



Genetic diversity of Horvath's Rock Lizard meets current environmental restrictions

Walter Cocca¹ · Anamarija Žagar^{1,2} · Neftalí Sillero³ · Michael J. Jowers¹ · Miha Krofel⁴ · Martina Lužnik⁵ · Martina Podnar⁶ · Nikola Tvrković⁷ · Miguel A. Carretero^{1,8} · Angelica Crottini^{1,8}

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Abstract

The Horvath's rock lizard *Iberolacerta horvathi* (Méhely, 1904) is an understudied lacertid species, which is geographically isolated from its congeners and currently classified as Near Threatened under IUCN red listing criteria. Due to its limited distribution and narrow environmental preferences, we expect that the species had been affected by past climatic oscillations. By combining all available data on species occurrences and our own records, we produced an updated distribution map. We used ecological niche models to identify the current environmental factors underlying the species range and developed a habitat suitability map. We sequenced one mitochondrial and one nuclear marker to characterize the distribution of the genetic variability and infer the historical demography of the species. The habitat suitability map identified areas where targeted field searches should be prioritized, as well as unsuitable habitats coinciding with likely barriers to gene flow. We found considerable genetic variability suggesting that the species probably survived the Pleistocene glaciations in at least two main refugia, one in the South and the other in the central/northern portion of its distribution. While southern populations show a moderate demographic decrease starting at the onset of the Eemian interglacial, the northern populations underwent an expansion during Late Pleistocene. We provide a revised species distribution and a first characterization of its genetic variability across its distribution to guide conservation priorities for this endemic and spatially restricted species.

Keywords *Iberolacerta horvathi* · Balkans · Phylogeography · Species distribution · Habitat suitability · Pleistocene refugia

Introduction

The Mediterranean peninsulas are well known to have acted as glacial refugia and promoted intraspecific diversity during the Pleistocene climatic oscillations (e.g., Hewitt 1996, 2011; Stewart 2009). In contrast, central and northern

Europe were predominantly re-colonized during interglacial periods (e.g., Taberlet et al. 1998; Hewitt 2000, 2004). Reptiles are ectotherms with often limited dispersal abilities and viable populations frequently survive in small refuges (e.g., Avise 2000; Zeisset and Beebe 2008). Therefore, by characterizing their genetic diversity it is possible to infer events of isolation, dispersal and admixture, gaining insights into

Walter Cocca and Anamarija Žagar have contributed equally to the development of this study and the preparation of the manuscript.

✉ Walter Cocca
walter.cocca85@gmail.com

- ¹ CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, No. 7, 4485-661 Vairão, Portugal
- ² NIB National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
- ³ CICGE: Centro de Investigação em Ciências Geo-Espaciais, Faculdade de Ciências da Universidade do Porto, Alameda do Monte da Virgem, 4430-146 Vila Nova de Gaia, Portugal

- ⁴ Department of Forestry and Renewable Forest Resources, Biotechnical Faculty, University of Ljubljana, Večna pot 83, 1001 Ljubljana, Slovenia
- ⁵ Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška 8, 6000 Koper, Slovenia
- ⁶ Croatian Natural History Museum, Demetrova 1, 10000 Zagreb, Croatia
- ⁷ Natura, Society of Nature Protection, Alagovićeva 21, 10000 Zagreb, Croatia
- ⁸ Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre, s/n, 4169-007 Porto, Portugal

species-specific responses to paleogeography and paleoclimatology (e.g., Hewitt 2000). In the Mediterranean peninsulas some species remained in their refugia during climatic oscillations (e.g., *Dalmatolacerta oxycephala*; Podnar et al. 2014), others dispersed through postglacial colonization and re-colonization events and undergone lineage admixture (e.g., *Coronella austriaca*; Sztencel-Jablonka et al. 2015), while others dispersed from one or multiple refugia (e.g., Crottini et al. 2007; Canestrelli et al. 2007, 2008), micro-refugia (e.g., Rull 2009), persisted in northern refugia (e.g., Salvi et al. 2013), in coastal refugia (e.g., Salvi et al. 2014) or persisted in refugia-within-refugia (e.g., Gómez and Lunt 2007; Maura et al. 2014). The latter scenario implies the survival of isolated populations in separate glacial refugia of suitable habitats included within a larger refugial area (Gómez and Lunt 2007; Abellán and Svenning 2014).

