



# A survival story: evolutionary history of the Iberian *Algyroides* (Squamata: Lacertidae), an endemic lizard relict

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## Abstract

The Iberian *Algyroides* (*Algyroides marchi*) is a lacertid lizard with one of the narrowest distribution ranges in continental Europe, restricted to a minute area in the Subbaetic mountains in SE Spain. Due to specific habitat requirements, this species is considered threatened by climate change and habitat degradation. Here, an improved and time-calibrated multilocus phylogenetic analysis, combining two mitochondrial, three nuclear markers as well as a battery of 12 microsatellite loci, was performed. Moreover, ancestral changes in effective population size were determined under Approximate Bayesian Computation (ABC) analysis. In parallel, past, present and future habitat suitability was inferred using Ecological Niche Models (ENMs). The diversification of *A. marchi* in the Iberian Peninsula began during the Upper-Pleistocene around 0.10 Mya. However, during the Last Interglacial the species had much larger suitable habitats along NE Iberia and/or the Cantabrian region. Indeed, ABC analysis indicates that not the Last Interglacial, but instead the Last Glacial Maximum led to a population bottleneck followed by a recovery/expansion. The footprint of this complex evolutionary history is reflected today in six monophyletic lineages, with little genetic differentiation and geographic coherency. This pattern most likely arises from the climatic oscillations during the Pleistocene, leading to a complete range shift and secondary contact, with very divergent haplogroups in sympatry and exchanging genes. Finally, the ENMs predict a considerable future retraction and shift in the area suitable for the species, which should be taken into account for conservation policies.

**Keywords** Iberian peninsula · Iberian *algyroides* · Phylogeography · Biogeography · Evolutionary history

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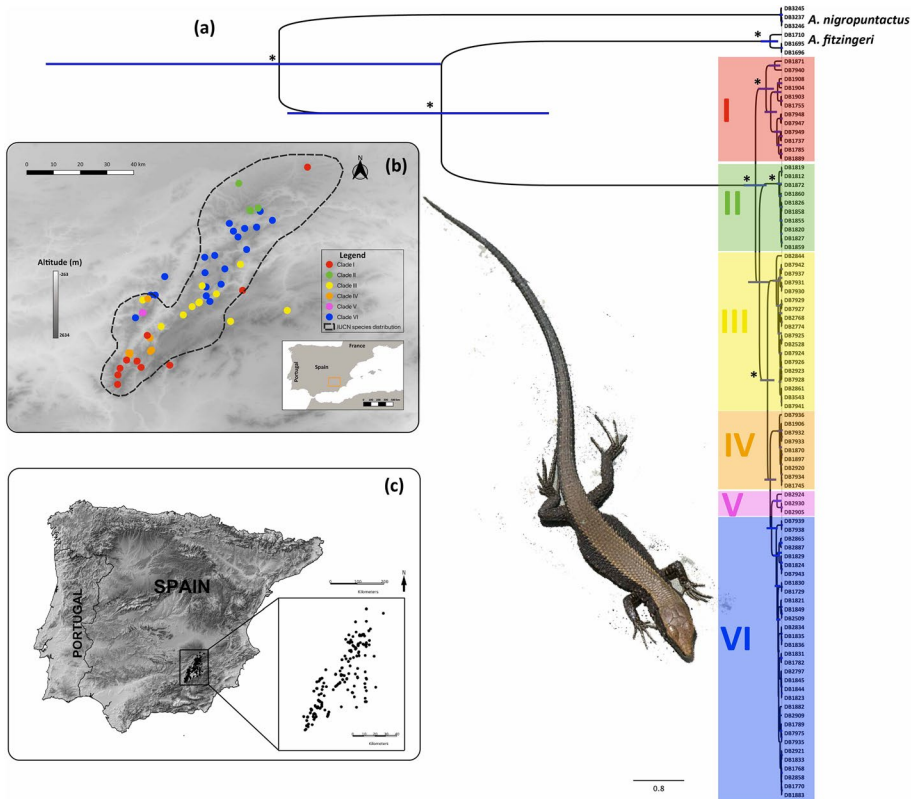
## Introduction

Southern Europe is well-known to retain a higher species richness and vertebrate fauna endemism levels when compared to central and northern Europe, both in part related to the existence of faunal refugia during Pleistocene glacial episodes (Hewitt 1996, 1999). Nonetheless, prior to the glaciations, the subtropical woodlands dominant in Europe during the Miocene were replaced by drier and steppe-like environments in many of these regions during the transition to the Pliocene (Fauquette et al. 1999; Jiménez-Moreno et al. 2010). In this context, the warmer and subtropical conditions first facilitated the dispersal of several taxa of most ectothermic vertebrates (see in Böhme 2003), while drier and more open landscapes were only favourable to certain groups (e.g. *Podarcis* wall lizards, Böhme and Corti 1993). After that initial filtering, the Pleistocene brought a series of glacial and interglacial climatic episodes, with the Last interglacial (LIG) between 130 kyr and 116 kyr (Otto-Bliesner et al. 2006), and the Last Glacial Maximum (LGM) ca. 22 kyr (Hijmans et al. 2005). During colder periods, temperate species' ranges contracted to lower latitudes; in southern Europe, the Iberian, Italian and the Balkan peninsulas became refugia where species survived the LGM to later colonize northern regions during postglacial warming (Hewitt 2011).

The Iberian Peninsula hosts one of the most species rich reptile assemblages in Europe (Sillero et al. 2014), mainly because of the establishment of several “refugia-within-refugia” in the region that allowed the survival of a wide range of taxa during Pleistocene's climatic changes (Gómez and Lunt 2007). The effects of these environmental changes are more apparent at the intraspecific level. In some cases, the geographical ranges of certain taxa broke up during glacial periods and population groups remained isolated in distinct refugia from which the present ranges were built up through colonization during interglacial periods (e.g. Salvi et al. 2013; Teixeira et al. 2015). Since substantial parts of the Iberian Peninsula remained with mild climatic conditions during the Ice Ages, and many of them remained mutually isolated during long periods, Iberian reptile fauna contains many endemisms with restricted and/or substructured ranges, such as *Podarcis bocagei*, and *P. carbonelli* (Pinho et al. 2011), *Lacerta schreiberi* (Godinho et al. 2008), *Iberolacerta monticola* (Remón et al. 2013) and *I. bonnali* (Mouret et al. 2011), *Timon nevadensis* (Miraldo et al. 2013) and *Algyroides marchi* (Carretero et al. 2010).

The Iberian *Algyroides* (*Algyroides marchi* Valverde 1958; for others *A. hidalgoi* Boscá 1916, see Sánchez-Vialas et al. 2018) is an endemic lizard restricted to the Subbaetic Mountain Range (Alcaraz, Segura and Cazorla) of South-eastern Iberian Peninsula (Fig. 1c), with one of the narrowest distribution ranges (100\*40 km, Carretero et al. 2010) among continental European lacertids (Sillero et al. 2014). Within this range, it is also highly stenotopic (Fernández-Cardenete and García-Cardenete 2015; Rubio 2002; Rubio and Martín 2017), inhabiting geomorphologically enclosed, rocky, shaded, and humid valleys (Carretero et al. 2010; Rubio and Carrascal 1994). This reflects its high sensitivity to water loss (Carneiro et al. 2017; García-Muñoz and Carretero 2013; Rubio and Carrascal 1994). Due to these biogeographic and ecological restrictions, the species is considered as endangered (Pérez-Mellado et al. 2009, Carretero et al. 2010).

Although inhabiting the Mediterranean region, the Iberian *Algyroides* has, in fact, a closer Atlantic affinity (Sillero et al. 2009), which makes it now more vulnerable to climate variations. In particular, the aridification of southern Iberia, predicted in a longer term according to global climate change scenarios (Sumner et al. 2003), may have a major impact on *A. marchi* considering its dependency on humid habitats at a geographic scale



**Fig. 1** **a** Bayesian phylogenetic tree based on the combined mtDNA dataset (16S+ND4). Asterisks corresponds to posterior probabilities > 95%, and the 95% confidence intervals for estimated node ages (95% HPD) are represented by blue bars; **b** Map depicting the geographical distribution of each mitochondrial lineage, and of the entire species according to IUCN (Pérez-Mellado et al. 2009). Colours match the ones from the phylogenetic tree; and **c** species presence records used to calculate the ecological niche models

(Carvalho et al. 2010). Hence, a robust inference on the areas of habitat suitability and its driving factors is of paramount importance to help develop future conservation plans. Apart from that, several human actions are threatening the species' habitat and survival, namely roads and forest track construction, touristic infrastructure and livestock grazing (Pérez-Mellado et al. 2009; Rubio and Martín 2017).

Nevertheless, the future responses to these forecasted disturbances will be strongly related with the evolutionary history of the species after past environmental changes, namely, those taking place during the Pleistocene (Sillero and Carretero 2013). In this regard, the only phylogenetic study on the Iberian Algyroides performed to date already revealed high levels of mitochondrial diversity, with three clades presumably separated since the Early Pleistocene, although it lacked proper tree calibration (Carretero et al. 2010). Moreover, both the limited genetic and ecological niche modelling analyses precluded the suggestion of hypothetical biogeographical scenarios.

In this study, a more complete molecular analysis was performed including not only previous mitochondrial and nuclear markers, but also an additional nuclear marker (Reln) and a battery of 12 microsatellite loci, in order to generate a robust hypothesis regarding

past and present population connectivity, respectively. All phylogenetic inferences were time-calibrated to determine approximate divergence times, and effective population size changes. In parallel, ecological niche models for the Iberian *Algyroides* in the current conditions were projected to past and future climate scenarios to examine the impact of several Pleistocenic events (Last Interglacial, Last Glacial Maximum and Mid-Holocene), and the forecasted shifts due climate change on the dynamics of the suitable habitats for the species. By following this multidisciplinary approach, we intend to draw a robust biogeographical scenario responsible for the current range and genetic diversity in a species where historical geographic shifts and extinctions are expected to be dominant.

