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Slippery slopes: Montane isolation and elevational shifts shape the evolution and diversity of *Iberolacerta* lizards

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ABSTRACT

Understanding the processes driving the diversity of mountain herpetofauna requires a comprehensive examination of species diversification across evolutionary scales. Here, we investigate the phylogeography of *Iberolacerta*, a genus of eight lizard species mainly restricted to high elevations in southwestern Europe. Using genomic data, we reconstructed a nuclear phylogeny that aligns with mitochondrial evidence in supporting the divergence of all currently recognized species. Notably, we detect historical nuclear gene flow between *I. cyreni* and *I. martinezricai* in Central Spain, suggesting past range overlap, reminiscent of previously observed mitochondrial introgression between *I. galani* and *I. monticola*, and the lack of divergence between disjoint populations of *I. monticola*. Bioclimatic projections accordingly depict broader historical ranges during the last glacial maximum compared to interglacial and current conditions. At the intraspecific level, genomic analyses of four high-elevation species reveal that genetic structure is mainly shaped by isolation-by-distance and, in *I. cyreni*, by separation among mountain ranges, while heterozygosity generally decreases with elevation. These findings are consistent with the impact of glacial-interglacial cycles on the genetic diversity of montane taxa: populations experience genetic isolation and altitudinal bottlenecks during interglacial periods, but are reconnected and admix in lowland areas during glacial periods. These processes are expected to leave contrasting signatures between the mitochondrial and nuclear genomes, as well as between slow- and fast-evolving molecular markers. From a conservation perspective, our results highlight that the genetically richest – and potentially most adaptive – populations occur at the lowland edges of the species' ranges, where they are also most vulnerable to climate change.

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1. Introduction

High-elevation mountains are some of the most challenging environments on Earth, characterized by low oxygen levels, high ultraviolet radiation, extreme temperature fluctuations, and rugged terrain with physical barriers between patchy habitats. While these conditions led mountain regions to foster unique biodiversity through both neutral (e.g., genetic drift) and selective processes (e.g., favoring specific adaptations), they also make species vulnerable to genetic erosion and extinction through the effects of climate change, small population sizes and population isolation (Schmitt, 2009, 2017; Rubidge et al., 2012; Rahbek et al., 2019).

Amphibians and reptiles are valuable models for studying the evolutionary processes that shape the genetic variation of species in mountain environments, and thus their capacity to respond to future environmental change (e.g., Oromi et al., 2012; van Buskirk & Jansen van Rensburg, 2020; Dufresnes et al., 2022). The ectothermic physiology, often limited dispersal ability, and microhabitat specificity of herpetofauna increase their sensitivity to habitat fragmentation and elevational environmental gradients, promoting local adaptation and genetic differentiation (Muir et al., 2014; Bachmann et al., 2020). In the Northern Hemisphere, the genetic diversity of populations has been further affected by historical patterns of contraction and recolonization following the Quaternary glaciations (Schmitt, 2007, 2009, 2017). Unlike temperate and warm-adapted species, which can permanently persist in areas that have remained climatically suitable throughout episodes of climatic change (e.g., sanctuary-type refugia *sensu* Recuero & García-París, 2011), mountain endemic species are expected to have shifted most of their distributions between the lowlands and the highlands to track appropriate glacial and interglacial habitats (Schmitt, 2007, 2009, 2017). As these species retracted into lowland refugia during ice ages (e.g., valleys), they potentially reached adjacent mountain ranges that were previously inaccessible to them, promoting gene flow between their respective populations. Conversely, as species moved towards higher elevations to escape the warming lowland climate during interglacial periods, mountain populations became isolated again and experienced founder events associated with the changes in distributions (Rahbek et al., 2019).

These processes have several implications for the genetic diversity of species. In particular, species should display strong contemporary genetic structure reflecting the current disconnection of populations found in separate peaks (Ursenbacher et al., 2009; Ferchaud et al., 2011; Dufresnes et al., 2022), and their genetic variation is expected to decrease along elevational gradients following the recent elevational shifts (Van Buskirk & Jansen van Rensburg, 2020; Oromi et al., 2012). However, these disjunct populations may lack a strong phylogeographic structure if they experienced prolonged and recurrent contact during their glacial history (Dufresnes et al., 2020,2024), or if a single refugial lineage contributed to the extant diversity (Carranza & Arribas, 2008; Mouret et al., 2011; Dufresnes et al., 2022; Graham et al., 2023). Conversely, the divergence should be more pronounced if the mountain populations remained isolated throughout the glacial stages, for instance, if lineages persisted in geographically separate lowland refugia, with consequently limited historical gene flow (Bonato et al., 2018; Horreo et al., 2018; Jansen van Rensburg et al., 2021), ultimately leading to speciation (Rahbek et al., 2019).

Between these two extremes, phylogeographic patterns inherently depend on the topography and environmental complexity of their mountain ranges, in respect to the ecology and life-histories of the species, but also on the molecular markers analyzed. Discordances may notably emerge between the diversity measured at mitochondrial vs. nuclear DNA loci, due to demographic factors (e.g., sex-biased dispersal) and past hybridization events (Toews & Brelsford, 2012), as well as between loci evolving at different rates (e.g., microsatellites vs. conserved gene sequences) and that consequently react differently to current and past differentiation (Ferchaud et al., 2015). Comprehensive phylogenetic and population genetic assessments can now be routinely achieved by genome reduction methods, which, combined with mitochondrial analyses, allow to draw a fine-scale picture of the diversity, distribution and gene flow between inter- and intraspecific lineages in space and time (Vences et al., 2024; Peñalba et al., 2024). Complementary insights may be gained by projecting past species ranges using environmental niche modelling, both to locate potential glacial refugia and to evaluate whether present distributional gaps used to be favorable in the recent past (Pahad et al., 2019), which can then be associated with patterns of genetic structure.

Rock lizards of the genus *Iberolacerta* (Squamata: Lacertidae) stand out as a suitable study system for exploring how the historical and contemporary connectivity and divergence of populations in mountain environments have affected their genetic structure and diversity. *Iberolacerta* presently comprises eight cold-adapted, polygynous species mostly restricted to mountainous areas of the Iberian Peninsula (*I. monticola*, *I. galani*, *I. martinezricai*, *I. cyreni*), the Pyrenees (*I. aranica*, *I. bonnali* and *I. aurelioi*) and the southeastern Alps and Dinaric Alps (*I. horvathi*). Their narrow ranges, relatively small population sizes, and specific ecological requirements make them susceptible to many threats, potentially including global warming (Martín & López, 2013; Ortega et al., 2016a; Jiménez-Robles & De la Riva, 2019). Two species have accordingly been listed as "endangered" in the most recent assessments for the International Union for the Conservation of Nature (IUCN) Red List (*I. martinezricai* and *I. aurelioi*), while three others are considered "near threatened" (*I. bonnali*, *I. aranica*, and *I. galani*) (IUCN, 2024).

Mitochondrial studies on *Iberolacerta* show three distinct clades which correspond to the species found in the three regions (Carranza et al., 2004; Crochet et al., 2004; Arribas et al., 2006), as more recently suggested in a phylogenomic tree of Lacertidae (Garcia-Porta et al., 2019). The intraspecific diversity was detailed for three species (*I. monticola*, *I. bonnali*, *I. horvathi*), based on different mtDNA sequences e.g., the 3′ part of the control region (CR) + cytochrome *b* (cyt *b*) (Remón et al., 2013; Arribas et al., 2014), 5′ CR (Mouret et al., 2011), cyt *b* (Cocca et al., 2021), combined with a few nuclear loci, e.g., microsatellites (Remón et al., 2013, Ferchaud et al., 2015) or a gene fragment (Cocca et al., 2021). The results suggest a major impact of Quaternary range movements in shaping the phylogeographic patterns. In *I. monticola*, the south westernmost isolated populations are surprisingly undiverged despite being presently separated by hundreds of kilometers, suggesting a massive Holocene retraction (Remón et al., 2013). In Montes de León, the subspecies *I. monticola astur* partly carries mtDNA derived from an allopatric species, *I. galani*, testifying of past episodic contact and hybridization (Arribas et al., 2014). In the Pyrenean *I. bonnali*, population genetic analyses also suggested good historical connectivity, but with radically different patterns of microsatellite vs. mitochondrial variation (Ferchaud et al., 2015). Like in other polygynous species (Johansson et al., 2008), dispersal is expected to be male biased in *Iberolacerta*, which could putatively generate cyto-nuclear discordances (Remón et al., 2013). These elements thus call to examine the evolution and diversity of *Iberolacerta* with genomic loci and to link phylogeographic patterns to past range shifts.

In this study, we combine analyses of thousands of genomic loci obtained by double digest Restriction Site Associated DNA (ddRAD-seq) with mtDNA inferences and ecological niche modelling to re-assess the phylogeography of *Iberolacerta*. We aim to (1) retrace the evolution and shifts in the distributions of species, with emphasis on historical gene flow, both in the nuclear genome and in the form of cyto-nuclear discordance, and (2) investigate the genetic diversity in the high-elevation *I. cyreni*, *I. aranica*, *I. aurelioi* and *I. bonnali* in relation to population isolation and elevational patterns.

2. Methods

2.1. ddRAD-seq analyses

2.1.1. Library and assembly

Ninety-six samples were targeted for genomic analyses, including 95 *Iberolacerta* and one *Podarcis muralis* as outgroup (Table S1). Tissue samples consisted of muscle pieces excised from preserved specimens curated in the BEV CEFE collection, as well as tail tips or blood taken from field-captured individuals. These samples represented all species and subspecies except for the recently described micro-endemic *I. monticola astur* in Asturias (Arribas et al., 2014), and with a dense geographic coverage for the three Pyrenean species and the Central Spanish *I. cyreni*.

DNA was isolated using the Qiagen Blood & Tissue kit and a ddRAD-seq genomic library was prepared following the steps and reagents detailed by Dufresnes et al. (2025). A bench workflow is available at https://doi.org/10.17504/protocols.io.kxygx3nzwg8j/v1. This protocol, adapted from Brelsford et al. (2016), consists of enzyme restriction by *MseI* and *SbfI*, adapter ligation with individual barcodes, duplicate PCR amplification, and size selection of 400–500 bp fragments. The final library was sequenced on a NextSeq 550 (Illumina) with the 2 × 75 bp kit (Max Planck Institute for Evolutionary Biology, Plön, Germany), which yielded 1.007 billion reads. Read filtering, demultiplexing and assembly were performed with STACKS 2.60 (Catchen et al., 2013). Specifically, the function process_radtags was used for demultiplexing the paired-end reads, trimming to 65 bp (flag -t 65), removing adapter sequences (flags -adapter-1, -adapter-2), and discarding reads with uncalled bases (flag -c), with low quality scores (flag -q) and flagged by Illumina's purity filter (flag -filter-illumina). The denovo.pl pipeline was applied to stack and catalog the demultiplexed paired-end reads, with default mismatch parameters (flags -M, -n) and PCR duplicate removal (flag -rm-pcr-duplicates). The final catalog contained 218,760 loci, with a mean effective per-sample coverage of 39.7×. Sequence and SNP calling for downstream analyses was done with the populations module of STACKS, using the flags -p and -r to adjust the tolerance for locus dropout.