Why species follow a given pattern may depend on many factors including habitat, physiology, dispersal capacity and competitive abilities, as well as past and current topography and climatic conditions. Whatever the case, understanding how the genetic diversity is spatially distributed across a species' range allows us to determine areas of historical stability and admixture, identifying evolutionary significant units (ESUs), and to predict those species which will be more threatened by global change, all aspects of paramount importance to define conservation priorities (Crandall et al. 2000).

Within the Palearctic radiation of the Lacertini (Squamata: Lacertidae), the phylogenetic position of the lizard genus *Iberolacerta* Arribas 1999 has remained unresolved although recent phylogenomic evidence suggest a distant relationship with the Peloponnese endemic *Hellenolacerta* (García-Porta et al. 2019). Eight species are currently recognized (Uetz et al. 2019), seven distributed in the Pyrenees and in the northern and central mountains of the Iberian Peninsula, and one species distributed in South-eastern Europe: the Horvath's Rock lizard, *Iberolacerta horvathi* (Méhely, 1904) (Gasc et al. 1997; Arnold et al. 2007; Sillero et al. 2014). The phylogenetic relationships of *I. horvathi* within the genus *Iberolacerta* remain uncertain (Carranza et al. 2004; Crochet et al. 2004; Arnold et al. 2007).

Iberolacerta horvathi's distribution, intraspecific genetic diversity and potential threats are poorly described, although it is known to occur in several protected areas across its distributional range (e.g., National Parks in Slovenia and Croatia, regional parks in Slovenia and Italy, a Natural Park in Austria, several Natura 2000 sites and other local reserves in all four countries). *Iberolacerta horvathi* is currently classified as Near Threatened under IUCN Red listing criteria, and the isolation of the different populations is assumed to be the main threat to this species (Vogrin et al. 2009). However, focused research is required to confirm this assumption and to identify other potential threats and how these might vary across the species distribution (Vogrin et al. 2009).

Current biogeographic data indicate that the species is distributed in four countries: Italy, Austria, Slovenia and Croatia (Sillero et al. 2014), where it occupies a relatively small range in the eastern Alps and in the northern-west Dinaric Mountains, showing an increase in abundance with altitude (e.g., De Luca 1989; Žagar et al. 2013). The species inhabits rocky substrates with sparse vegetation, although it can also be found in the clearings of densely forested areas at low elevations (Arnold 1987; Arnold and Ovenden 2004; Arnold et al. 2007; Cabela et al. 2007; Žagar et al. 2013).

Due to these narrow habitat preferences and range, we expect that past climatic oscillations had a strong impact on the distribution and genetic variability of the species. In this study, we assembled all available information on *I. horvathi*'s distribution and combined it with our own distribution records to produce an updated map of occurrences. We used ecological niche models (Sillero 2011) to identify the current environmental factors underlying the species range and to obtain a map of suitable habitats. We characterized the phylogeographic structure and historical demography of the species and tested the role of possible barriers and paleoclimatic events in shaping the current genetic diversity of *I. horvathi*. The outcomes of this research will provide valuable information for an improved conservation assessment of the species, both in terms of defining its conservation status and potential threats.

Materials and methods

Distribution

To produce the updated map of species occurrence, we used locality data from several sources: (a) new locality records collected between 2006 and 2015 (N=32), resulting from surveys that included very remote places (which we believe significantly reduced sampling bias due to remoteness or sampling effort); (b) locality records from museum collections; (c) local and private databases; and (d) published literature (Fig. 1a; see Online Resource 1 for more details). Records with resolution higher than 1 × 1 km are presented as locality points and the remaining (resolution of 10 × 10 km) as UTM grid squares (N=388; see Online Resource 1 for more details). The population reported in Karwendel Gebirge in Southern Germany (Capula and Luiselli 1990) is strongly disputed (Schmidtler and Schmidtler 1996) and has not been recently confirmed (Cabela et al. 2004); thus, we did not include this record in the species distribution map. We also omitted the two southernmost points and one UTM square: one obtained from a specimen hosted in the collection of the Natural History Museum of Ljubljana (Durmitor, Zminje jezero; 43.1562 19.0713) with only partial collection information and in need of verification; two