## Materials and methods

### Molecular analyses

#### DNA extraction, amplification and sequencing

A total of 84 individuals of Iberian *Algyroides* were used in this part of the study, distributed across the Spanish provinces of Albacete, Granada, Jaén and Murcia, corresponding to the overall geographic range of the species (Carretero et al. 2010; Fernández-Cardenete and García-Cardenete 2015; Rubio 2002; Rubio and Martín 2017). Three specimens of *Algyroides fitzingeri* from Sardinia (Italy) and three *A. nigropunctatus* from Peloponnese (Greece) were also added to the dataset. Geographic location of each specimen genetically analysed is represented in Fig. 1b, and detailed information about the locality, amplified genes and GenBank accession codes in Table S1.

Tissue from tail tip muscle was collected from each individual and preserved in 96% ethanol. Genomic DNA was extracted using a standard high-salt protocol (Sambrook et al. 1989). A fragment including the terminal portion of the ND4 gene and the tRNAs for Serine, Histamine and Leucine was amplified by Polymerase Chain Reaction (PCR) using the primers published by Arévalo et al. (1994). Amplification conditions for the ND4 gene were the same as described in Pinho et al. (2006). Primers used for the 16SrRNA gene in both amplification and sequencing were 16Sa and 16Sb from Palumbi (1996) and amplification conditions were the same as described in Harris et al. (1998).

Three nuclear protein-coding gene fragments were also sequenced: the melanocortin 1 receptor (MC1R), the pyruvate kinase (PKM2int5) and Reelin (RELNint61). For amplification and sequencing of the MC1R, PKM2int5 and RELNint61 the primers MC1RF and MC1RR, PKSQF and PKSQR and RELN61F RELN62R (Pinho et al. 2010) were used, respectively.

Amplification of all three nuclear fragments was carried out in 25 µl volumes, containing 5.0 µl of 10× reaction Buffer, 2.0 mM of MgCl<sub>2</sub>, 0.5 mM each dNTP, 0.2 µM each primer, 1U of Taq DNA polymerase (Invitrogen), and approximately 100 ng of template DNA. PCR conditions were the same as described in Pinho et al. (2010). All amplified fragments were sequenced in a ABI3730XL automated sequencer.

The obtained sequences were imported into the software Geneious Pro v.5.5.9 (Drummond et al. 2010) where alignment was performed using MAFFT v.7.017 (Kato and Standley 2013) using the default parameters (auto algorithm; scoring matrix = 200PAM/k = 2; gap open penalty = 1.53; and offset value = 0.123). DnaSP v5 (Librado and Rozas 2009) was implemented to calculate several genetic diversity parameters for each genetic marker.

For all specimens of the Iberian *Algyroides*, 12 microsatellite loci were genotyped (3 dinucleotide, 4 trinucleotide and 5 tetranucleotide repeats) from a battery developed for *Podarcis hispanica* (Ph12010, Ph124, Ph152, Ph2014, Ph30, Ph412, Ph50, Ph8311 in Costa et al. 2013), *Zootoca vivipara* and *Podarcis muralis* (Lv-4–72, Lv-4- $\alpha$  and B4, C9, respectively in Runemark et al. 2008). All loci were amplified according to the described conditions in those studies. All amplifications were performed including negative controls. PCR products were separated by size on an ABI3100xl genetic analyser using the 350ROX size standard. Allele sizes were determined using GENEMAPPER v.4.0 (Chatterji and Pachter 2006) and checked manually.

Microsatellite diversity was evaluated based on the number of alleles ( $N_a$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities per locus using ARLEQUIN v.3.5.2.2 (Excoffier and Lischer 2010). This same software was used to assess deviations from the Hardy–Weinberg equilibrium (HWE), and to test for pairwise Linkage Disequilibrium for all loci (10,000 permutations), based on the exact test of Guo and Thompson (1992). Significance levels were corrected using a False Discovery Rate procedure (Benjamini and Hochberg 1995), using R (R Core Team 2018). Since HWE deviations could be due to the presence of null alleles, a survey to detect these scoring errors was carried out using MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004).

All sequences generated in this study were submitted to GenBank with accession numbers ranging from X to Y (included upon acceptance of this manuscript).

### Mitochondrial phylogenetic analysis

Phylogenetic analysis based on the mitochondrial dataset (16S + ND4) was performed with Bayesian Inference (BI) methods, using *Algyroides nigropunctatus* as an outgroup (Garcia-Porta et al. 2019; Mendes et al. 2016), and three individuals of *A. fitzingeri*. Only specimens sequenced for both the 16S and ND4 were used in this analysis.

In order to determine the best fitting nucleotide model, the software PartitionFinder v.1.1 was implemented (Lanfear et al. 2012), where tests were performed among schemes with genes unpartitioned (to avoid over-parameterized models). Parameters used were *branchlengths* = linked and *modelselection* = BIC. Implemented models are as described in Table S2.

Bayesian Inference was implemented with the software BEAST v.1.8.4 (Bouckaert et al. 2019), and the analysis was run twice for  $25 \times 10^5$  generations with a sampling frequency of 1000. Models and prior specifications applied were as follows (otherwise by default): Trees linked, Strict Clock; Coalescent with Constant Population Size; random starting tree; alpha Uniform (0, 10); clock.rate for ND4 was normal, and for 16S was exponential (default values). Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v.1.7.1 (Rambaut et al. 2018) after obtaining an effective sample size (ESS) > 200. The initial 10% of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using FigTree v.1.4.4 (Rambaut 2009).

### Species tree and divergence time estimation

A multispecies model was implemented with \*BEAST (Bouckaert et al. 2019), not only to determine the age of the most recent common ancestor (MRCA), but also to assess the

relationships between the main clades recovered from the mtDNA phylogenetic analyses. This was performed using both mitochondrial and phased nuclear loci, and the best nucleotide models inferred using PartitionFinder (see Table S2). All markers were run with unlinked trees (mtDNA trees were linked), sites and clock models, so that each locus and respective priors used would not constrain the calculation of the parameters for the other marker, such as mutation rate, tree topology or branch length.

The lack of specific internal calibration points for *Algyroides* precluded the direct estimation of the timing of cladogenetic events in the phylogeny. Therefore, for this purpose the mean substitution rates and standard errors for the same ND4 gene region calculated for *Podarcis* wall lizards in Pinho et al. (2007) were used here, under a strict molecular clock. The *clock.rate* prior parameter for ND4 was set as a normal distribution with a mean of 0.0226 and a standard deviation of 0.0031, so that mutation rate varied between 0.0278 and 0.0174 mutation/site/million years. A Coalescent with Constant Population Size prior was selected for the trees, with a random starting one. Two individual runs of  $2 \times 10^8$  generations were performed with a sampling frequency of 10,000. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer (Version 1.7.1; Rambaut et al. 2018) after obtaining an effective sample size (ESS) > 200. Runs were combined using LogCombiner (discarding 10% of the initial runs), and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using FigTree (Version 1.4.4; Rambaut 2009).

## Haplotypes networks

The phylogenetic relationships among haplotypes for the combined mitochondrial dataset and for each single nuclear DNA loci were inferred using a Median Joining network (Bandelt et al. 1999). To construct these haplotype networks, the software PopART (Leigh and Bryant 2015) was used, with the parameter epsilon set to 0.

Prior to this analysis, phase reconstruction of the nDNA dataset was performed. This was inferred using the program PHASE v.2.1.1 (Stephens and Donnelly 2003; Stephens et al. 2001), considering a threshold of 60% ( $p=q=0.6$ ), since this has been demonstrated to reduce the number of genotype uncertainties with little or no increase in false positives (Garrick et al. 2010). PHASE input files were created in SeqPHASE (Flot 2010), and all polymorphic sites with a probability of <0.6 were coded in both alleles with the appropriate IUPAC ambiguity code.

## Population structure

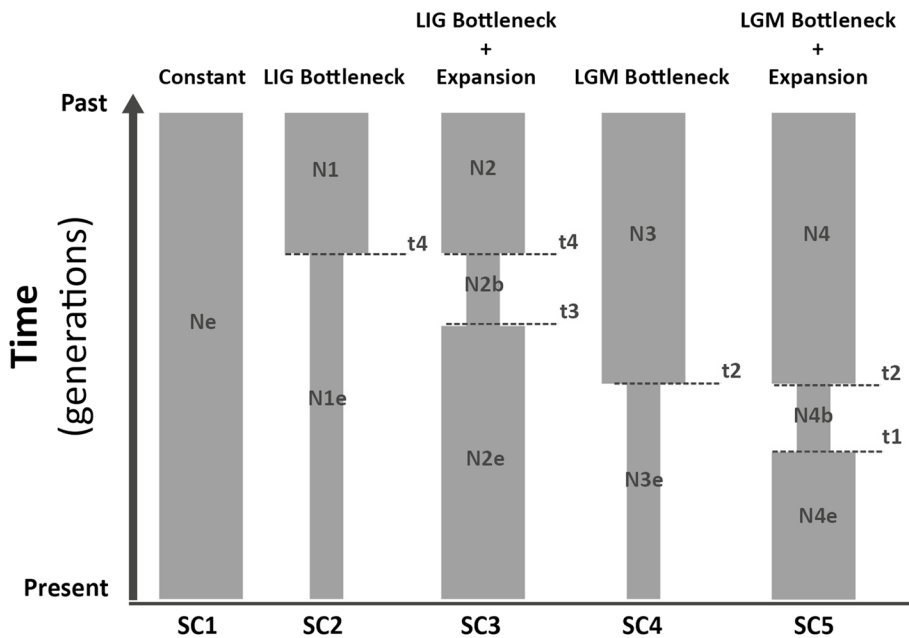
In order to explore contemporary patterns of intraspecific variability in the target species, a Bayesian clustering analysis was performed, implemented in the software STRUCTURE v.2.3.4 (Falush et al. 2003; Pritchard et al. 2000), only for the microsatellite loci in equilibrium. STRUCTURE was run for five iterations with a burn-in length of  $50 \times 10^3$  steps, followed by  $55 \times 10^4$  MCMC reps, for K between 1 and 8 (number of mtDNA clades plus 2). This analysis was performed using the admixture model with correlated allele frequencies and was tested under a supervised (with LOCPRIOR; Hubisz et al. 2009) and an unsupervised (without LOCPRIOR) learning algorithms. In the supervised method, sampling locality was provided as a priori information, whereas it was not provided in the unsupervised method. Determination of the number of clusters (K) was based on the method

described by Evanno et al. (2005), and graphical plotting of STRUCTURE results were implemented with the online software CLUMPAK (Kopelman et al. 2015).