2.1.2. Phylogenomics

Fourteen samples with very few reads, and that accordingly featured high proportions of missing data (>80 %) in preliminary analyses, were discarded (Table S1), which excluded one of the sampled taxa (*I. m. monticola*). For the remaining 82 samples (81 *Iberolacerta* + 1 outgroup), we first obtained a concatenated alignment of 10,557 RAD loci (1,356,505 bp) genotyped in at least 70 samples (−p: 70). A maximum-likelihood (ML) phylogeny was inferred with IQ-TREE 2.3.6 (Minh et al., 2020), using ModelFinder for model selection and 1000 ultrafast bootstrap pseudoreplicates. The tree was visualized and annotated with the R package ggtree (Yu et al., 2017). Samples were mapped in respect to their taxonomic identity in QGIS 3.24.3.

Second, to confirm phylogenetic relationships and estimate divergence times, we focused on a reduced subset of 19 samples (18 *Iberolacerta* + 1 outgroup; Table S1) chosen to represent the diversity of *Iberolacerta* species and their intraspecific diversity, without admixture between the retrieved clusters (see Results). For this subset, we obtained an alignment of 6668 RAD loci (854,774 bp) genotyped in all but one sample (−p: 18). An ML tree was inferred with IQ-TREE, and a Bayesian timetree was reconstructed with BEAST 2.7.7 (Bouckaert et al., 2019). For the latter, we used the GTR + G + I model, an optimized relaxed clock, a birth–death tree prior, and normally distributed time to the most recent ancestor (tMRCA) priors. In the absence of fossil or geological calibrations directly relevant to *Iberolacerta*, we set up the tMRCA priors according to the timeframes obtained by the lacertid phylogenomic study of Garcia-Porta et al. (2019), as follows: (1) the split between *P. muralis* and *Iberolacerta* to 37.6 Mya (σ = 5.0; covering a 95 % prior distribution of 27.8–47.4 Mya); (2) the diversification of *Iberolacerta* to 14.7 Mya (σ = 2.0; 10.8–18.6); (3) the diversification of the Pyrenean clade (*I. bonnali*, *I. aranica* and *I. aurelioi*) to 5.8 Mya (σ = 1.0; 3.8–7.8). The σ values were chosen to also approach the *Iberolacerta* mitochondrial timetree of Arribas et al. (2006), which however relied on a geological calibration relevant to a distant outgroup and yielded the youngest estimates reported for this genus on timetree.org (Kumar et al., 2022). The BEAST analysis was run for 100 million iterations, sampling every 10,000. Chain stationarity and effective size of parameters were monitored in Tracer 1.7 (Rambaut et al., 2018). A maximum clade credibility tree was produced with the TreeAnnotator module of BEAST (with a burnin of 20 %), and visualized with FigTree 1.4.3 (https://github.com/rambaut/figtree/releases).

To mitigate potential biases arising from concatenated genomic alignments, which assume a single shared evolutionary history among loci, we additionally conducted a time-calibrated multispecies coalescent analysis with SNAPPER (Stoltz et al., 2021), as implemented in BEAST 2, which accounts for gene tree heterogeneity when reconstructing phylogenetic relationships. To this end, the 19 samples were assigned to nine groups corresponding to the eight *Iberolacerta* species and the *P. muralis* outgroup. SNAPPER XML input files were generated using the Ruby script *snapp_prep.rb* (https://github.com/mmatschiner/snapp_prep), and the same calibration constraints as above were applied. We performed three independent MCMC runs, each consisting of 1 million iterations. Convergence was confirmed from the trace profiles in Tracer, and log and tree files were combined using the LogCombiner module of BEAST. The resulting trees were visualized in DensiTree v3.0.2 (Bouckaert, 2010), with a burnin of 10 %, and species trees from the posterior were summarized in a maximum clade credibility tree with TreeAnnotator.

Third, we evaluated the effect of historical gene flow on the diversification of *Iberolacerta* using TreeMix (Pickrell & Pritchard, 2012), following an *ad hoc* pipeline (https://github.com/carolindahms/TreeMix). This analysis was restricted to the 18 *Iberolacerta* samples of the above subset, assigned to eight groups (i.e., taxa), and for which we obtained 5348 RAD loci genotyped in all groups and samples (−p: 8 −r: 1). To minimize the effect of physical linkage, only a single SNP per RAD locus was retained (−write-random-snp flag). TreeMix was run allowing for zero to ten migration events, with 10 replicates and 1000 bootstrap replicates for each setting. The optimal number of migration edges was identified using linear modeling estimation in OptM (Fitak, 2021). Finally, TreeMix was rerun with the optimal number of migration edges, performed across 100 iterations and 1000 bootstrap replicates. For this final run, migration weight (i.e., % of admixture) for each migration event was averaged across all runs, and jackknifing was used to estimate the standard errors.

2.1.3. Intraspecific diversity

To examine the genetic structure and diversity within the four species for which we had proper geographic coverage, we filtered the RAD loci genotyped and polymorphic among the n samples of each (−p: n), retaining a single SNP per locus (−write-random-snp). This approach maximizes the number of loci informative within each species, discards any missing data and avoids obviously linked SNPs. The obtained datasets (alignments and SNP matrices) featured 3975 RAD loci for *I. cyreni* (n = 31), 1967 RAD loci for *I. bonnali* (n = 11), 3971 RAD loci for *I. aurelioi* (n = 11) and 8502 RAD loci for *I. aranica* (n = 10). To compare diversity levels, we exported a fourth matrix based on 3236 RAD loci present among the samples of all three Pyrenean species (n = 32).

For each species, the alignment dataset was used to build a phylonetwork with SplitsTree (Huson & Bryant, 2006). The SNP matrix was used to estimate observed heterozygosity (H_o) with the R package *hierfstat* (Goudet, 2005), perform a Principal Component Analysis (PCA) on allele frequencies with the R package *adegenet* (Jombart, 2008), and assign individuals into genetic clusters with the R package LEA (Frichot & François, 2015). For the latter, we ran chains for K = 1–10 clusters (20

replicates for each), retaining 10 % of the data for the cross-entropy estimation. To assess how many clusters best summarize the data, we compared the cross-entropy across Ks (Frichot & François, 2015), the most likely K being reflected by the lowest cross entropy. For a thorough exploration, all Ks with a cross-entropy lower than the cross-entropy of K = 1 (no structure) were reported, and if K = 1 had the lowest cross-entropy, K = 2 was reported. For the Pyrenean species, H_o was also computed from the SNP dataset for the loci shared across all three species.

Two geographical patterns of diversity were further assessed. First, we tested for isolation-by-distance (IBD) by relating net pairwise divergence *D* (computed in MEGA 11; Tamura et al., 2021) to geographic distances *d* (computed with Geographic Distance Matrix Generator; Ersts, 2006), using Mantel tests with 1000 permutations. Second, we related H_o to elevation using linear regressions. Finally, we gathered published heterozygosity estimates obtained from microsatellite markers analyzed in two studies, as follows. First, to test if diversity relates to elevation in a fifth species, *I. monticola*, we used the expected heterozygosity (H_e) estimates reported for 14 populations by Remón et al. (2013). Second, to assess whether comparing microsatellite vs. ddRAD-seq diversity is reliable, we related our H_o estimates of *I. bonnali* with those obtained for that species by Ferchaud et al. (2015), for nine localities analyzed by both studies.

2.2. Mitochondrial DNA analyses

2.2.1. MtDNA barcoding

To depict the mitochondrial diversity and evolution of *Iberolacerta*, we mined publicly available GenBank sequences generated by previous studies (Harris et al. 1998; Beyerlein & Mayer 1999; Fu, 2000; Mayer & Arribas, 2003; Carranza et al., 2004; Crochet et al., 2004; Arribas et al., 2006; Galán et al., 2007; Pavlicev & Mayer, 2009; Greenbaum et al., 2011; Mouret et al., 2011; Remón et al., 2013; Arribas et al., 2014; Mendes et al., 2016; Carvalho et al., 2017; Garcia-Porta et al., 2019; Zangl et al., 2020; Cocca et al., 2021). These included fragments of seven different markers, namely 12S rRNA (12S), 16S rRNA (16S), ND4, COI, cyt *b*, the 5′ part (CR1) and the 3′ part (CR2) of the control region, for a total of 900 sequences obtained from 597 different specimens. In addition, we generated new sequences of ND4 for 24 specimens, using primers ND4 and LEU (Arèvalo et al. 1994) to amplify a fragment of ca 900 bp. PCRs were run in volumes of 25 µl, composed of 12.5 µl of GoTaq® Green Master Mix (Promega), 0.5 µl of each primer (10 µM), 10.5 µl of nuclease-free water and 1 µl of template DNA. PCR conditions consisted of an initial denaturation at 94 °C for 5′, followed by 35 cycles of denaturation (94 °C for 45″), annealing (48 °C for 45″), and extension (72 °C, 90″), with a final extension step at 72 °C during 7′. Amplified fragments were purified with ExoSAP-IT (Applied BiosystemsTM) and Sanger sequenced in both directions at Macrogen (Madrid, Spain). The final dataset thus comprised 924 sequences from 621 specimens, including 586 that could be geo-referenced to 137 different sampling sites (Table S2).

For each fragment, sequences were aligned manually in Seaview 5 (Gouy et al., 2021) and exploratory neighbor joining trees were produced with PhyML 3.0 (Guindon et al., 2010) to identify the lineages corresponding to each species and subspecies. Within taxa, major mitogroups were initially identified from the most informative fragments, both in terms of phylogenetic resolution and sampling coverage, namely cyt *b* for *I. monticola*, *I. horvathi* and *I. bonnali*, and ND4 for *I. cyreni*. Correspondence across fragments was then possible based on geographic distributions and sequences of additional markers for the same samples. The obtained barcoding dataset allowed to map the distribution of each lineage, as well as to verify and eventually update taxonomic assignments.

2.2.2. MtDNA phylogenetics

To build a high-resolution mitochondrial phylogeny, we prepared a supermatrix alignment (7658 bp) of the seven fragments concatenated for 18 samples representative of the diversity of *Iberolacerta* (Table S3). Sequences from a mitogenome of *Podarcis muralis* (FJ460597) were used for the outgroup. As for ddRAD-seq, a ML tree was first inferred with IQ-TREE, and a time calibrated tree was inferred with BEAST, using the same parameters and calibrations described above.

2.3. Ecological niche modelling

2.3.1. ENMs

Ecological niche models (ENMs) were built with MaxEnt 3.4.4 (Phillips et al., 2006) based on bioclimatic data to predict current and past range distributions. To this end, we gathered a total of 4299 localities with confirmed *Iberolacerta* occurrence (≤1 km accuracy), comprising records from museum collections, the scientific literature, citizen-science platforms (GBIF, INPN) and new observations (Table S4). Nineteen bioclimatic layers (bio1–19; 30 arc second resolution) representative of the climate over a period covering the years ~1950–2000 were extracted from the WorldClim 1.4 database (https://www.worldclim.org).