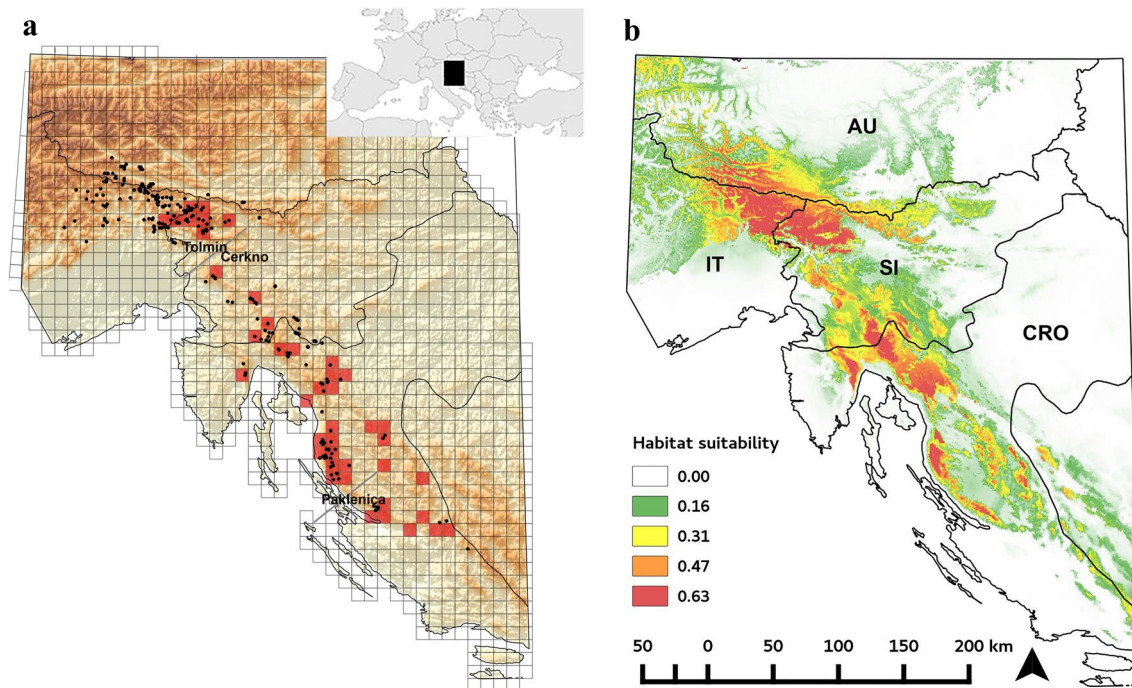


Fig. 1 **a** Updated occurrence map of *Iberolacerta horvathi* (N=388 records). Data of 10×10 km UTM precision is represented by red squares, higher precision data by black dots. The lines represent the

sampling gaps identified in this study. **b** Maxent model computed with presence records with 1×1 km (or higher) precision (N=343)

from the Croatian herpetological database [Biokovo, planinarski dom podno sv. Jure; 43.3497 17.0691 and the grid cell WJ73, corresponding to the unverified locality “Šibenik” from Pozzi (1966) for which De Luca (1989) noted no voucher evidence]. These localities have not been recently confirmed (e.g., Jelić et al. 2015) and are distant from the currently known species’ distribution; therefore they have been conservatively excluded from this compilation.

Ecological niche modelling

For the realised niche model (RNM; sensu Sillero 2011), we used only localities with a resolution of 1×1 km or higher (N=343, Online Resource 1). We delimited the study area for the RNM using the criteria of Anderson and Raza (2010): we excluded any area where the species cannot disperse, based on our expert opinion. The study area includes the south-eastern part of Alps and the north-western part of the Dinaric Mountains, covering an area of 324,809 km² (Fig. 1b). The study area encompasses a wide array of climate regimes, influenced by the Adriatic Sea and by the large elevational differences of the heterogeneous topography (the highest in the Alpine area). Two major climatic areas can be distinguished: (1) the Mediterranean climate extending along the coast of the Adriatic Sea, characterized by either dry or humid months during the summer and mild rainy winters; and (2) the Alpine climate of the pre-Alpine

and Alpine area with relatively high precipitation year around and cool winters (Flohn and Fantechi 1984).