Additionally, ARLEQUIN v.3.5.2.2 was implemented on the microsatellite loci to estimate  $F_{ST}$  values between all identified mitochondrial clades, and their significance assessed with 100 permutations.

### Demographic history

A comprehensive approach to understand the demographic history of the Iberian Algyroides was performed using the Approximate Bayesian Computation (hereafter ABC), where alternative demographic scenarios were modelled using DIYABC v.2.1.0 (Cornuet et al. 2010, 2014). This analysis was carried out using microsatellite loci together with the mtDNA markers. This allowed testing explicit scenarios about the timing and intensity of a genetic bottleneck during the Last Glacial Maximum (LGM) and the Last Interglacial (LIG) by simulating scenarios within set priors of time and population size, and comparing summary statistics from the simulated to observed datasets. To run ABC, the generation time of the species was set to 3 years, since females attain sexual maturity at this age (Palacios et al. 1974; Rubio and Palacios 1986) and assuming a 1:1 female to male sex ratio (well supported for the sister species *A. fitzingeri* in Capula et al. 2002; indirect data for the species in Palacios et al. 1974). A “control” was simulated considering that the population has kept demographically stable across time (Fig. 2, SC1), and a LIG and LGM bottlenecks without and with recovery/expansion (Fig. 2; SC2, SC3 and SC4, SC5 respectively).



**Fig. 2** The five demographic histories tested on the microsatellite and mtDNA data of the Iberian Algyroides using Approximate Bayesian Computation in DIYABC.  $N_x$  variables represent effective population sizes, and  $t_x$  variables represent the timing of the demographic events. Details for the prior distributions for each of these parameters is provided in Table S3



Details on the summary statistics used, demographic priors and mutational models are described in Supplement 3. Each demographic scenario was simulated 1 million times (as recommended in Cornuet et al. 2008, 2010). The posterior probabilities of the five ABC scenarios were estimated using polychotomous logistic regression. The selected scenario was the one with the highest probability value with a nonoverlapping 95% confidence interval. Confidence in scenario choice was evaluated by computing type I and type II errors in the selection of scenarios. For more information on priors, type I and type II error calculations, and model checking procedures see Supplement 3.

## Ecological Niche modelling

### Species dataset

Species distribution data (Fig. 1c) were collected from the chorological database of the Spanish Herpetological Society (Asociación Herpetológica Española: [www.herpetologica.org/basedatos.asp](http://www.herpetologica.org/basedatos.asp)); several bibliographic sources (Ceacero et al. 2007; Eikhorst et al. 1979; Gil-Sánchez 1992; Lafuente and Roca 1993; Otero et al. 1978; Palacios et al. 1974; Ple-guezuelos et al. 1989; Sánchez-Videgaín and Rubio 1996; Valverde 1958); personal communications from herpetologist colleagues; and fieldwork (performed during 2009 in 35 journeys between 25th of March and 13th of September, with 12 researchers, completing 52 h per researcher).

### Study areas

The models were calculated in two study areas: the Iberian Peninsula with a spatial resolution of 1 km<sup>2</sup>; and a smaller area comprising all records in the Subbaetic Range with a spatial resolution of 30 m<sup>2</sup>.

### Environmental data

Two sets of environmental data were used for modelling the species' realised niches (sensu Sillero 2011) in the Iberian Peninsula and the Subbaetic Range.

For the Iberian Peninsula, altitude and climatic data were downloaded from WorldClim 1.4 (Hijmans et al. 2005; <http://www.worldclim.org/>). As present models were projected to several past and future climatic scenarios (see below), the variables BIO3, BIO14 and BIO15 were excluded from the set of 19 bioclimatic variables (Booth et al. 2014) available in WorldClim, as they are biased when projected to past and future scenarios (Bedia et al. 2013; Varela et al. 2015). Slope was also included in the models as it represents the enclosed, rocky, shaded, and humid valleys where the species occurs (Carretero et al. 2010). Slope was derived from altitude, which was previously projected to a projected coordinate system (EPSG: 23,030) as it should not be calculated in a geographical system (e.g. WGS84) (Sillero and Barbosa 2021). Six bioclimatic variables plus slope were selected with a Pearson correlation lower than 0.70. Slope was included in all past and future climatic scenarios.

For projections to past climates, five scenarios were used: one scenario for the Last Interglacial (LIG: ~120,000–140,000 years BP); three scenarios (CCSM4, MIROC-ESM, MPI-ESM-P) for the Last Glacial Maximum (LGM: ~21,000 years BP); and one scenario (CCSM4) for the Mid Holocene (~5000 years BP).



For the projections to future climates, three atmosphere–ocean general circulation models (GCMs: CCSM4, HadGEM2-ES, MRI-CGCM3) were used, with four greenhouse gas scenarios (representative concentration pathways: RCP 2.6, 4.5, 6.0 and 8.5), and two time periods: 2050 (average for 2041–2060) and 2070 (average for 2061–2080). The four RCPs are based on different levels of greenhouse gas concentration: in RCP 2.6 very low concentrations by the end of the twenty-first century are expected; in RCP 4.5 and RCP 6.0 these levels stabilise; and in RCP 8.5 an increase of gas emissions over time is forecasted. Future climate scenarios were selected considering their good modelling performance in Europe, following McSweeney et al. (2015).

All the variables of past, present and future had a spatial resolution of 30 arc-seconds (approximately 1 km<sup>2</sup>), except for one past scenario (LGM) that had a spatial resolution of 2.5 arc-minutes (approximately 5 km<sup>2</sup>). As the Iberian Peninsula had a larger extent during the LGM due to the lower sea level, the LGM variables were clipped to the current extent of the Iberian Peninsula to avoid projection artefacts due to excessive extrapolation.

For the Subbaetic Range, topographical data were obtained from the Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model (ASTER GDEM; <http://www.gdem.aster.ersdac.or.jp/>). The ASTER GDEM is a project formed by the Ministry of Economy, Trade and Industry of Japan (METI) and the USA's National Aeronautics and Space Administration (NASA) to produce the first DEM covering all continents, between latitudes 83° N and 83° S, with a spatial resolution of 30 m. ASTER GDEM are freely available on the Internet (Sillero and Tarroso 2010). Six variables at 30m<sup>2</sup> were selected with a Pearson correlation lower than 0.70: altitude, plus five topographic variables derived from altitude (slope in percentages, cardinal orientation of the surface or aspect, number of hours with sunlight during 2009, solar radiation during 2009, and index of surface curvature). The aspect (measured in degrees, from 0° to 360°) cannot be used as a continuous variable, because the values are circular (e.g. north is representing by both 0° and 360°). Therefore, it was transformed into a categorical variable, by reclassifying the values in the range of 40° (Sillero and Barbosa 2021). All topographical variables were derived in a projected coordinate system (EPSG: 23,030) (Sillero and Barbosa 2021).

## Ecological niche models

We calculated the realised niche models with Maximum Entropy (Maxent 3.4.1 software; [www.cs.princeton.edu/~schapire/maxent](http://www.cs.princeton.edu/~schapire/maxent)), a general-purpose machine learning method that uses presence-only occurrence and background data (it does not use pseudo-absence data; Guillera-Aroita et al. 2014; Sillero and Barbosa 2021), is particularly suited to noisy or sparse information, and works with continuous and categorical variables (Phillips et al. 2017, 2006). Background corresponds to the whole study area (Phillips et al. 2009): therefore, background data points were obtained from the area delimited as our study areas (i.e. the Iberian Peninsula and the Subbaetic Range). Maxent chooses the model with the maximum entropy, i.e. the one that produces the most uniform distribution but still infers the observed data as accurately as possible. It estimates the range of a species with the constraint that the expected value of each variable (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 species-target distribution. We deleted duplicated points at the resolution of 1 km<sup>2</sup> and 30 m<sup>2</sup>; thus, only one point per cell was used in the analysis. We ran Maxent in clog-log format with default parameters using 70% of presence records from each dataset as training

data and 30% as test data. The model was replicated 10 times as Maxent is a machine learning algorithm with a probabilistic component (Sillero and Barbosa 2021). Therefore, we calculated the arithmetic mean and the standard deviation of a set of 10 models through an iterative process, as a compromise among statistical power, computing time, and storage. Maxent identified the importance of each environmental variable by the following factor analyses: (1) jackknife analysis of the average area under the curve (AUC) with training and test data; and (2) average percentage contribution of each variable to the models. For this purpose, variables were excluded in turn and a model was created with the remaining variables; then a model was created using each individual variable.

