE FOR THE MOST CONTRIBUTING BIOCLIMATIC VARIABLES

Variable contributions for *Berberomeloe insignis* current species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
Bio12	52.3	41.7
Bio17	37.3	52.5
Bio4	6.6	3.9
Bio1	3.8	1.9

Variable contributions for *Berberomeloe insignis* 4.5 future scenario species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
Bio4	39.7	47
Bio1	32.6	29.5
Bio12	27.2	23.5
Bio17	0.6	0

Variable contributions for *Berberomeloe insignis* 8.5 future scenario species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
Bio4	53.5	63.8
Bio1	37	32.7
Bio12	8.6	3.5
Bio17	0.8	0

Variable contributions for *Berberomeloe tenebrosus* current species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
1	77.8	88
4	21	11.7
12	1.2	0.3
17	0	0

Variable contributions for *Berberomeloe tenebrosus* 4.5 future scenario species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
1	83.8	92.5
4	15.8	7.3
12	0.4	0.3
17	0	0

Variable contributions for *Berberomeloe tenebrosus* 8.5 future scenario species distribution model with MaxEnt.

Variable	Percent contribution	Permutation importance
1	85	93.4
4	14.9	6.6
12	0.1	0
17	0	0