To describe this environmental diversity, we downloaded climatic data from WorldClim 1.4 (Hijmans et al. 2005; <http://www.worldclim.org/>) with a resolution of 1×1 km, and selected seven bioclimatic variables with a Pearson correlation lower than 0.75 (Table 1). We calculated the RNMs with Maximum Entropy (Maxent 3.4.1 software; www.cs.princeton.edu/~schapire/maxent), a general-purpose machine learning method that uses presence-only occurrence and background data (Phillips et al. 2017). Maxent does not use pseudo-absence data (Guillera-Arroita et al. 2014). Maxent is particularly well suited to noisy or sparse information, and works with continuous and categorical variables (Phillips et al. 2004, 2006; Phillips and Dudík 2008). We used default settings to select 10,000 randomly placed background points from the entirety of the delimited study area (324,809 km² rectangular area seen in Fig. 1b). 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 (e.g., maximizes entropy for a given chi-squared value). 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 1×1 km; thus,

Table 1 List of climatic variables with a Pearson correlation lower than 0.75 obtained from WorldClim data series (Hijmans et al. 2005; <http://www.worldclim.org/>) and their contributions and permutation values in Maxent models of *Iberolacerta horvathi*

Variables	Contributions (%)	Permutations (%)
Precipitation of wettest quarter	39.4	19.7
Altitude	30.1	39.9
Mean temperature of driest quarter	15.0	17.5
Precipitation of driest quarter	4.7	2.1
Precipitation of coldest quarter	4.2	9.9
Temperature annual range	3.4	1.3
Mean temperature of wettest quarter	3.2	9.7

only one point per 1 km 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. The model was replicated ten times as Maxent is a machine learning algorithm with a probabilistic component. Therefore, we calculated the arithmetic mean and the standard deviation of a set of ten models through an iterative process. Maxent identified the importance of each environmental variable as follows: (1) jack-knife analysis of the average AUC with training and test data; and (2) average percentage contribution of each variable to the models (Online Resource 2). 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 receiver operating characteristics (ROC) plots. The area under the curve (AUC) of the ROC plot was taken as a measure of the overall fit of the models (Liu et al. 2005). Random models have an AUC equal to 0.5; the closer to an AUC of 1, the better the model is. AUC was selected because it is independent of prevalence (the proportion of presence in relation with the total dataset size; VanDerWal et al. 2009; but see Lobo et al. 2008). We calculated a set of null models in R (R Core Team 2020) for each modelling method, following Raes and Steege (2007). For this, we created 100 different datasets with 50 random points following a Poisson distribution. We calculated a RNM with each dataset and obtained the AUC values. Therefore, AUC were compared between the ten Maxent models and the null models.

Characterization of species distribution area

We delimited the area of habitat suitability of the species based on the predictions by the Maxent model where the model values were above the threshold of maximum training sensitivity plus specificity logistic threshold (0.3206) (Online Resource 3). This area was used to clip the 1 × 1 km raster of the model for the three ecogeographical variables

contributing the most to the model: precipitation of wettest quarter of the year, altitude, and mean temperature of driest quarter of the year (Table 1). The clipped raster contained 19,513 1 × 1 km raster cells that were used as values to describe the environmental conditions of species occurrence.

Tissue samples, DNA extraction and sequencing

We collected 110 samples from 24 localities covering almost the entire species range (Figs. 2, 3; Table 2; Online Resource 4). Tail tips were collected opportunistically from 2010 to 2015 during several field expeditions across the pre-Alps and Alps in north-eastern Italy and Northern Slovenia, and across the Dinaric Mountains from Southern Slovenia to Croatia and several tissue samples were obtained from museum collections (see Online Resource 4 for more details). Samples were stored in 96% EtOH.