All models were tested with the receiver operated characteristics (ROC) plots and the True Skill Statistics (TSS; Allouche et al. 2006). The AUC of the ROC plot was taken as a measure of the overall fit of the models (Liu et al. 2005). AUC is used to discriminate a species' model from a random model. Random models have an AUC equal to 0.5; the closer to an AUC of 1, the better the model. TSS is a classification evaluation metric and is equal to sensitivity + specificity - 1. TSS ranges from -1 to 1, with 0 corresponding to a random classification power (Allouche et al. 2006). TSS tends to be correlated with AUC (Allouche et al. 2006).

Maxent models of the Iberian Peninsula for the present climate were projected to the LIG, LGM, Mid-Holocene, 2050, and 2070 using the same set of variables corresponding to each period, as indicated above. Maxent models for the Subbaetic Range were not projected to other scenarios as these models included only topographical variables.

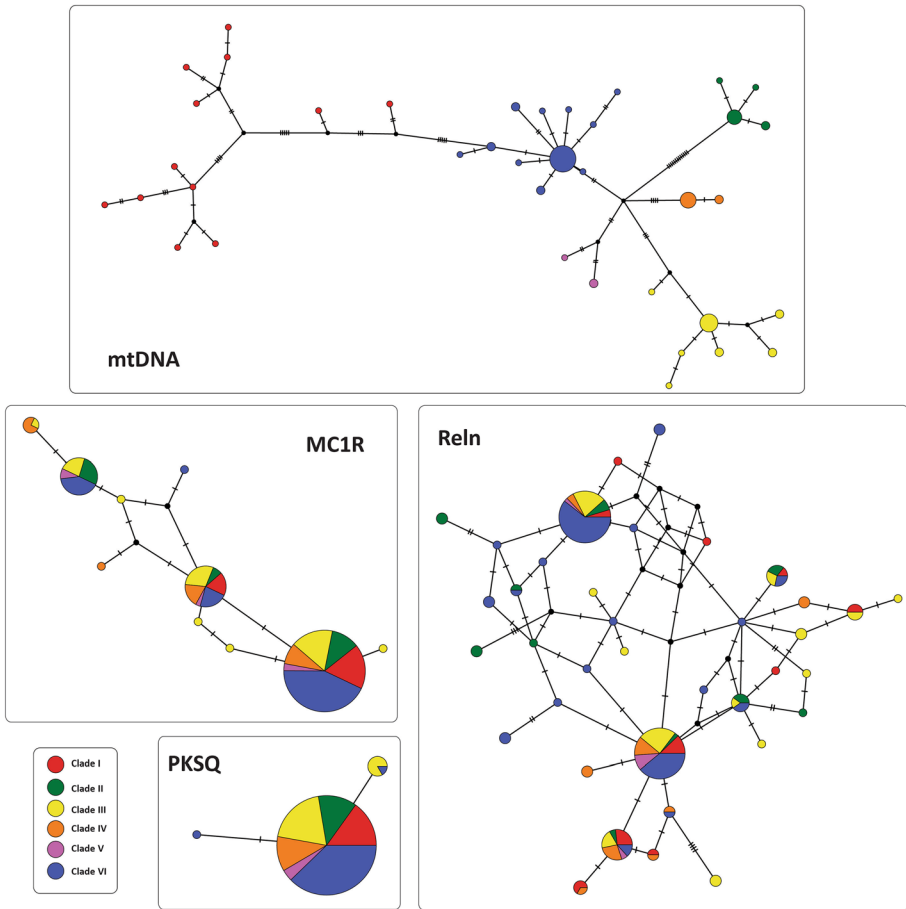
To check if our models do not change with the type of algorithm used, we calculated them again with the Multivariate Adaptive Regression Spline (MARS) algorithm (Friedman 1991). MARS is a non-parametric regression technique that introduces a hinge function in the linear relationship. A hinge function is a change in the slope of the linear regression (Friedman 1991). We chose MARS because MAXENT also uses the hinge function. Therefore, we calculated past, present and future MARS models in the Iberian Peninsula, and a model at 30 m of spatial resolution in the Subbaetic Range. We used in MARS the same variables, presences, and parameters than Maxent. MARS was also evaluated with AUC and TSS.

## Results

### Phylogeographic assessment and age estimation

Mitochondrial DNA results from both the haplotype network and phylogenetic tree (Figs. 1a and 3), suggest the existence of six distinct monophyletic groups within *Algyroides marchi* (= *A. hidalgoi*), although support is lower than 95% for most nodes. Remarkably, these groups have mostly no geographic coherency; for instance, Clade I has a patchy distribution with the majority of individuals occurring in the southernmost part of the species' distribution, plus two other isolated individuals in the central (Venta Ticiano, Jaén, DB7940) and the northernmost (El Berro, Albacete, DB1871) parts of the range. This dissociation between genetic proximity and geographic distance is also seen in clades III, IV and VI. Only in clades II and V, which have narrower geographic distributions, genetic distance and geography corresponded better.

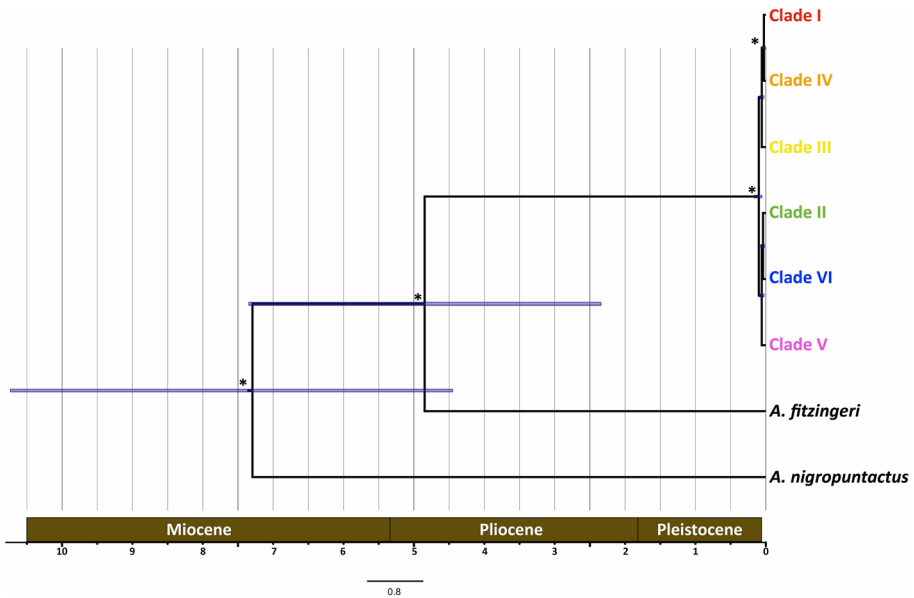
The phylogenetic relationships obtained from the multilocus species tree are quite different from the ones using only the combined mtDNA dataset (Fig. 4). However, and



**Fig. 3** Haplotype networks for the combined mitochondrial dataset (16S+ND4) and for each phased nuclear marker (MC1R, Reln and PKSQ). The transversal bars represent the number of mutational steps and the black circles the missing haplotypes. Colours and clades match the ones from Fig. 1a

in a similar fashion, it does not provide high posterior support for most of the mtDNA clades as being genetically different units. According to this topology, the diversification of the species in the Iberian Peninsula began during the Upper-Pleistocene around 0.10 Mya, with the separation of clades I, IV and III's common ancestor from the remaining extant groups. This took place a long time after its separation from the closest relative *A. fitzingeri*, approximately 4.85 Mya.

A common feature to all nuclear haplotype networks is the lack of correspondence with the groups obtained from the mtDNA analyses, and the sharing of many haplotypes among clades (Fig. 3). The pattern from PKSQ is mainly due to a lack of genetic diversity of this marker (Table 1), with only one exclusive haplotype out of a total of three. On the contrary, in Reln's case, the genetic diversity was higher with most of the haplotypes being exclusive, but the relationships among them unclear. The pattern of MC1R was intermediate in diversity but still showing lack of correspondence



**Fig. 4** Multilocus species tree calibrated based on the ND4 mutation rate, following Pinho et al. (2007). Asterisks corresponds to Bayesian posterior probabilities > 95%, and the 95% confidence intervals for estimated node ages (95% HPD) are represented by blue bars. Colours and clades match the ones from Fig. 1a

**Table 1** Nucleotide and haplotype diversity (mean  $\pm$  standard deviation) calculated with DnaSP for each genetic marker

Locus	Nucleotide diversity	Haplotype diversity
<i>mtDNA</i>		
16S	0.00390 $\pm$ 0.00033	0.842 $\pm$ 0.026
ND4	0.00897 $\pm$ 0.00067	0.909 $\pm$ 0.022
<i>nDNA</i>		
MC1R	0.00156 $\pm$ 0.00015	0.543 $\pm$ 0.039
PKSQ	0.00022 $\pm$ 0.00008	0.082 $\pm$ 0.029
Reln	0.00495 $\pm$ 0.00019	0.852 $\pm$ 0.019

with the mitochondrial clades (Fig. 3). Regarding the diversity indexes for mtDNA, the ND4 marker shows higher haplotype and nucleotide diversities when compared to 16S (Table 1).