The bioclimatic envelope was modelled independently for each species (except for the micro-endemic *I. martinezricai*, for which too few localities exist), and for all species altogether. Analyses were conducted under the WGS 84 projection, with different masks for each species, designed to encompass their respective range and adjacent areas (Table S5). Autocorrelation among variables was assessed by Pearson correlation coefficients in ENMTools 1.3 (Warren et al., 2010), and for variable pairs with coefficients above 0.8, only a single variable was retained. The occurrence dataset was filtered to remove duplicate localities (<30 arc seconds apart), and 1368 localities were retained for the models.

For each envelope, ten replicates were performed, keeping 30 % of localities for random testing, and a number of background points of 20,000. In each run, 372 candidate models were produced with distinct regularization multipliers (0.5–6 at intervals of 0.5) and feature classes (representing all combinations of linear, quadratic, product, threshold, and hinge response types), and the one with the best parameter settings was selected based on statistical significance (partial ROC), predictive power (omission rates E = 5 %), and complexity level (AICc) using the R package kuenm (Cobos et al., 2019). Model performance was evaluated using the Area Under the Curve (AUC; ranging 0–1) and the True Skill Statistic (TSS; ranging from −1 to +1) of the 10-percentile training omission threshold. A ClogLog output format (ranging 0–1) was chosen for downstream processing (Phillips et al., 2017), and the relative contributions of variables were estimated by a jackknife analysis.

2.3.2. Projected distributions

Based on the ENMs, we projected the probabilities of occurrence according to the current climatic conditions, as well as conditions during the Last Interglacial (LIG; ca 120,000–140,000 years ago) and the Last Glacial Maximum (LGM; ca 22,000 years ago), extracted from the WorldClim 1.4 database (https://www.worldclim.org/data/v1.4/paleo1.4.html). Maps were produced in QGIS 3.24.3. For the LGM, we considered two general atmospheric circulation models, the Community Climate System Model (CCSM4) and the Model for Interdisciplinary Research on Climate (MIROC-ESM), and averaged their occurrence probabilities using the raster calculator of QGIS.

To assess changes in predicted distributions between the three periods, we reported the STATISTICS_MEAN of the raster layers. This statistic corresponds to the sum of occurrence probabilities over the whole mask divided by the number of cells, making it comparable between layers of different resolutions. We then computed the cumulated occurrence probability relative to the present by dividing the STATISTICS_MEAN of the LIG and LGM conditions to the STATISTICS_MEAN of the current conditions. These relative estimates are then comparable between species despite different mask extents.

3. Results

3.1. Genomic phylogeography

Based on 10,557 RAD loci (~1.4 Mb) and 81 ingroup samples, the IQ-TREE ML phylogeny is robustly supported and recovers all eight *Iberolacerta* species as monophyletic (Fig. 1), being arranged in three major clades. The first clade features the Alpine-Dinaric species *I. horvathi*, showing little divergence among our scattered samples from Croatia, Slovenia and Italy. The second clade features the Pyrenean species *I. aurelioi*, *I. aranica* and *I. bonnali*, each forming a deeply diverged lineage, with again little intraspecific diversity. The third clade comprises the Central Iberian species *I. cyreni* as a deeply diverged lineage, sister to a clade that regroups the closely related Western Iberian species *I. monticola* (subspecies *I. m. cantabrica*), *I. galani* and *I. martinezricai*. *Iberolacerta cyreni* features shallow lineages that can be geographically associated with the Sierra de Guadarrama (*I. c. cyreni*) and the Sierra de Gredos and Sierra de Béjar (*I. c. castiliana*), in which *I. c. castiliana* is paraphyletic.

The ML analysis of stringently filtered 6,668 RAD loci (~855 kb) among a subset of 18 representative *Iberolacerta* samples (free of admixture between identified intraspecific clusters; see 3.3) yielded an identical topology (Fig. S1), confirming the monophyly of all species, and suggesting the paraphyly of *I. c. castiliana* with partial support.

Using secondary calibrations adapted from Garcia-Porta et al. (2019), the ddRAD-seq BEAST timetree recovered an initial diversification of *Iberolacerta* during the Middle Miocene, with the early split of *I. horvathi* from Ibero-Pyrenean ancestors (15.5 Mya) (Fig. 2A). The Late Miocene was marked by the divergence between the Pyrenean and Iberian species (13.8 Mya), followed by the early split of *I. cyreni* (11.4 Mya) and *I. aurelioi* (6.9 Mya) from their respective Iberian and Pyrenean clades. The remaining species emerged since the Mio-Pliocene transition, namely *I. aranica* and *I. bonnali* (5.5 Mya), *I. monticola* (4.4 Mya) and *I. galani* and *I. martinezricai* (3.0 Mya). Intraspecific splits were not older than the Pleistocene, the oldest corresponding to the diversification of *I. cyreni* (2.0 Mya).

The species tree obtained by SNAPPER recovered a similar topology and divergence time estimates as the IQ-TREE and BEAST analyses, with the noticeable exception of the Western Iberian clade (Fig. 3). In the SNAPPER tree, *I. monticola* and *I. galani* are grouped as sister species, and all three species are markedly younger (1.6 My for *I. martinezricai*, 1.2 My for *I. monticola* and *I. galani*) (Fig. 3).

The TreeMix analysis suggested that the data was best described by a bifurcating tree with a single admixture event (Fig. S2). The obtained

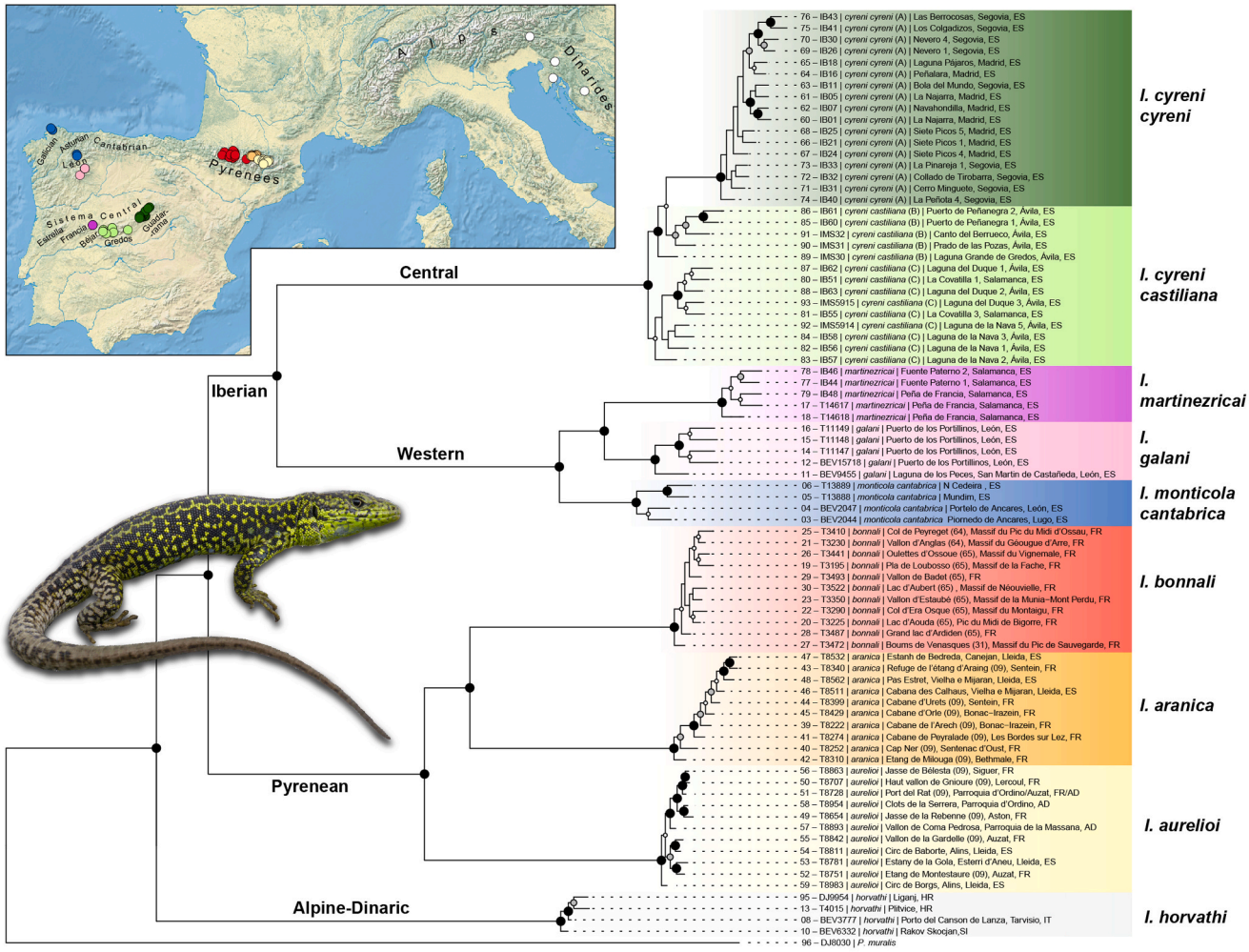

Fig. 1. Maximum-likelihood tree of *Iberolacerta* inferred from ddRAD-seq sequences and geographic origins of samples. The phylogenetic analysis (IQ-Tree) is based on 10,557 concatenated RAD tags (totaling 1.4 Mb) and 82 samples (81 *Iberolacerta* + 1 outgroup). Branch support based on 1000 ultrafast pseudoreplicates are given by large-size black circles (100 %), medium-size grey circles (95–99 %), small-size white circles (70–94 %), or no circles (<70 %). Tip labels indicate sample identifiers (Table S1), taxonomic assignment (as well as lineage letters for *I. cyreni*), and locality. Colors distinguish taxa. The main mountain ranges discussed are labelled on the map. Photo credit: Birgit & Peter Oefinger (https://www.eurolizards.com/).