Total genomic DNA was extracted using a proteinase K (10 mg/μL) digestion following a standard saline method (Bruford et al. 1992). PCR was used to amplify a fragment of the mitochondrial cytochrome b (Cytb) gene of ca. 600 nucleotides and a fragment of the exonic region of the nuclear melanocortin 1 receptor (MC1R) gene of ca. 700 nucleotides. MC1R has been previously used to infer phylogeographic patterns in multiple other lacertids and it provided excellent phylogeographic resolution (e.g. Pinho et al. 2010; Buades et al. 2013; Salvi et al. 2014; Freitas et al. 2016). Primers and amplification conditions for both fragments are available in Online Resource 5. All fragments were sequenced using an ABI 3730XL automated sequencer at Macrogen Inc.

Sequence alignment and data analysis

Chromatograms were checked and sequences were edited, where necessary, and automatically aligned using the CLUSTAL option of the sequence alignment editor BioEdit (V.7.2.0; Hall 1999). Newly generated sequences were deposited in GenBank (Cytb: MN096384–MN096493; MC1R: MN096494–MN096546; Online Resource 4). We determined the number, nature and distribution of base substitutions using DnaSP (V6; Rozas et al. 2017) for both fragments. No stop codons or indels were detected in the Cytb and MC1R sequence datasets. We computed nucleotide (Π , the average proportion of nucleotides that differ from random pairs of sequences) and haplotype (Hd, the gene diversity providing the probability that two random sampled alleles are different) diversities (Nei 1987) for each locality and main group (Northern group and Southern group; see SAMOVA for more details) and for both fragments (Table 2).