## Population structure

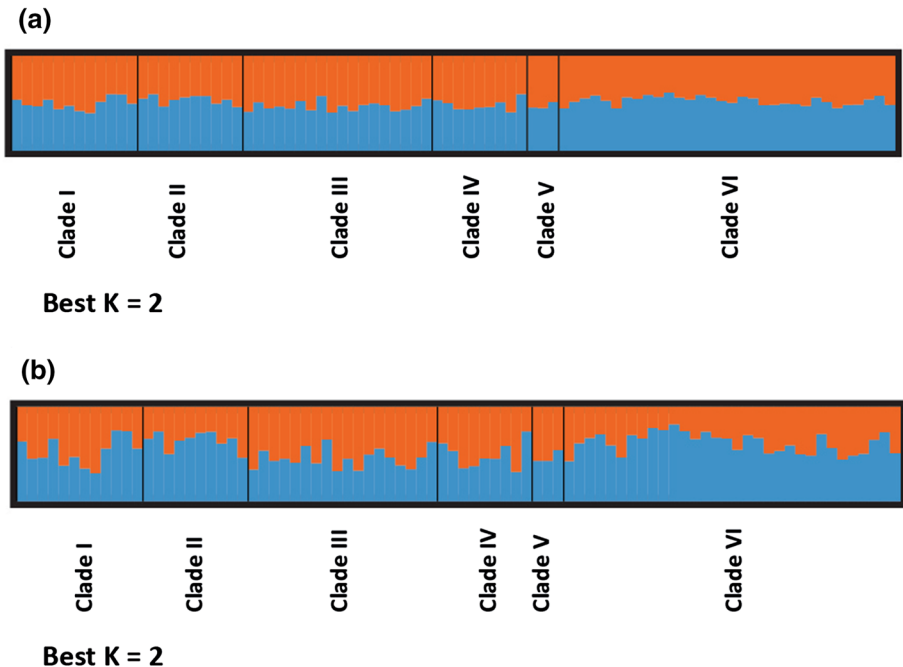
Of the 12 microsatellite loci studied, only seven (B4, Ph12010, Ph124, Ph2014, Ph412, Ph50 and Ph8311; Table S4) seemed to be in HWE and with no null alleles. Hence, only these were used in further analyses. All loci were polymorphic, but allelic richness was not especially high when compared to other studies using these same markers (Costa et al. 2013; Runemark et al. 2008), ranging from three (C9) to 15 (Ph30). STRUCTURE results revealed that the best K was 2. Surprisingly, and independently of considering or not the

locality as prior, the Iberian Algyroides' population seems to have no genetic structure (Fig. 5), even when examining the results from other Ks (data not shown).

$F_{ST}$  values were all quite low between all mtDNA assigned clades ( $F_{ST} < 0.2$ ), varying from 0.00143 (Clade II vs Clade III) to 0.06749 (Clade IV vs Clade VI) (Table S5). Regarding microsatellite diversity, Clade III presents the highest values for all three indexes ( $N_a = 5.714$ ;  $H_e = 0.64951$ ;  $\Theta(H) = 1.69638$ ), but Clade V has the lowest number of alleles ( $N_a = 2.571$ ) and Clade II the lowest values for the remaining diversity indexes ( $H_e = 0.51760$ ;  $\Theta(H) = 1.50248$ ).

## Demographic history

Results from the Approximate Bayesian Computation, indicate that scenario 5 (LGM bottleneck with expansion) was the one with the highest posterior probability (0.40) with a 95% confidence interval not overlapping with the one from other scenarios (Table 2). This scenario was also the one presenting the lowest type I error (0.39), meaning that it was consistently chosen when posterior data were simulated under this scenario. The second-best scenario was SC3 (LIG bottleneck with expansion), although in this case the type I error was relatively high (0.74). Error rate testing showed that both SC3 and SC5 were consistently selected even when posterior data were simulated under other scenarios. Consequently, type II error rates for these scenarios were relatively high. Nevertheless, the posterior probability values and low type I error rate supported scenario 5 as the most likely. Both the pre-evaluation of the scenarios tested and the model checking step for the most



**Fig. 5** Bayesian clustering results using the software STRUCTURE for the microsatellite dataset, implementing the admixture model with correlated allele frequencies, under an unsupervised (without LOCPRIOR) (a), and a supervised (with LOCPRIOR) learning algorithm (b). The best K in both analyses was 2

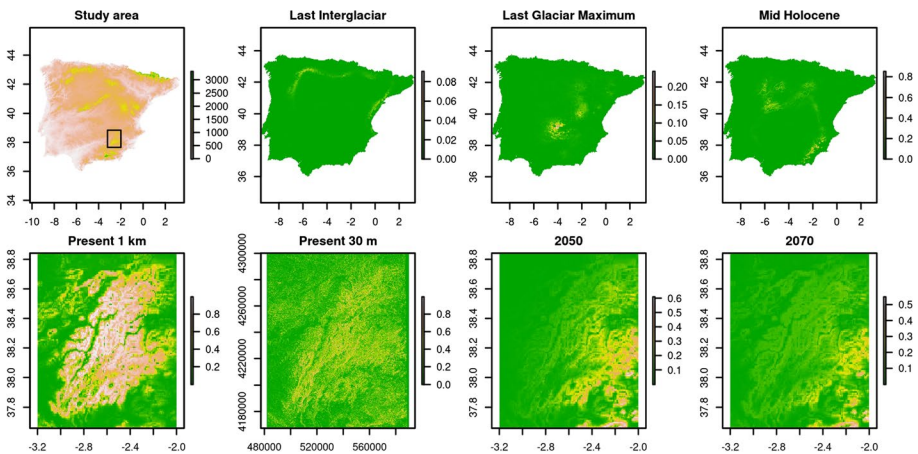
**Table 2** DIYABC error rates for the competing scenarios selected by the logistic regression method, based on the simulation of 500 pseudo-observed datasets (PODs – the “True Scenario”) derived from the posterior distribution. The total number of PODs for a given scenario is represented in brackets ( ) beside the scenario number. Posterior probability of the tested demographic scenarios and 95% confidence intervals (CI), under the logistic regression approach using 1% of the data closest to the observed dataset (1000)

True Scenario used for simulation	Type II error rate					Type I error rate	Posterior probability [95% credible interval]
	1	2	3	4	5		
1 (236)	–	0.17	0.23	0.15	0.30	0.86	0.20 [0.19, 0.22]
2 (132)	0.06	–	0.11	0.22	0.31	0.70	0.09 [0.08, 0.10]
3 (184)	0.14	0.14	–	0.06	0.41	0.74	0.23 [0.22, 0.24]
4 (104)	0.09	0.31	0.10	–	0.23	0.72	0.08 [0.07, 0.08]
5 (344)	0.07	0.10	0.14	0.08	–	0.39	0.40 [0.38, 0.42]

probable scenario (SC5) showed a good fit between the observed and simulated datasets under the chosen priors (Fig. S1).

## Ecological niche models

Suitable habitats based on climatic variables and slope for the extant populations of *Algyroides marchi* (= *A. hidalgoi*) were located not only across the current Subbaetic Range but also included the eastern Penibaetic Mountains, Central System, the Iberian System, Sierra of Cuenca, the Cantabrian Mountains and the Pyrenees (Fig. 6). Temperature Annual Range was the variable with the highest contribution to the model (60.5%, Table S6), and is also the variable explaining more the model when used alone, affecting its performance when removed (Fig. S2). Slope and Precipitation were also important contributing



**Fig. 6** On top, Maxent ecological niche models of *Algyroides marchi* are projected to the Present, and to past scenarios during the Last Interglacial (~130–116 kyr years BP), the Last Glacial Maximum (~22 kyr years BP) and the Mid Holocene (~6kyr years BP), for the entire Iberian Peninsula. Below, maps represent the projections to the Present at 1 km and 30 m of spatial resolution, and Future (2050 and 2070), in more detail for the study area

variables for these ecological niche models. The species selected positively intermediate values of slope, temperature and precipitation (avoiding extreme values) (Fig. S3).

Regarding the ecological niche models projected to the past, the predicted habitat suitability during the Last Interglacial was reduced to the Cantabrian Mountains, the Pyrenees and to the Catalan coastal range, that is, not encompassing the current range. Afterwards, during the Last Glacial Maximum the areas with suitable habitats included the Iberian System and Sierra of Cuenca, the Southern Castilian Plateau and scattered areas along the Subbaetic Range. Finally, and very similar to the ecological niche models predicted to the present, the suitable habitats during the Mid Holocene included the current Subbaetic Range, but also the Iberian Mountains, the Central System and the Northern Castilian Plateau.

In the future climate change scenarios, the forecasted suitable habitat gradually shrinks and shifts towards the south-east of the Subbaetic mountains (Fig. 6). The model at 30 m of spatial resolution of the Subbaetic range (Fig. 6) provided a very similar result than the Iberian model for the present at 1 km of spatial resolution.

MARS models had very high values of evaluation metrics, in the Iberian Peninsula (AUC:  $0.98 \pm 0.03$ ; TSS:  $0.94 \pm 0.05$ ), and in the Subbaetic Range (AUC:  $0.85 \pm 0.015$ ; TSS:  $0.57 \pm 0.05$ ). Both models were very similar to Maxent models (Fig. S4).

## Discussion

Currently, only a small proportion of the Earth's land surface contain large numbers of species with small geographical ranges (Lamoreux et al. 2006; Orme et al. 2005), making these regions of particular conservation concern (Malcolm et al. 2006). At least for some animal and plant groups, such areas coincide with regions that have rare climates, are higher and colder compared to neighbouring cells (see in Ohlemüller et al. 2008). Small-range species tend to occur in climatically diverse regions, where they were likely buffered from extinction in the past. These areas represent interglacial relict regions where species were able to survive in the last 10,000 years (the "Climatic Relict Hypothesis", Ohlemüller et al. 2008).