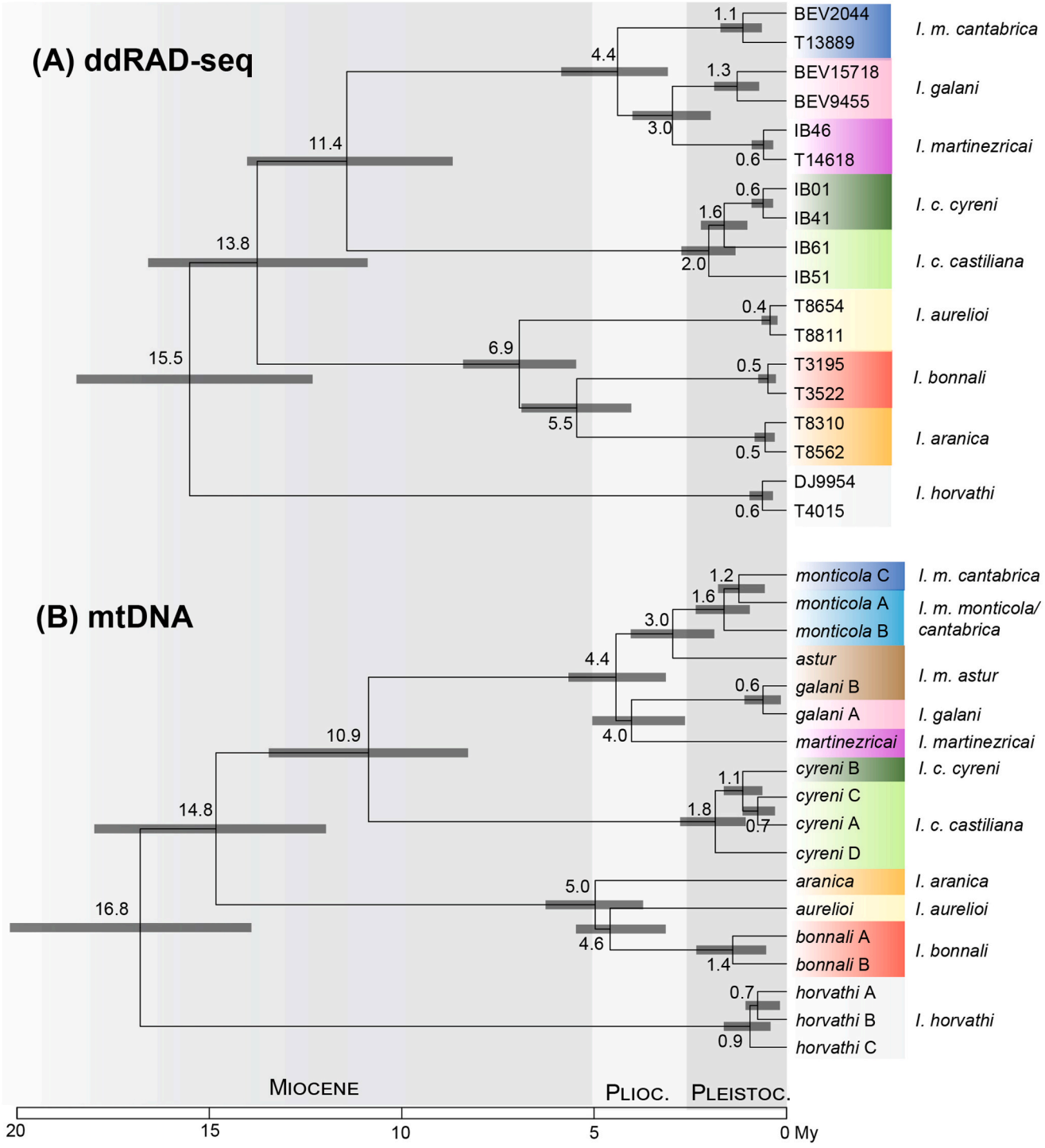

Fig. 2. Bayesian timetrees for *Iberolacerta* inferred from ddRAD-seq and mitochondrial sequences. (A) Timetree analysis based on 6,668 concatenated RAD tags (totaling 855 kb) and 19 selected samples (18 *Iberolacerta* + 1 outgroup). (B) Timetree analysis based on seven concatenated mitochondrial gene fragments (totaling 7.7 kb) and 19 identified lineages (18 *Iberolacerta* + 1 outgroup). Node values are median age estimates (in My), with dark bands representing 95 % highest posterior densities (HPD). Samples and lineages are colored by taxonomic assignments.

tree corresponded to the phylogenetic tree with strong statistical support, and traced 6.8 % (± 1.7 %) of the ancestry of *I. martinezricai* to *I. cyreni* (Fig. 4).

3.2. Mitochondrial phylogeography

Among 924 mtDNA sequences representative of 621 individuals (582 geo-referenced to 137 sites) and seven gene fragments, we distinguished 18 phylogeographic lineages in *Iberolacerta* (Fig. 5). The trees of each gene fragment are provided in Figs. S3–S9. Using concatenated sequences representative of each lineage (ca 7.7 kb), the mitochondrial ML phylogeny yielded a generally similar topology as the ddRAD-seq trees, although some internal nodes lack support (Fig. 5). Specifically, the sister relationship between *I. galani* and *I. martinezricai* is not robust, and the diversification pattern among the three Pyrenean species (early split of *I. aranica*), also unsupported, differs from the pattern retrieved by the

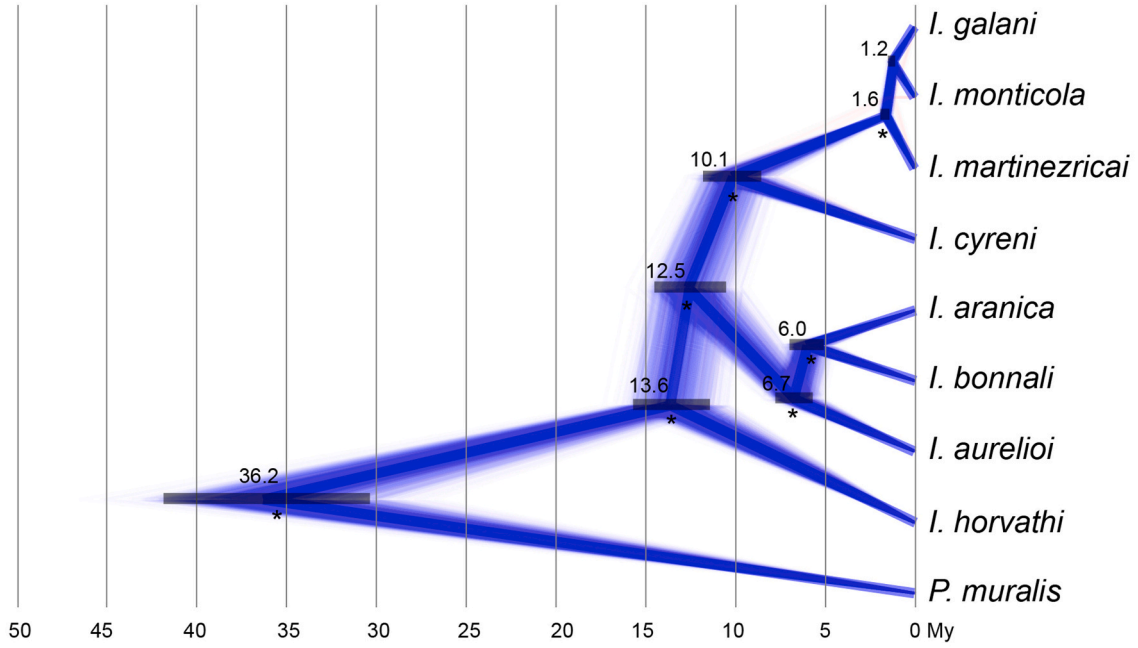

Fig. 3. Time-calibrated multispecies coalescent species tree inferred with SNAPPER. Sampled posterior trees are shown in blue, overlaid by branch support values (* = full support), median node ages, and 90 % highest posterior density intervals (grey bars) for the maximum clade credibility tree (MCCT). The only node without full support is the sister relationship between *I. galani* and *I. monticola*, with a posterior probability of 0.99. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

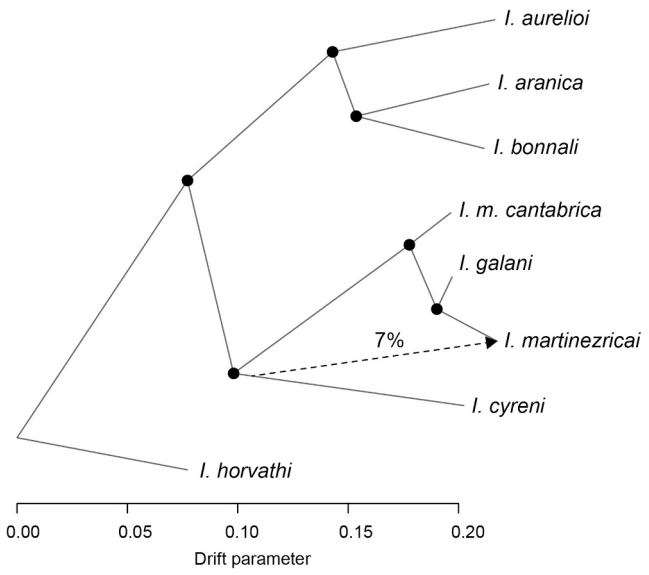

Fig. 4. TreeMix analysis of divergence with gene flow in *Iberolacerta*. The analysis assumes one migration event (the most meaningful solution, Fig. S2) and is based on 5348 SNPs genotyped in 18 *Iberolacerta* samples. The migration event is indicated by the arrow, with its weight given as %. Dark circles mark branches with >90 % support.

ddRAD-seq analyses (early split of *I. aurelioi*), which instead received strong support. The mitochondrial timetree analysis recovered broadly similar divergence times as the ddRAD-seq BEAST timetree (Fig. 2B).

Within species, the mitochondrial diversity of *I. horvathi* studied by Cocca et al. (2021) is summarized by three mitogroups of Pleistocene age (<1.0 My), two restricted to the Dinarides (inland: *horvathi* A; coastal: *horvathi* B), and one spread across the eastern Alps (*horvathi* C) (Fig. 5). In *I. monticola cantabrica*, the available sequences, mostly from Remón et al. (2013), can also be divided in three Pleistocene mitogroups (< 1.6 My), successively found across the Asturian (*monticola* A), the Galician (*monticola* B) and the Cantabrian ranges (*monticola* C). Two of these mitogroups (*monticola* A and B) co-occur in the isolated population of Serra da Estrela in Portugal (*I. m. monticola*). In Montes de León (southern Asturias), we retrieved the patterns reported by Arribas et al. (2014), namely the deep mitochondrial divergence of *I. m. astur* and the matriline it captured from *I. galani* (*galani* B), which we dated to the Pliocene (3 My) and the Middle Pleistocene (0.6 My), respectively (Fig. 2). In *I. cyreni*, our new sequences combined with those from Crochet et al. (2004) correspond to four Pleistocene lineages (<1.9 My) in which *I. c. castiliana* is paraphyletic (albeit with low support). In the Pyrenees, *I. aurelioi* and *I. aranica* each corresponds to a single lineage, while *I. bonnali* features two diverged mtDNA lineages (sequences from Arribas et al., 2006) that we dated to the Pleistocene (1.4 My) (Fig. 2B).