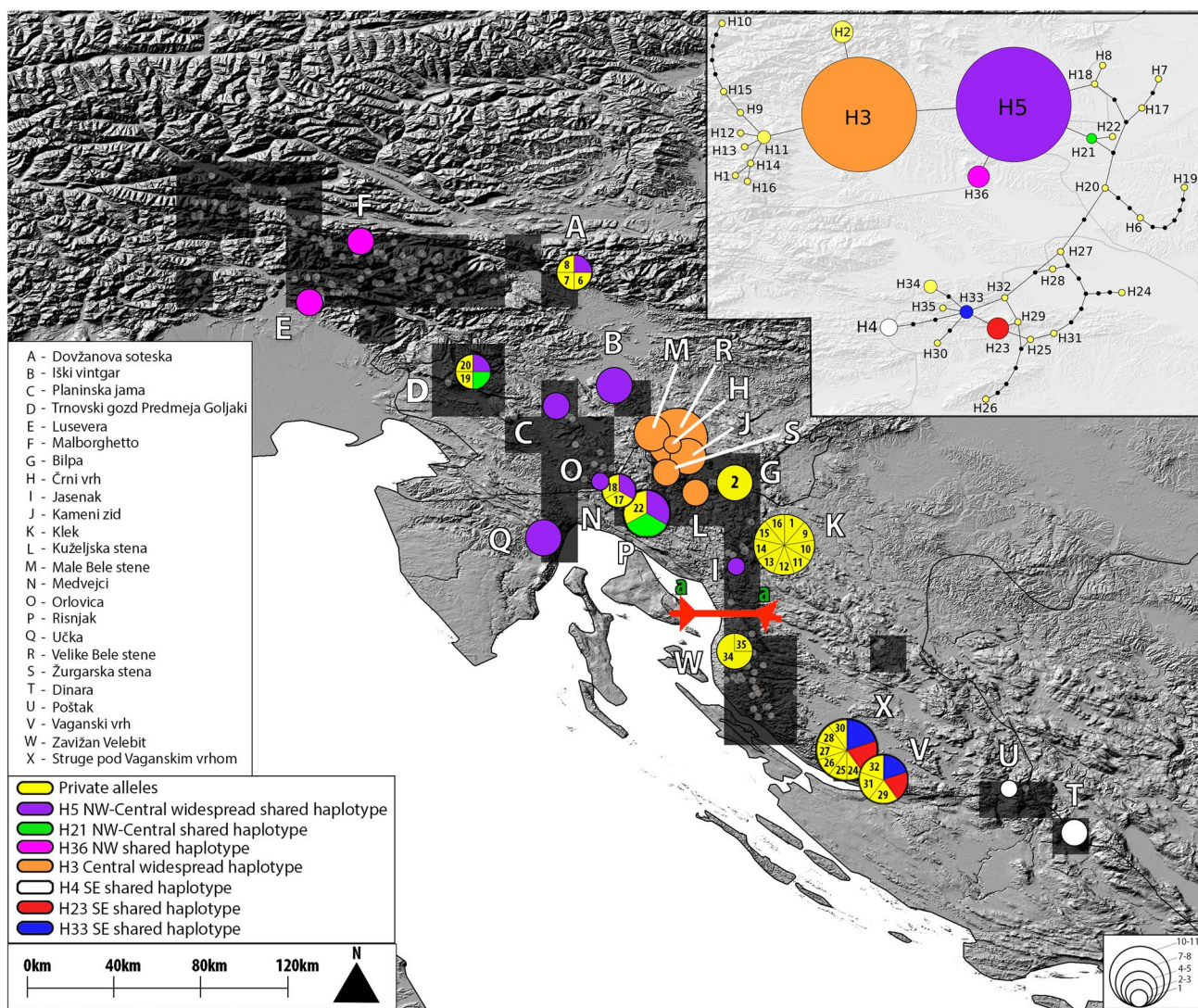


Fig. 2 Haplotype network reconstruction of the Cytb mitochondrial fragment and geographical distribution of haplotype frequencies across the analyzed sampled localities of *Iberolacerta horvathi*. The dark-grey squares and the small light-grey circles represent the updated distribution of the species. In the network, the black dots

represent extinct or unsampled haplotypes. The red symbol represents the most likely barrier to gene flow inferred using the software BARRIER, and it coincides with the geographical structure of genetic variation inferred with the Spatial Analysis of MOlecular VAriance (SAMOVA)

Haplotype network reconstruction

Individual Cytb sequences were collapsed into haplotypes using the online web tool DnaCollapser 1.0 available on the FaBox platform (<http://users-birc.au.dk/biopv/php/fabox/>). The alignment of the MC1R fragment contained heterozygous positions, therefore we phased genotypes to identify the haplotypes for further analyses using the PHASE algorithm (version 2.1.1) with default settings (Stephens et al. 2001),

as implemented in the software DnaSP. PHASE parameters were 1000 iterations, one thinning interval and 100 burn-in iterations and a posterior probability threshold of 0.9 to determine the most probable inferred haplotypes for each nuclear sequence. Analyses were repeated three times using different seed values. We used TCS version 1.21 (Clement et al. 2000) to explore the intraspecific relationships of the haplotypes, using the statistical parsimony criterion (Templeton et al., 1992) (Figs. 2, 3). This algorithm estimates the