The Iberian *Algyroides* is not only the Iberian lizard with the smallest distribution range (Fernández-Cardenete and García-Cardenete 2015; Rubio 2002; Rubio and Martín 2017), but also part of a genus whose relatives are disconnectedly spread across the Mediterranean Basin. *Algyroides* species are spatially very distant from each other, some of them with equally restricted geographic ranges. The phylogeny of the genus and its relations suggest that the common ancestor to all *Algyroides* appeared somewhere in the Eastern Mediterranean, colonizing Europe during the Miocene (Harris et al. 1999; Mendes et al. 2016). This has been recently corroborated by a phylogenomic study supporting the Balkan genus *Dinarolacerta* as sister group to all *Algyroides* (García-Porta et al. 2019). During this period, subtropical forest environmental conditions prevailed in the region (Böhme 2003; Cavazza and Wezel 2003), but soon after, by the end of the Miocene beginning of the Pliocene, aridity increased creating drier and more open environments (Cavazza and Wezel 2003; Fauquette et al. 1999). This may have pushed *Algyroides* taxa into several small remnants of suitable habitat across the Mediterranean Basin where they evolved in allopatry. In fact, these environmental conditions were most likely followed by extensive extinction within *Algyroides* lineages (Harris et al. 1999; Mendes et al. 2016). This extinction hypothesis is evidenced by the long phylogenetic branches obtained in Mendes et al.



(2016) and corroborated by our phylogenetic topologies (Figs. 1a and 4). Moreover, palynological records indicate that such processes caused a vegetation turnover within the Iberian Peninsula making Mediterranean biomes to prevail (Jiménez-Moreno et al. 2010). This suggests that even before the Pleistocene, a substantial part of the Iberian Peninsula was already unsuitable for *Algyroides*.

The results from the ecological niche model projections to the past (Fig. 6) demonstrate that during the LIG, the climatic conditions in the current species range were highly unfavourable, only improving slightly during the LGM. Remarkably, from the LIG to the LGM, the ecological niche models identified a massive geographic shift of suitable habitats from the coastal Iberian Peninsula towards inland. Afterwards, the climate during the Mid-Holocene (even warmer than today; Kitoh and Murakami 2002), seemed to have offered extremely favourable environmental conditions for this lizard species. In other words, models suggest that during the LIG the Iberian *Algyroides* was not present in the Subbaetic mountains but most likely distributed along the Catalan coast and/or the Cantabrian mountains. The colonization of the Subbaetic range should have taken place sometime later, between the LIG and the LGM. It is worth noting that the climatic variables included in the models will hardly represent correctly the environmental conditions of *Algyroides* in the Iberian Peninsula, i.e. enclosed, rocky, shaded, and humid valleys, since Worldclim climatic variables are created by interpolation from meteorological stations data (Hijmans et al. 2005, 2017). Although these interpolations are performed with altitude as co-variable, they might fail to correctly represent the microclimate at the bottom of the valleys, since variables should correspond most likely to the valley tops. This bias might increase when considering past climatic scenarios. Therefore, our models are probably failing in detecting deep valleys where the species might have survived during warm periods.

The footprint of this microgeographic dynamics is seen today by six mitochondrial lineages, most of them with little node support and geographic coherency, a pattern most likely resulting from full admixture or survival of admixed populations due to the climatic oscillations during the Pleistocene (e.g. Godinho et al. 2008). The low node support recovered by all phylogenetic analyses, allied with the lack of structure and population differentiation evidenced by the microsatellite loci, are a strong evidence for past and current gene flow among populations of *A. marchi*. Hence, these clades can hardly be considered as genetically distinct units.

Indeed, Pleistocene's cyclical ice ages caused extinction and repeated changes in the ranges of several extant taxa, depending on the latitude and topography. Such fast latitudinal and altitudinal range shifts had noticeably stochastic and selective effects on genetic variation and architecture: populations and lineages went extinct, alleles were lost in bottlenecks and founder effects, mutations occurred through time and were spread by selection and population expansions (Hewitt 2000, 2004). In a variety of phylogenetically unrelated herpetofaunal groups across the Mediterranean Basin, population expansion was followed by the establishment of secondary contact zones, with divergent haplogroups found today in sympatry (e.g. Gonçalves et al. 2012; Martínez-Freiria et al. 2017; Miraldo et al. 2011; Rosado et al. 2017; Salvi et al. 2011; Velo-Anton et al. 2018), similarly to the mitochondrial phylogeographical pattern observed here.

This same pattern is also supported by the results from the Approximate Bayesian Computation analyses; the most likely demographic scenario is the one describing a population bottleneck followed by a genetic recovery (i.e. expansion) during the LGM (Table 2) and establishment of secondary contact. This biogeographical hypothesis accounts for the existence of several distinct haplogroups in the same region and the high levels of genetic diversity observed (Table 1). In fact, inside its minute range, the Iberian *Algyroides* contains as much or

even more genetic diversity than other endemic Iberian lizards with substantially larger ranges (e.g. *Psammodromus hispanicus* in Fitze et al. 2011; *Timon nevadensis* in Miraldo et al. 2011; *Podarcis bocagei* in Pinho et al. 2007), or even than its sister species *Algyroides fitzingeri* (Salvi et al. 2011).

The fact that the cold/dry climate during the LGM caused a drastic decline of this species' geographic range is not surprising, considering the environmental requirements of the species. In fact, the extant populations of the Iberian *Algyroides* are known to be highly sensitive to water loss (García-Muñoz and Carretero 2013; Rubio and Carrascal 1994), inhabiting geomorphologically enclosed, rocky, shaded and humid areas (Carretero et al. 2010; Rubio and Carrascal 1994). Indeed, these ecophysiological and habitat specificities were likely behind the most contributing variables when building the present ecological niche models; temperature, slope and precipitation were the variables that explained and affected more the model performance.

Unfortunately, such environmental conditions are expected to be dramatically modified, mostly due to climate change (see results), but also due to human-induced habitat degradation (Pérez-Mellado et al. 2009; Rubio and Martín 2017). Due to its small geographic range, as well as reduced and fragmented area of occupation and small population size, *A. marchi* has already been attributed the highest level of conservation category; classified as Endangered by IUCN with the same recommendation for Spain (Pérez-Mellado et al. 2009). However, climate change in the current range certainly increases that level of threat. Consistent with our future niche model predictions, in 2050 and 2070 the distribution of *A. marchi* will undergo a substantial range reduction and shift towards the southeast, evidencing that the Subbaetic mountains will no longer harbour favourable environmental conditions for the maintenance of this lizard species.

According to Sumner et al. (2003), by the late twenty first century the far south of Spain will undergo a continued spread of aridity of the climate, extending westwards from the extreme west of Almeria province to include the whole Andalusia. Specifically, it is predicted an annual precipitation reduction of between 6% and 14% in this Spanish region. This is already the hottest and one of the most arid parts of Iberia, and an area with a great dependence on the sufficiency of the winter rains. A study on 30 years of daily rainfall analyses have demonstrated a clear trend towards a decrease in the total rainfall in Andalusia (Romero et al. 1998), followed by another study from Sumner et al. (2001) denoting a clear shortening of the winter rainfall season in this same region. Overall, these predicted environmental changes will be disastrous to several taxa of the region, the Iberian *Algyroides* included. A study by Carvalho et al. (2010) on the impact of future climate change on 37 endemic and nearly endemic herptiles of the Iberian Peninsula, predicts that *A. marchi* will have a smaller distribution until 2050. Generally, up to 13 species may lose their entire potential distribution by 2080 (Carvalho et al. 2010). The Atlantic region will decrease in extent while the Mediterranean region will expand northwards (Sousa-Guedes et al. 2020). These results are actually not surprising considering the hydric requirements of the studied species, as mentioned above (Carretero et al. 2010; García-Muñoz and Carretero 2013; Rubio and Carrascal 1994); in fact, the highest among the lacertids studied to date (García-Porta et al. 2019).

## Conclusions

Overall, the stenotopic lizard *Algyroides marchi* (= *A. hidalgoi*) has a surprisingly high mitochondrial and nuclear genetic variability considering its reduced geographic distribution. Nevertheless, during the Last Glacial Maximum it underwent a considerable shrinking in effective population size followed by an expansion and secondary contact, which led to the establishment of several divergent haplogroups found today in sympatry and exchanging genes. Despite this, the future of this species is not bright since the not so far predicted environmental conditions will certainly cause a new reduction of this species' range apart from the locally induced threats. In this context, linking current and future ranges and conserving suitable habitats (Carretero et al. 2010) seem the most reasonable measures in situ to allow geographic dispersal and maintenance of genetic diversity, something that the species has sufficiently proved to be able to achieve. Overall, the implementation of such conservation plan for this relict Iberian endemic species is of paramount importance to prevent that a long and successful story of survival may not have an abrupt end.

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**Author contributions** Catarina Rato: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing—Original Draft, Writing—Review & Editing. Neftali Sillero: Methodology, Software, Validation, Formal analysis, Investigation, Writing—Review & Editing. Francisco Ceacero: Investigation, Writing—Review & Editing. Enrique García-Muñoz: Investigation. Miguel A. Carretero: Conceptualization, Investigation, Resources, Writing—Review & Editing, Supervision, Project administration, Funding acquisition.

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**Data availability** All sequences generated in this study were submitted to GenBank with accession numbers ranging from X to Y (included upon acceptance of this manuscript).