3.3. Genetic structure and diversity

3.3.1. Iberolacerta cyreni

The analyses of 3,975 SNPs genotyped in *I. cyreni* (n = 31) confirmed genetic structure between the two main ranges of that species, namely Sierra de Guadarrama and Sierra de Gredos (Fig. 6). The lowest-entropy LEA runs consist of two genetic clusters (K = 2; File S10A). These, along with the first axis of the PCA (PC1) and the phylonetwork, separate the samples from the two ranges (Fig. 6). Runs with three (K = 3) and four (K = 4) clusters, which still feature lower cross-entropy values than runs with no structure (K = 1) (File S10A), reflect population differentiation within each range. In Sierra de Gredos, populations from the main Gredos mountains (pastel green) are distinguished from those of the nearby Sierra de Béjar (lime green). The westernmost Gredos samples (= the closest to Sierra de Béjar) show potential signs of introgression, namely intermediate ancestry coefficients and middle positions along PC2 (which discriminates both groups) and on the phylonetwork (Fig. 6). In Sierra de Guadarrama, the northernmost samples are distinguished at K = 4, noting that these are not particularly divergent on the phylonetwork and the PCA (Fig. 6) – even across higher dimensions. Genetic diversity (H_o) is twice higher in Sierra de Gredos than

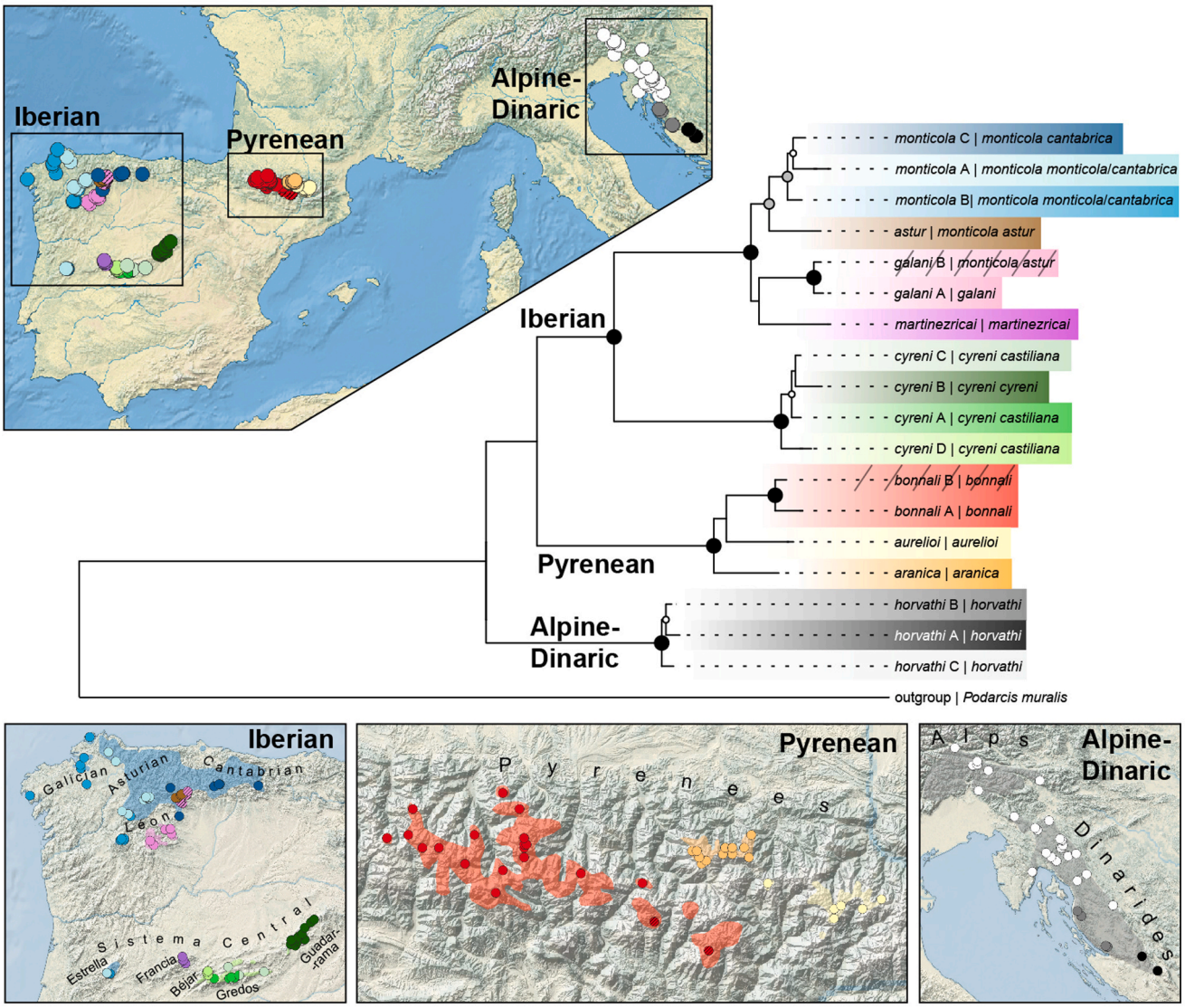

Fig. 5. Maximum-likelihood tree of *Iberolacerta* inferred from mitochondrial sequences and geographic distributions of lineages. The phylogenetic analysis is based on seven gene fragments (totaling 7.7 kb). Branch support based on 1000 ultrafast pseudoreplicates are given by large-size black circles (100 %), medium-size grey circles (95–99 %), small-size white circles (70–94 %), or no circles (<70 %). Tip labels indicate lineage identifiers and their associated taxa. Colors/dash patterns distinguish mitochondrial lineages. The distributions of the three main clades are detailed in separate maps, with indication of the discussed mountain ranges.

in Sierra de Guadarrama (Fig. 6). The relationship between genetic (D) and geographic (d) distances is highly significant (Fig. S11; $P < 0.001$, $r = 0.93$, Mantel test on log-transformed data), supporting that IBD largely contributes to the patterns of differentiation.

3.3.2. Pyrenean species

The analyses of 1,967 SNPs in *I. bonnali* ($n = 11$), 3,971 SNPs in *I. aurelioi* ($n = 11$) and 8,502 SNPs for *I. aranica* ($n = 10$) revealed shallow genetic differentiation across their respective ranges. In all three cases, the LEA runs with K = 1 have the lowest cross-entropy (File S10B–D), which is indicative of no significant structure. Nevertheless, the PCAs, phylonetworks and ancestry estimates for runs with K = 2 highlight some geographic segregation of the genetic diversity, notably along longitudinal gradients (Fig. 7). In *I. bonnali* and *I. aurelioi*, all samples appear evenly related, despite range discontinuities, and H_o was generally higher in the core area than the sampled edges of their distributions (Fig. 7). In *I. aranica*, the two easternmost samples have the highest diversity and divergence, the rest being genetically more homogeneous (Fig. 7). All three species show significant association between genetic divergence and geographic distances suggestive of IBD (Fig. S11), with $r = 0.77$ for *I. aranica*, $r = 0.61$ for *I. bonnali* and $r = 0.70$ for *I. aurelioi* ($P < 0.001$, Mantel tests on log-transformed data).

3.3.3. Diversity and elevation

H_o significantly decreased with elevation in the well-sampled *I. cyreni* ($P = 0.001$, $R^2 = 0.30$, $n = 31$; Fig. 8A), but not in the lesser sampled *I. aranica* ($P = 0.53$, $n = 10$), *I. bonnali* ($P = 0.41$, $n = 11$) and *I. aurelioi* ($P = 0.52$, $n = 11$). Considering the three Pyrenean species together to increase statistical power, and using H_o estimated from the loci shared among the three species, a significant decrease with elevation emerges ($P = 0.001$, $R^2 = 0.30$, $n = 32$; Fig. 7B). In *I. monticola*, H_e estimates obtained from microsatellites by Remón et al. (2013) do not associate with elevation ($P = 0.73$; Fig. 8C).

Finally, for nine localities of *I. bonnali* shared across both studies, our ddRAD-seq H_o estimates significantly co-varied with the microsatellite H_o estimates of Ferchaud et al. (2015) ($P = 0.02$, $R^2 = 0.52$, $n = 9$; Fig. S12), suggesting comparability between the genetic methods.

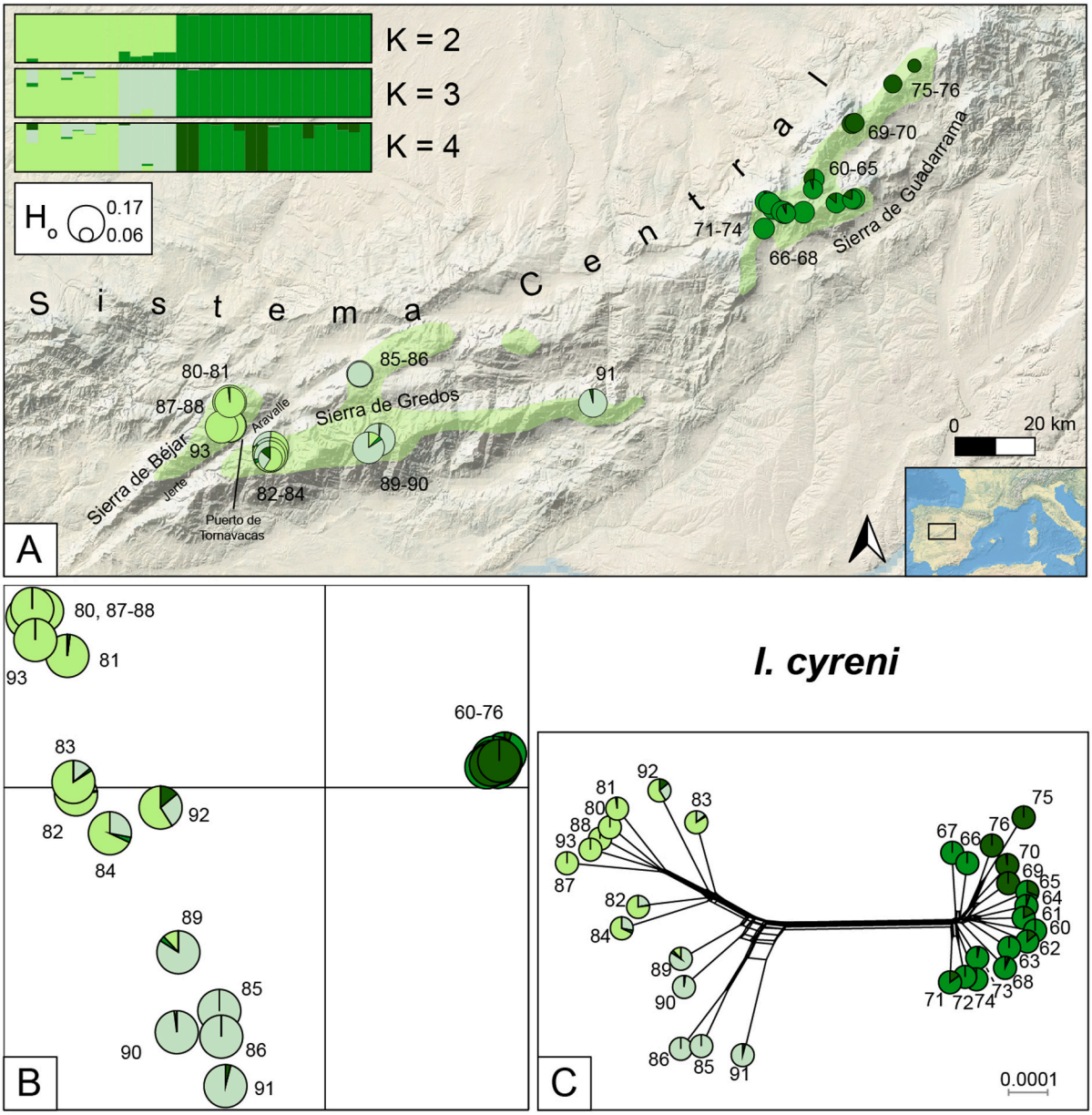

Fig. 6. Genetic diversity and structure in *Iberolacerta cyreni*. Analyses are based on 3975 SNPs genotyped in 31 samples. (A) Individual ancestry coefficients obtained by LEA for the best runs for K = 2–4 (barplots), geographic distributions of clusters (pie charts), and observed heterozygosity H_o (relative symbol size). (B) Scatterplots of the first two PCA dimensions. (C) Phylogenetic networks of relative sample divergence. Pie charts show the best K = 4 run; K = 2 has the lowest cross-entropy (= most meaningful structure solution) and K = 3–4 have cross-entropy lower than K = 1 (= more meaningful than no structure). Mountain ranges and other mentioned geographic places are labelled.