### Declarations

**Conflict of interest** The authors declare no conflicts of interest.

**Ethical approval** Collection permits were provided by Junta de Castilla-La Mancha and Junta de Andalucía.

## References

Allouche O, Tsoar A, Kadmon R (2006) Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *J Appl Ecol* 43:1223–1232

- Arévalo E, Davis SK, Sites JW Jr (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Syst Biol* 43:387–418
- Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48
- Bedia J, Herrera S, Gutiérrez JM (2013) Dangers of using global bioclimatic datasets for ecological niche modeling. Limitations for future climate projections. *Global Planet Change* 107:1–12
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statistical Soc B* 57:289–300
- Böhme MJ (2003) The miocene climatic optimum: evidence from ectothermic vertebrates of Central Europe. *Palaeogeogr Palaeoclimatol Palaeoecol* 195:389–401
- Böhme W, Corti C (1993) Zoogeography of the lacertid lizards of the western Mediterranean Basin. In: Valakos E, Böhme W, Pérez-Mellado V, Maragou P (eds) *Lacertids of the Mediterranean Region*. Hellenic Zoological Society, Athens, A Biological approach, pp 13–33
- Booth TH, Nix HA, Busby JR, Hutchinson MF (2014) BIOCLIM: the first species distribution modelling pack-age, its early applications and relevance to most current MaxEnt studies. *Divers Distrib* 20:1–9
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 15:e1006650
- Capula M, Luiselli L, Filippi E, Ceccarelli A (2002) Habitat characteristics and demography of high-altitude populations of the lacertid lizard, *Algyroides fitzingeri*. *Ital J Zool* 69:33–36
- Carneiro D, García-Muñoz E, Žagar A, Pafilis P, Carretero MA (2017) Is ecophysiology congruent with the present-day relictual distribution of a lizard group? Evidence from preferred temperatures and water loss rates. *Herpetol J* 27:47–56
- Carretero MA, Ceacero F, García-Muñoz E, Sillero N, Olmedo MI, Hernández-Sastre PL, Rubio JL (2010) Seguimiento de *Algyroides marchi*. Informe final. Monografías SARE. Asociación Herpetológica Española – Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid
- Carvalho SB, Brito JC, Crespo EJ, Possingham HP (2010) From climate change predictions to actions - conserving vulnerable animal groups in hotspots at a regional scale. *Glob Change Biol* 16:3257–3270
- Cavazza W, Wezel FC (2003) The Mediterranean region – a geological primer. *Episodes* 26:160–168
- Ceacero F, García-Muñoz E, Pedrajas L, Hidalgo A, Guerrero F (2007) Actualización herpetológica de la provincia de Jaén. *Munibe* 25:130–139
- Chatterji S, Pachter L (2006) Reference based annotation with GeneMapper. *Genome Biol* 7:R29
- Cornuet JM, Pudlo P, Veysier J, Dehne-Garcia A, Gautier M, Leblois R, Marin J-M, Estoup A (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* 30:1187–1189
- Cornuet JM, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics* 11:401
- Cornuet JM, Santos F, Beaumont MA, Robert CP, Marin JM, Balding DJ, Guillemaud T, Estoup A (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics* 24:2713–2719
- Costa D, Lopes S, Pereira C, Luís C, Couto A, Pinho C (2013) Isolation and characterization of microsatellite loci in *Podarcis hispanica* morphotype 1A (Squamata: Lacertidae) and cross-amplification in closely-related species. *Mol Ecol Resour* 13:966–968
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2010). Geneious [Computer software]. Retrieved from <http://www.geneious.com/>
- Eikhorst W, Eikhorst R, Nettmann HK, Rykena S (1979) Beobachtungen an der Spanischen Kieledachse. *Algyroides marchi* Valverde, 1958 (Reptilia: Sauria: Lacertidae). *Salamandra* 15:254–263
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–567
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587
- Fauquette S, Suc JP, Guiot J, Diniz F, Feddi N, Zheng Z, Bessais E, Drivaliari A (1999) Climate and biomes in the West Mediterranean area during the Pliocene. *Palaeogeogr Palaeoclimatol Palaeoecol* 152:15–36

- Fernández-Cardenete JR, García-Cardenete L (2015) Lagartija de Valverde – *Algyroides marchi*. In: Salvador A, Marco A (Eds), Enciclopedia Virtual de los Vertebrados Españoles. Museo Nacional de Ciencias Naturales, Madrid. <http://www.vertebradosibericos.org/>
- Fitze PS, Gonzalez-Jimena V, San-Jose LM, San Mauro D, Aragón P, Suarez T, Zardoya R (2011) Integrative analyses of speciation and divergence in *Psammodromus hispanicus* (Squamata: Lacertidae). *BMC Evol Biol* 11:347
- Flot JF (2010) SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Mol Ecol Resour* 10:162–166
- Friedman JH (1991) Multivariate adaptive regression splines. *Ann Stat* 19:1–67
- García-Muñoz E, Carretero MA (2013) Comparative ecophysiology of two sympatric lizards. Laying the groundwork for mechanistic distribution models. *Acta Herpetologica* 8:123–128
- García-Porta J, Irisarri I, Kirchner M, Rodríguez A, Kirchhof S, Brown JL, MacLeod A, Turner AP, Ahmadzadeh F, Albaladejo G, Crnobrnja-Isailovic J, De la Riva I, Fawzi A, Galán P, Göçmen B, Harris DJ, Jiménez-Robles O, Joger U, Jovanović Glavaš O, Karış M, Koziel G, Künzel S, Lyra M, Miles D, Nogales M, Oğuz MA, Pafilis P, Rancilhac L, Rodríguez N, Rodríguez Concepción B, Sanchez E, Salvi D, Slimani T, Skhifa A, Qashqaei AT, Žagar A, Lemmon A, Moriarty Lemmon E, Carretero MA, Carranza S, Philippe H, Sinervo B, Müller J, Vences M, Wollenberaler KC (2019) Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nat Commun*. <https://doi.org/10.1038/s41467-41019-11943-x>
- Garrick RC, Sunnucks P, Dyer RJ (2010) Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *BMC Evol Biol*. <https://doi.org/10.1186/1471-2148-1110-1118>
- Gil-Sánchez JM (1992) Dos nuevas localidades de *Algyroides marchi* (Reptilia, Lacertidae). Granada
- Godinho R, Crespo EG, Ferrand N (2008) The limits of mtDNA phylogeography: Complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. *Mol Ecol* 17:4670–4683
- Gómez A, Lunt DH (2007) Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: Weiss S, Ferrand N (eds) Kluwer Academic Publishers. The Netherlands, Dordrecht, pp 155–188
- Gonçalves DV, Brito JC, Crochet PA, Geniez P, Padial JM, Harris DJ (2012) Phylogeny of North African *Agama* lizards (Reptilia: Agamidae) and the role of the Sahara desert in vertebrate speciation. *Mol Phylogenet Evol* 64:582–591
- Guillera-Arroita G, Lahoz-Monfort JJ, Elith J (2014) Maxent is not a presence-absence method: a comment on Thibaud et al. *Method Ecol Evol* 5:1192–1197
- Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–372
- Harris DJ, Arnold EN, Thomas RH (1998) Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proc Royal Soc London B* 265:1939–1948
- Harris DJ, Arnold EN, Thomas RH (1999) A phylogeny of the European lizard genus *Algyroides* (Reptilia: Lacertidae) based on DNA sequences, with comments on the evolution of the group. *J Zool* 249:49–60
- Hewitt GM (1996) Some genetic consequences of the ice ages and their role in divergence and speciation. *Biol J Lin Soc* 58:247–266
- Hewitt GM (1999) Postglacial recolonization of the European biota. *Biol J Lin Soc* 68:87–612
- Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913
- Hewitt GM (2004) Genetic consequences of climatic oscillations in the quaternary. *Philosophical Trans Royal Soc B Biol Sci* 359:183–195
- Hewitt GM (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica* 139:617–638
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978
- Hijmans RJ, Phillips S, Leathwick J, Elith J (2017) Package ‘dismo’ - species distribution modeling. CRAN repository
- Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Mol Ecol Resour* 9:1322–1332
- Jiménez-Moreno G, Fauquette S, Suc JP (2010) Miocene to Pliocene vegetation reconstruction and climate estimates in the Iberian Peninsula from pollen data. *Rev Palaeobot Palynol* 162:403–415
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780