3.4. Projections of present and past distributions

Ecological niche models (ENMs) received high AUC (≥0.96) and TSS (≥0.88) scores, suggesting good predictive fit (Table S5). Accordingly, range projections under current bioclimatic conditions encompass most of the present distributions of species, and reveal additional potentially suitable areas (Fig. 9). The final models selected for each species, including their variable contributions, are detailed in Table S5. Models retained from 5 to 11 variables, some being shared by most models (bio2, bio4, bio8, bio9, and bio15).

LGM range projections revealed vast areas of bioclimatic suitability for the Iberian species *I. cyreni*, *I. galani*, *I. monticola*, as well as the Pyrenean *I. bonnali* (Fig. 9), which were larger by several orders of magnitude relative to the projections under current conditions (Fig. 10). According to the LGM projections, *I. cyreni* could have potentially reached the micro-endemic *I. martinezricai*, *I. galani* could have been in contact with *I. monticola astur*, and *I. monticola* could have been continuously distributed across northwestern Iberia (Fig. 9). The projection aggregating all species also suggested large areas of potential presence (Fig. 9). In contrast, more restricted glacial ranges are suggested for the Alpine *I. horvathi*, namely near its southern Adriatic boundary, and no suitable areas are identified for the Pyrenean *I. aranica* and *I. aurelioi* (Fig. 9). These predicted absences should be considered with caution given that the projections are constrained by the species currently realized bioclimatic niche.

Potentially suitable areas during the last interglacial period are close to the currently suitable areas for *I. cyreni*, *I. aurelioi*, *I. horvathi*, and to some extent, *I. galani* (with low probability estimates), while no areas

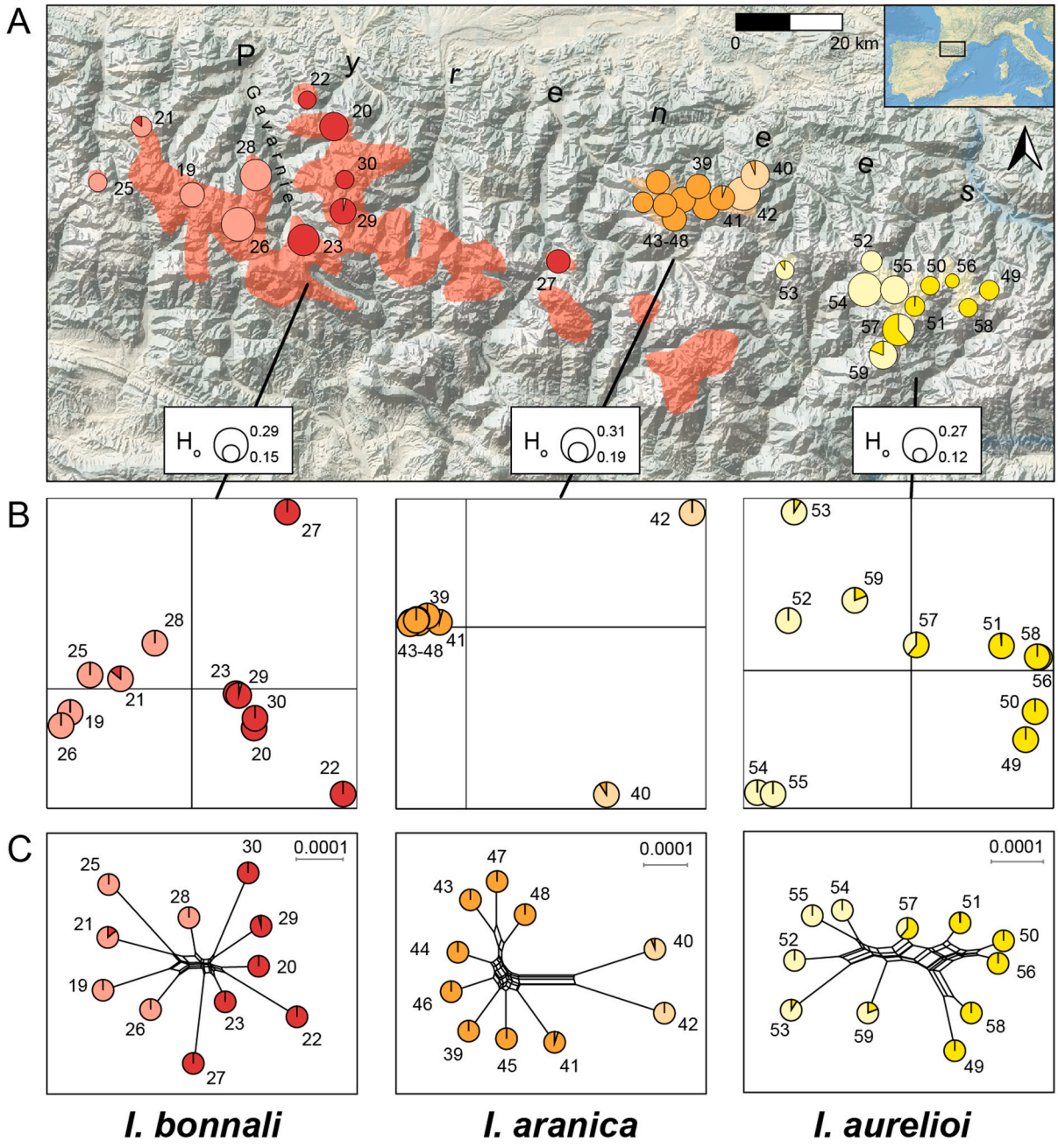

Fig. 7. Genetic diversity and structure in the three Pyrenean species. Analyses are based on 1967 SNPs genotyped in 11 samples for *I. bonnali*, 8502 SNPs genotyped in 10 samples for *I. aranica*, and 3971 SNPs genotyped in 11 samples for *I. aurelioi*. (A) Geographic distribution of clusters obtained by LEA (pie charts) and observed heterozygosity H_o (relative size). (B) Scatterplot of the first two PCA dimensions. (C) Phylogenetic network of relative sample divergence. Pie charts show the best K = 2 runs, although K = 1 (no structure) yielded the lowest cross-entropy in all cases.

could be identified for *I. monticola*, *I. bonnali* and *I. aranica* (Fig. 9). Accordingly, the LIG projections generally suggest more restricted ranges than the LGM projections (Fig. 10).

Discussion

4.1. Phylogeographic signatures of dynamic mountain diversifications

Phylogenomic analyses of *Iberolacerta* largely corroborate the mitochondrial phylogeny, both in terms of the general tree topology and the relative divergence of species. We confirmed the early divergence of rock lizards in the eastern Alps – preceding the diversification of the extant Iberian species (see also Garcia-Porta et al., 2019) – which was not always recovered by earlier mitochondrial phylogenies based on shorter sequences (Carranza et al., 2004; Crochet et al., 2004). According to the results, the evolutionary history of the genus thus follows a pattern of progressive restriction of ancestral populations to major systems: the Alps (*I. horvathi*), the Pyrenees (*I. aurelioi, I. bonnali, I. aranica*), the Sistema Central mountains of Iberia (*I. cyreni*), and finally, the Cantabrian, Asturian and León mountains in northwestern Spain (*I. monticola* and *I. galani*). Evidence of subsequent dispersal events between these ranges is reflected by *I. martinezricai*, endemic to Peña de Francia in the Sistema Central, yet phylogenetically related to the northwestern Iberian species, as well as by the southernmost occurrence of *I. monticola* in the Serra da Estrela (subspecies *I. m. monticola*) (Fig. 5). These patterns suggest long-term isolation between the Alpine/Dinaric,

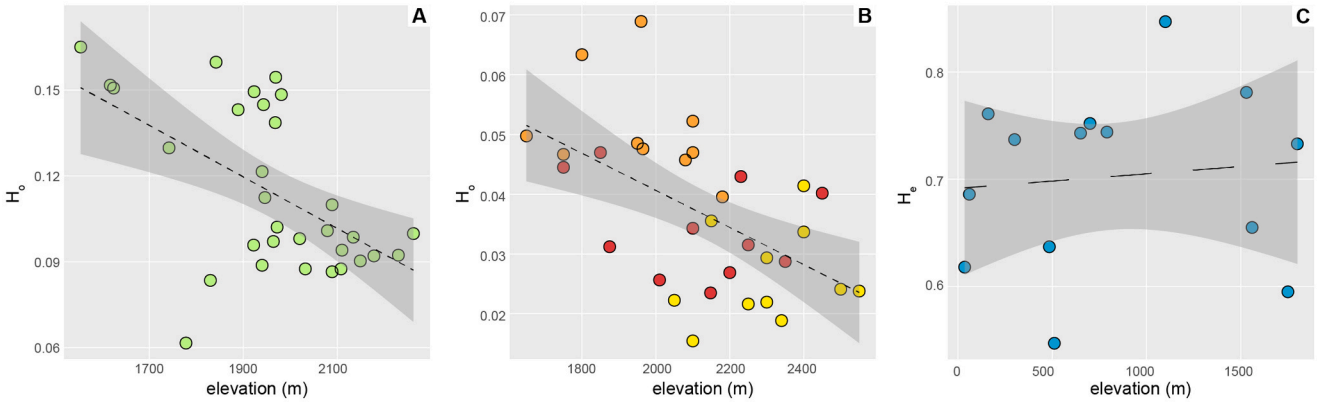

Fig. 8. Relationship between heterozygosity and elevation in *Iberolacerta* species. (A) In *I. cyreni* (green), based on the observed heterozygosity (H_o) computed from 3,975 SNPs genotyped in 31 samples. (B) Among the Pyrenean species *I. aranica* (orange), *I. bonnali* (red) and *I. aurelioi* (yellow), based on H_o computed from 3236 loci genotyped in 32 samples; (C) In *I. monticola* (blue), based on expected heterozygosity (H_e) computed from 10 microsatellite loci genotyped in 14 populations by Remón et al. (2013). Linear regressions are shown with their confidence interval; tight and loose dash lines indicate significance and non-significance based on log–log regressions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pyrenean and Iberian ranges, but episodic connections between the Sistema Central and northwestern Iberia during the Quaternary.

Differences in the topology and divergence times retrieved for the Western Iberian clade across mitochondrial, concatenated ddRAD, and multispecies coalescent analyses highlight the complementary strengths of each approach. The species-tree results, which show partial discordance in the placement of *I. monticola*, *I. martinezricai*, and *I. galani*, likely reflect both model sensitivity to historical interspecific gene flow (Mars et al., 2025) – *I. martinezricai* seemingly having introgressed with *I. cyreni* (Fig. 4) – and inherent differences in how coalescent vs. concatenation models handle incomplete lineage sorting. This underscores the value of integrating multiple phylogenetic frameworks when reconstructing complex evolutionary histories, and cautions against over-interpreting divergence times – not only because of uncertainties in calibration settings, but also when estimates are derived from a single analytical framework.

The drivers of speciation in *Iberolacerta* have been widely discussed in previous studies. A commonly invoked hypothesis posits competition with lowland lacertid lizards, which may have confined *Iberolacerta* to isolated high-elevation habitats (Carranza et al., 2004; Jiménez-Robles & De la Riva, 2019). In parallel, the Alpine orogeny (Eocene–Miocene, ca. 40–20 Ma), which formed the Pyrenees, Cantabrian, and Central Iberian ranges, yielded extensive montane environments and fragmented the western European landscape (Muñoz 1992, De Vicente et al., 2007, Teixell et al., 2016). Our molecular dating places the crown diversification of *Iberolacerta* in the Miocene, coinciding with these orogenic phases, suggesting that topographic uplift and associated habitat heterogeneity provided the initial geographic and ecological template for the divergence of ancestral lineages. Evolution within these mountain systems likely promoted adaptation to cold, alpine environments, reinforcing isolation and enabling persistence in montane refugia independently of competitive exclusion (Monasterio et al., 2010, Ortega et al., 2016a, 2016b). Subsequently, Pliocene and Quaternary climatic oscillations, together with continued minor uplift in the Sistema Central and the Alps (Andrés et al., 2019; Nocquet et al., 2016) – but seemingly not in most of the Pyrenees (Teixell et al., 2016; Bosch et al. 2016; Ford et al., 2022) – likely amplified population isolation, fostering intraspecific diversification and shaping the current pattern of mountain micro-endemism.

In the Pyrenees, the nuclear and the mitochondrial trees are discordant in respect to the first species to have diverged i.e., *I. aurelioi* in the nuclear trees and *I. aranica* in the mtDNA tree (but see e.g., Carranza et al., 2004; Garcia-Porta et al., 2019). Given that i) the topology of the Pyrenean clade is not robustly supported in the mitochondrial phylogeny, ii) no historical nuclear gene flow was detected among these species, and iii) all phylogenomic analyses – either based on concatenated or multispecies coalescent approaches – were congruent, this pattern probably results from a lack of resolution of the mtDNA sequences to resolve the divergence events (which seemingly occurred within a short evolutionary timespan), rather than ancestral hybridization. The fact that all three species have remained evolutionarily independent suggests that they have continuously subsisted in disconnected ranges, or alternatively, that reproductive barriers have effectively prevented introgressive hybridization if their ranges did overlap in the past. At the intraspecific level, the Pyrenean species are genetically homogenous, and their genetic variation is essentially mediated by isolation by distance (IBD), implying a unique origin for each of them, e.g., from single glacial refugia. This is not unexpected for the geographically restricted *I. aranica* and *I. aurelioi*, and it was previously hypothesized for the more widespread *I. bonnali*, whose main distribution revolves around a single network of valleys (Gavarnie) (Ferchaud et al., 2015). Nevertheless, the western group of isolated *I. bonnali* populations features a distinct mitochondrial lineage that could reflect a separate Pleistocene refugium (Arribas et al., 2006; 1.4 Mya of divergence in our timetree), which should be examined with nuclear loci. Accordingly, the LGM projections implied potentially large suitable glacial ranges for *I. bonnali*, but no clear glacial refugium for *I. aranica* and *I. aurelioi*, which could indicate that the microclimate space currently occupied by these species is not well represented in the relatively low-resolution climate layers used in the ENMs, or that these species may be capable of tolerating a broader spectrum of bioclimatic conditions than their present realized niche suggests.

Several other species feature geographically segregated mitochondrial lineages, namely in the eastern Alps and the Dinarides for *I. horvathi* (Cocca et al., 2021), in the Galician, Asturian, León and Cantabrian ranges for *I. monticola* (Remón et al., 2013), and in the Sierra de Béjar, Sierra de Gredos and Sierra de Guadarrama for *I. cyreni* (this study) (Figs. 2 and 5). While the nuclear diversity of these species broadly associates with the mitochondrial diversity, it also largely reflects IBD, thus implying range-wide gene flow despite the local population disconnections and the phylogeographic breaks suggested by mtDNA. Nearly all the genomic variance in *I. cyreni* was explained by IBD, and some of its defined clusters show widespread admixture, e.g., between Sierra de Béjar and Sierra de Gredos, even though these massifs are now isolated by the Aravalle and Jerte river valleys and the Puerto de Tornavacas mountain pass (Fig. 6). The lower diversity retrieved in Sierra de Guadarrama could likewise suggest an historical eastward colonization from Sierra de Gredos, after which the intermediate populations in the Alberche valley (the lowland corridor between these mountains) disappeared. In fact, a similar pattern was documented in

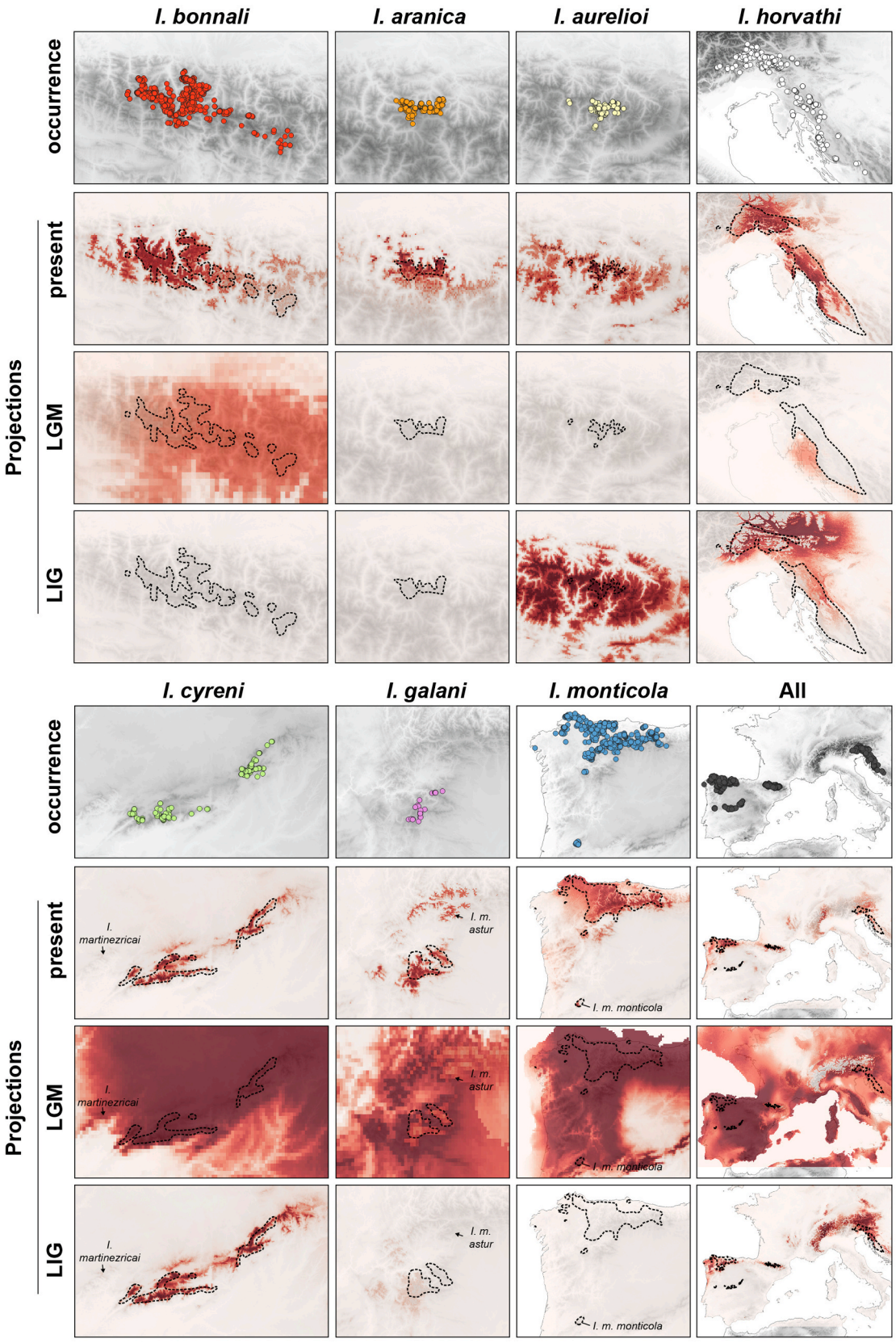

Fig. 9. Occurrence datasets and projected distributions of *Iberolacerta* species under current (present), last glacial maximum (LGM) and last interglacial (LIG) conditions. Bioclimatic suitability is reflected by the same color gradient (white: 0; dark red: 1). Current distributions are emphasized by dash lines. Background shading and lines reflect topography and coastlines. For some species, the present-day ranges of relevant additional taxa are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

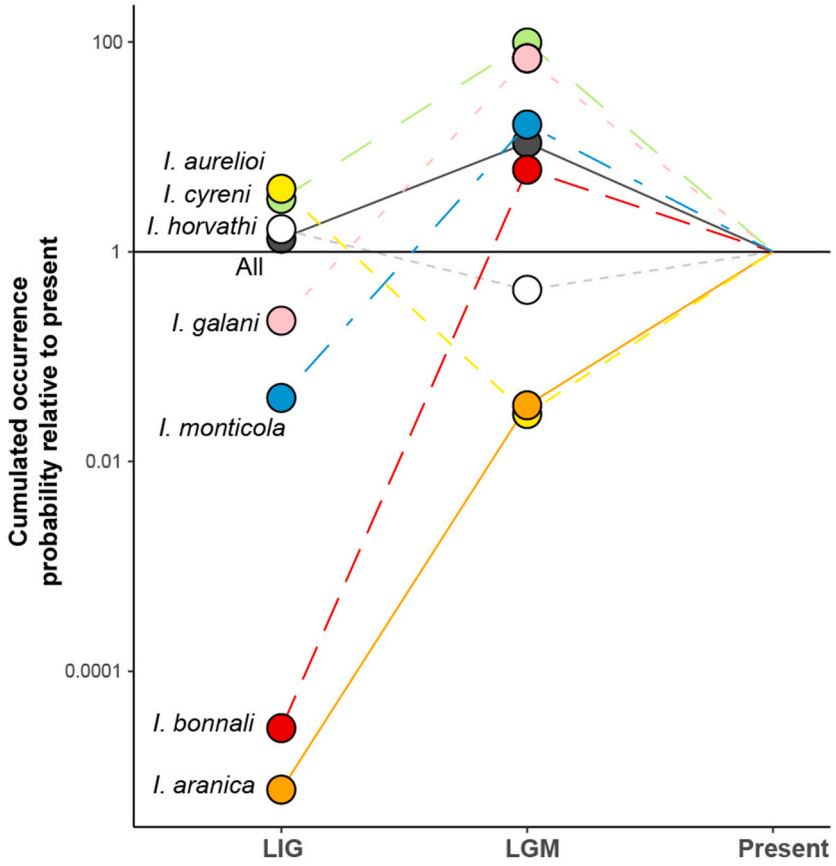

Fig. 10. Relative changes in the projected ranges of *Iberolacerta* between the last interglacial (LIG), the last glacial maximum (LGM) and present conditions. A log-scaled y-axis is used to facilitate comparison between species.