- Kitoh A, Murakami S (2002) Tropical Pacific climate at the mid-Holocene and the Last Glacial Maximum simulated by a coupled ocean-atmosphere general circulation model. *Paleoceanography*. <https://doi.org/10.1029/2001PA000724>
- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Mol Ecol Resour* 15:1179–1191
- Lafuente M, Roca V (1993) Helminthofauna of *Algyroides marchi* Valverde, 1958 (Sauria, Lacertidae) en las Sierras de Alcaraz y Cazorla. *Miscelanea Zoológica* 17:268–271
- Lamoreux JF, Morrison JC, Ricketts TH, Olson DM, Dinerstein E, McKnight MW, Shugart HH (2006) Global tests of biodiversity concordance and the importance of endemism. *Nature* 440:212–214
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 29:1695–1701
- Leigh JW, Bryant D (2015) PopART: full-feature software for haplotype network construction. *Method Ecol Evol* 6:1110–1116
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452
- Liu C, Berry PM, Dawson TP, Pearson RG (2005) Selecting thresholds of occurrence in the prediction of species distributions. *Ecography* 28:385–393
- Malcolm JR, Liu CR, Neilson RP, Hansen L, Hannah L (2006) Global warming and extinctions of endemic species from biodiversity hotspots. *Conserv Biol* 20:538–548
- Martínez-Freiria F, Crochet PA, Fahd S, Geniez P, Brito JC, Velo-Anton G (2017) Integrative phylogeographical and ecological analysis reveals multiple Pleistocene refugia for Mediterranean *Daboia* vipers in north-west Africa. *Biol J Lin Soc* 122:366–384
- McSweeney CF, Jones RG, Lee RW, Rowell DP (2015) Selecting CMIP5 GCMs for downscaling over multiple regions. *Clim Dyn* 44:3237–3260
- Mendes J, Harris DJ, Carranza S, Salvi D (2016) Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenation approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees. *Mol Phylogenet Evol* 100:254–267
- Miraldo A, Faria C, Hewitt GM, Paulo OS, Emerson BC (2013) Genetic analysis of a contact zone between two lineages of the ocellated lizard (*Lacerta lepida* Daudin 1802) in south-eastern Iberia reveal a steep and narrow hybrid zone. *J Zool Syst Evol Res* 51:45–54
- Miraldo A, Paulo OS, Hewitt GM, Emerson BC (2011) Phylogeography and demographic history of *Lacerta lepida* in the Iberian Peninsula: multiple refugia, range expansions and secondary contact zones. *BMC Evol Biol*. <https://doi.org/10.1186/1471-2148-1111-1170>
- Mouret V, Guillaumet A, Cheylan M, Pottier G, Ferchaud AL, Crochet PA (2011) The legacy of ice ages in mountain species: post-glacial colonization of mountain tops rather than current range fragmentation determines mitochondrial genetic diversity in an endemic Pyrenean rock lizard. *J Biogeogr* 38:1717–1731
- Ohlemüller R, Anderson BJ, Araújo MB, Butchart SHM, Kudrna O, Ridgely RS, Thomas CD (2008) The coincidence of climatic and species rarity: high risk to small-range species from climate change. *Biol Lett* 4:568–572
- Orme CDL, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ, Ding TS, Rasmussen PC, Ridgely RS, Stattersfield AJ, Bennett PM, Blackburn TM, Gaston KJ, Owens IPF (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature* 436:1016–1019
- Otero C, Castien E, Senosiain R, Portillo F (1978) Fauna de Cazorla. Ministerio de Agricultura, Madrid
- Otto-Bliesner BL, Marshall SJ, Overpeck JT, Miller GH, Hu A, members CLIP (2006) Simulating arctic climate warmth and icefield retreat in the Last Interglaciation. *Science* 311:1751–1753
- Palacios L, Ayarzagüena J, Ibañez C, Escudero J (1974) Estudio sobre la lagartija de Valverde *Algyroides marchi* (Reptilia, Lacertidae). *Doñana Acta Vertebrata* 1:5–31
- Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In Hillis D, Moritz C, Mable BK (Eds). Sinauer, Sunderland, MA, pp. 205–247
- Pérez-Mellado V, Marquez R, Martínez-Solano I (2009) *Algyroides marchi*. The IUCN Red List of Threatened Species 2009 e.T840A13082975
- Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME (2017) Opening the black box: an open-source release of Maxent. *Ecography* 40:887–893
- Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distribution. *Ecol Model* 190:231–259
- Phillips SJ, Dudík M, Elith J, Graham CH, Lehmann A, Leathwick J, Ferrier S (2009) Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecol Appl* 19:181–197



- Pinho C, Ferrand N, Harris DJ (2006) Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. *Mol Phylogenet Evol* 38:266–273
- Pinho C, Harris DJ, Ferrand N (2007) Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation. *Mol Ecol* 16:1191–1205
- Pinho C, Kaliontzopoulou A, Harris DJ, Ferrand N (2011) Recent evolutionary history of the Iberian endemic lizards *Podarcis bocagei* (Seoane, 1884) and *Podarcis carbonelli* Pérez-Mellado, 1981 (Squamata: Lacertidae) revealed by allozyme and microsatellite markers. *Zool J Linn Soc* 162:184–200
- Pinho C, Rocha S, Carvalho BM, Lopes S, Mourão S, Vallinoto M, Brunes TO, Haddad CFB, Gonçalves H, Sequeira F, Ferrand N (2010) New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. *Conserv Genet Resour* 2:181–185
- Pleguezuelos JM, Moreno M, Ruíz I (1989) Nuevas citas de anfibios y reptiles para el SE de la Península Ibérica. *Doñana Acta Vertebrata* 16:300–305
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- R Core Team (2018) R: a language and environment for statistical computing. Vienna, Austria
- Rambaut A (2009) Figtree [Computer Software]
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* syy032. <https://doi.org/10.1093/sysbio/syy1032>
- Remón N, Galán P, Vila M, Arribas O, Naveira H (2013) Causes and evolutionary consequences of population subdivision of an Iberian mountain lizard *Iberolacerta monticola*. *PLoS One* 8:6034
- Romero R, Guijarro JA, Ramis C, Alonso S (1998) A 30-year (1964–93) daily rainfall data base for the Spanish Mediterranean regions: first exploratory study. *Int J Climatol* 18:541–560
- Rosado D, Rato C, Salvi D, Harris DJ (2017) Evolutionary history of the Morocco lizard-fingered geckos of the *Saurodactylus brossei* complex. *Evol Biol*. 10.100
- Rubio JL (2002) *Algyroides marchi* Valverde, 1958. Lagartija de Valverde. In: Pleguezuelos JM, Márquez R, Lizana M (eds) Atlas y libro rojo de los anfibios y reptiles de España. DGCN-AHE, Madrid, pp 193–195
- Rubio JL, Carrascal LM (1994) Habitat selection and conservation of an endemic Spanish lizard *Algyroides marchi* (Reptilia, Lacertidae). *Biol Cons* 70:245–250
- Rubio JL, Martín VS (2017) Habitat use by peripheral populations of a lizard with a highly restricted distribution range, the Spanish algyroides, *Algyroides marchi*. *Salamandra* 53:405–412
- Rubio JL, Palacios F (1986) Reproductive cycle of *Algyroides marchi* (Reptilia: Lacertidae). In: Roček Z (ed), Studies in herpetology, Prague, pp. 473–478
- Runemark A, Gabirot M, Bensch S, Svensson EI, Martín J, Pafilis P, Valakos ED, Hansson B (2008) Cross-species testing of 27 pre-existing microsatellites in *Podarcis gaigeae* and *Podarcis hispanica* (Squamata: Lacertidae). *Mol Ecol Resour* 8:1367–1370
- Salvi D, Harris DJ, Kaliontzopoulou A, Carretero MA, Pinho C (2013) Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evol Biol* 13:147
- Salvi D, Harris DJ, Perera A, Bologna MA, Carretero MA (2011) Preliminary survey on genetic variation within the Pygmy Algyroides, *Algyroides fitzingeri*, across Corsica and Sardinia. *Amphibia-Reptilia* 32:281–286
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbour Press, New York
- Sánchez-Vialas A, Calvo-Revuelta M, Rubio JL, Palacios F, García-París M (2018) Taxonomic and nomenclatural status of Iberian *Algyroides* (Lacertidae). *Amphibia-Reptilia*. <https://doi.org/10.1163/15685381-20181016>
- Sánchez-Videgán J, Rubio JL (1996) Atlas preliminar de los anfibios y reptiles de las sierras prebéticas albacentenses. Al-Basit: Revista de estudios albacetenses. Segunda Época, Año XXI 38:5–30
- Sillero N (2011) What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecol Model* 222:1343–1346
- Sillero N, Barbosa AM (2021) Common mistakes in ecological niche models. *Int J Geogr Inf Sci*. <https://doi.org/10.1080/13658816.2020.1798968>
- Sillero N, Brito JC, Skidmore AK, Toxopeus B (2009) Biogeographical patterns derived from remote sensing variables: the amphibians and reptiles of the Iberian Peninsula. *Amphibia-Reptilia* 30:185–206
- Sillero N, Campos J, Bonardi A, Corti C, Creemers R, Crochet PA, Isailović JC, Denoël M, Ficetola GF, Gonçalves J, Kuzmin S, Lymberakis P, de Pous P, Rodríguez A, Sindaco R, Speybroeck J, Toxopeus



- B, Vieites DR, Vences M (2014) Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia* 35:1–31
- Sillero N, Carretero MA (2013) Modelling the past and future distribution of contracting species. The Iberian lizard *Podarcis carbonelli* (Squamata: Lacertidae) as a case study. *Zoologischer Anzeiger*
- Sillero N, Tarroso P (2010) Free GIS for herpetologists: free data sources on Internet and comparison analysis of proprietary and free/open source software. *Acta Herpetologica* 5:63–85
- Sousa-Guedes D, Arenas-Castro S, Sillero N (2020) Ecological niche models reveal climate change effect on biogeographical regions: the Iberian Peninsula as a case study. *Climate* 8:42
- Stephens M, Donnelly P (2003) A comparison of bayesian methods for haplotype reconstruction. *Am J Hum Genet* 73:1162–1169
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978–989
- Sumner GN, Homar V, Ramis C (2001) Precipitation seasonality in Mediterranean Spain: character and future prospects. *Int J Climatol* 21:219–247
- Sumner GN, Romero R, Homar V, Ramis C, Alonso S, Zorita E (2003) An estimate of the effects of climate change on the rainfall of Mediterranean Spain by the late twenty first century. *Clim Dyn* 20:789–805
- Teixeira J, Martínez-Solano I, Buckley D, Tarroso P, García-Paris M, Ferrand N (2015) Genealogy of the nuclear  $\beta$ -fibrinogen intron 7 in *Lissotriton boscai* (Caudata, Salamandridae): concordance with mtDNA and implications for phylogeography and speciation. *Contributions Zool* 84:193–215
- Valverde JA (1958) Una nueva lagartija del género *Algyroides* Bibron procedente de la Sierra de Cazorla (Sur de España). *Archivos Del Instituto De Aclimatción* 7:127–134
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Resour* 4:535–538
- Varela S, Lima-Ribeiro MS, Terribile LC (2015) A short guide to the climatic variables of the last glacial maximum for biogeographers. *PLoS One* 10, e0129037
- Velo-Anton G, Martínez-Freiria F, Pereira P, Crochet PA, Brito JC (2018) Living on the edge: ecological and genetic connectivity of the spiny-footed lizard, *Acanthodactylus aureus*, confirms the Atlantic Sahara desert as a biogeographic corridor and centre of lineage diversification. *J Biogeogr* 45:1031–1042

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