the co-distributed Iberian frog *Rana iberica* (Martínez-Solano et al., 2005) and the fire salamander *Salamandra salamandra* (Antunes et al., 2021). Using microsatellites, Remón et al. (2013) also reported overwhelming IBD ($r \geq 0.8$) for the main range of *I. monticola* (the Cantabrian and Asturian mountains), namely across the allopatric distributions of two of its mitochondrial lineages (*monticola* C and A). In *I. horvathi*, the nuclear fragment (MC1R) analyzed by Cocca et al. (2021) features closely related haplotypes (single mutational steps) across the whole range, with their respective distributions roughly matching the main mtDNA haplogroups, which is in principle also consistent with a single evolutionary lineage which diversity is segregated by geography. Consistent with this hypothesis, the projected LGM range of *I. horvathi* indicates a single refugial area, located close but outside of the southern edge of the current distribution, where the genetic diversity of the species is higher (Cocca et al., 2021) – noting again that the spatial resolution used for the ENMs may not suffice to capture the spatial complexity of the microclimates favored by the species.

These observations are in line with the expectation that mountain populations may have retained some historical connectivity that has potentially homogenized their genomes. The phenomenon is more obvious from nuclear than mitochondrial loci due to differences in effective sizes, especially when dispersal is male-biased, in which case female-inherited mitochondrial divergence can persist even when nuclear gene flow is pervasive (Toews & Brelsford, 2012). In polygynous lizards, gene flow is expected to be male-biased, which was shown in some species (Johansson et al., 2008), and was indirectly suggested for *Iberolacerta* based on the size of home ranges (Aragón et al., 2001; Remón et al., 2013). If females do disperse less than males, the mitochondrial lineages thus may have potentially accumulated the divergence of several episodic interglacial range fragmentations, even though that divergence may have been repeatedly diluted at the nuclear level by gene flow during the glacial stages via lowland refugial connections. In parallel, some of the intraspecific mtDNA diversity seems to reflect real phylogeographic breaks, as seen from the micro-endemic *I. m. astur* (Arribas et al., 2014), although this remains to be confirmed by phylogenomic data.

A compelling example of former connection and subsequent range shrinkage is the lack of mitochondrial divergence (and weak microsatellite differentiation) between the southernmost population of *I. monticola* (subspecies *I. m. monticola* in Serra da Estrela) and the northwestern Spanish populations (*I. m. cantabrica*), despite a distributional gap of several hundred kilometers (Remón et al., 2013). This pattern indicates the presence of *I. monticola* across Galicia and northern Portugal until very recently, followed by a generalized range retraction. Accordingly, our projections suggested a drastic increase in the potential area occupied by this species during the LGM compared to the previous interglacial stage and the present-day conditions (as also retrieved by Sousa-Guedes et al., 2020), and molecular analyses inferred genetic bottlenecks in several of its isolated northwestern populations, indicating recent population declines (Remón et al., 2013).

Shared historical connections are further highlighted from signatures of past interspecific hybridization between two pairs of nearby species. First, we traced nuclear alleles derived from *I. cyreni* into *I. martinezricai*, implying a secondary contact between their adjacent massifs. Second, past mitochondrial introgression was documented in *I. monticola* from Montes de León (subspecies *I. m. astur*), which acquired variants from *I. galani*, suggesting that these species were once connected across the León massif (Arribas et al., 2014), namely during the Middle Pleistocene, according to the age of captured mtDNA. The past range projections of these species accordingly predicted potentially overlapping

distributions during glacial stages but range fragmentation during the interglacial stages. However, unlike the intraspecific instances of gene flow, here the species maintained most of their genetic integrity, either because the former contacts were occasional, i.e., species had time to purge signs of introgression during periods of allopatry, or due to the limited occurrence and success of hybridization caused by pre- and post-zygotic barriers, respectively.

Patterns of diversification and shifts in suitable bioclimatic conditions in rock lizards thus imply dynamic biogeographic processes in which recurrent secondary contacts alternate with population divergence as ranges expanded and retracted with the climatic changes. In turn, the high historical but contemporarily limited dispersal leads to weak phylogenetic divergence, despite strong genetic structure at local scales. Accordingly, molecular studies on mountain herpetofauna typically retrieve shallow mitochondrial divergence but substantial population differentiation, as measured from F-statistics computed from microsatellites or RFLP markers, e.g., in the Pyrenean brook newt *Calotriton asper* (Milá et al., 2010), the midwife toad *Alytes obstetricans* in the Pyrenees (Lucati et al., 2022), the vipers *Vipera ursinii* and *V. berus* in the western Alps (Ursenbacher et al., 2009; Ferchaud et al., 2011), and the Alpine salamander *Salamandra atra* in the Dinarides (Razpet et al., 2016; Šunje et al., 2021). These phylogenetic and population genetics results should not be viewed as contradictory, as they reflect distinct processes acting at different timescales (evolutionary divergence vs. ongoing demographic dynamics), even more so when the compared markers differ in their mode of inheritance and effective size (mitochondrial vs. nuclear DNA). In this respect, genomic approaches appear useful to determine whether the nuclear differentiation of populations reflects long-term evolutionary divergence or recent population fragmentation (as indicated by distinct or shallow phylogenetic clades), as well as to verify that the mitochondrial diversity corresponds to extant phylogeographic diversity. An extreme but increasingly reported form of cyto-nuclear discordance are the so-called “ghost” lineages, i.e., mitochondrial lineages initiated by past allopatric divergence and that continue to persist long after the corresponding populations have merged back their nuclear genomes, sometimes reaching species-level divergence and mimicking deep phylogeographic breaks (Hinojosa et al., 2019; Dufresnes et al., 2020). Several such cases have accordingly been identified in mountain herpetofauna of the Iberian Peninsula, especially in Cantabria (Dufresnes et al., 2020) and the Sistema Central (Ambu et al., 2025). This could be the fate of some of the mitochondrial lineages retrieved in *I. cyreni* and *I. monticola*, unless nuclear gene flow becomes weaker during future glacial periods (promoting the evolutionary divergence of populations) or female dispersal eventually homogenizes the matrilineal diversity.

4.2. Elevational bottlenecks

The observed decrease of heterozygosity with elevation in *I. cyreni*, and generally among the three Pyrenean species, is consistent with founder effects following post-glacial elevational shifts – in a similar fashion to that the temperate fauna of Europe features depauperated diversity at northern latitudes due to expansions from southern Mediterranean refugia (Schmitt, 2007). The narrow elevational ranges of each Pyrenean species could explain, besides sample size, why the relationships between elevation and H_o are not significant when calculated separately. Rather than post-glacial upward expansions, it is also possible that the lower diversity of the highest populations reflects their smaller population sizes, making them more susceptible to drift, especially if they suffer frequent declines due to more barren environmental conditions. These hypotheses could be tested with F-statistics, notably inbreeding coefficients, by genotyping multiple individuals per population. In contrast, the lack of association between genetic diversity and elevation in *I. monticola* (based on the microsatellite results of Remón et al., 2013) may reflect different processes, such as recent declines in the lowland populations (which are threatened by habitat fragmentation linked to human-induced landscape changes; Remón et al., 2013), or that even the highest populations of that species are not necessarily subjected to stronger drift, given these are still situated at lower elevations than the lowest populations of *I. cyreni*, *I. aranica*, *I. bonnali* and *I. aurelioi*. In other mountain herpetofauna, elevation does not always affect genetic diversity, having a significant effect in some cases (e.g., Preißler et al., 2020; Van Buskirk & Jansen van Rensburg, 2020), but not in others (e.g., Razpet et al., 2016; Dufresnes et al., 2022).

Whatever the cause for their elevated diversity, low and mid-elevation populations of Central Iberian and Pyrenean rock lizard species are also those most threatened by climate change, as increasingly warm conditions are expected to make their habitats less suitable and/or favor other competing and potentially more adaptable species (e.g., *Podarcis* wall lizards) in the near future. If so, the adaptive potential of *Iberolacerta* species could progressively erode, as it would rely only on the genetic diversity of the high elevation depauperated populations. Alternatively, physiological responses to environmental changes may rely on phenotypic plasticity, which was shown to be markedly high for high-elevation specimens of *I. cyreni* experimentally translocated downhill (Megía-Palma et al., 2020). Genetic diversity is being lost globally (Shaw et al., 2025) but is not sufficiently captured by current metrics of population threats, as currently used in Red List assessments (Schmidt et al., 2023).

Data Accessibility

The data used in this study is archived on NCBI SRA under BioProject PRJNA949685 (demultiplexed ddRAD-seq reads; accessions SRR35210073–SRR35210154), GenBank (ND4 sequences; accessions PX251204–PX251228), and Zenodo (doi: 10.5281/zenodo.17037714; SNP matrices and sequence alignments).

CRediT authorship contribution statement

Christophe Dufresnes: Writing – original draft, Visualization, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sven Gippner:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Sylvia Hofmann:** Formal analysis, Writing – review & editing. **Spartak Litvinchuk:** Writing – review & editing, Investigation, Formal analysis. **Anamarija Žagar:** Writing – review & editing, Resources, Data curation. **Daniel Jablonski:** Writing – review & editing, Resources, Investigation, Conceptualization. **Gilles Pottier:** Writing – review & editing, Resources, Investigation. **Rodrigo Megía-Palma:** Writing – review & editing, Resources, Investigation. **Gregorio Sánchez-Montes:** Writing – review & editing, Resources, Investigation. **Octavio Jiménez Robles:** Writing – review & editing, Resources, Investigation. **Enrique Ayllón:** Writing – review & editing, Resources, Investigation. **Pierre-André Crochet:** Writing – review & editing, Resources, Investigation. **Íñigo Martínez-Solano:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108502.

Data availability

The data has been uploaded on appropriate online archives, with accessions and doi provided in the manuscript

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