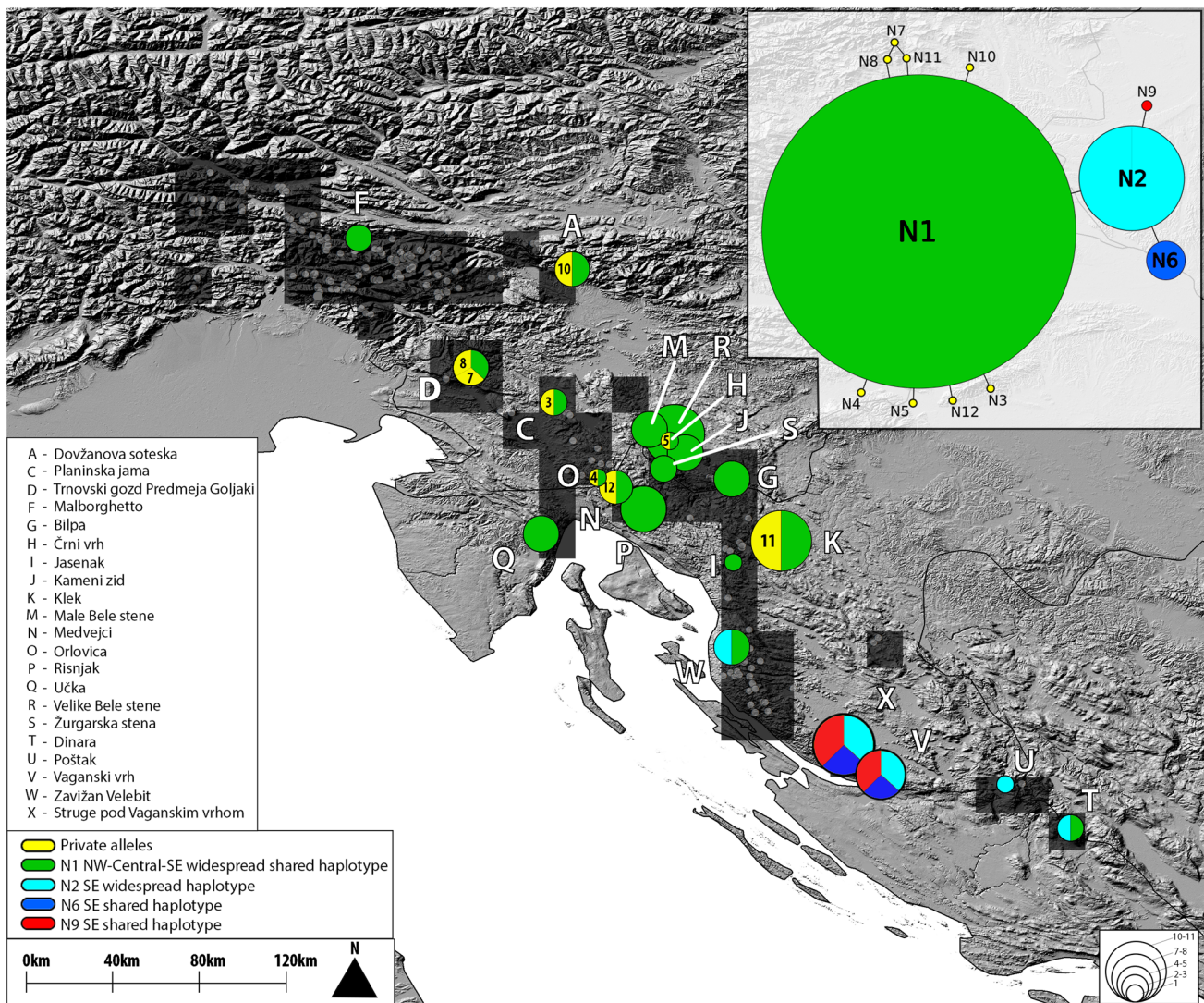


Fig. 3 Haplotype network reconstruction of the MC1R nuclear DNA fragment and geographical distribution of haplotype frequencies across the analyzed sampled localities of *Iberolacerta horvathi*.

The dark-grey squares and the small light-grey circles represent the updated distribution of the species

number of mutational steps by which pairwise haplotypes differ, computing the probability of parsimony for pairwise differences until the probability exceeds 0.95. There was no need to adjust the threshold to infer the number of mutational steps that needed to join all haplotypes into one single network for each marker. Loops that occurred in the network were resolved by (1) treating connections with singletons and/or rare haplotype to be less likely than connections with central and frequent haplotypes (Posada and Crandall 2001) and (2) using the geographical criterion, favouring connection between haplotypes from the same or proximate populations over connection with haplotypes from distant populations (Pfenninger and Posada 2002). Networks were graphically prepared using the online tool tcsBU (Santos et al. 2015).

Genetic structure and historical demography analyses

Due to the low number of available sequences and the limited variability observed in the nuclear marker we performed these analyses only with mtDNA data, aware of the possibility that phylogeographic patterns inferred from mtDNA can dramatically differ from those inferred from nuclear data (Toews and Brelsford 2012). We explored the geographical structure of Cytb genetic variation using the spatial analysis of molecular variance implemented in SAMOVA 2.0 (Dupanloup et al. 2002). This software defines groups of populations that are geographically homogeneous and maximally differentiated from each other. The method is based on a simulated annealing procedure that maximizes

Table 2 Locality names and ID, geographical location, number of analyzed individuals from each locality (N and 2N for phased nuDNA) followed by haplotype ID and their frequencies, the number of polymorphic sites (S), Nei's (1987) nucleotide diversity (Π) and Haplotype diversity [SD (Π) and SD (Hd)], computed separately for the Cytb and MC1R gene fragments

Locality	Latitude	Longitude	CYTB		MC1R							
			N	Haplo freq	S	Π ; SD (Π)	Hd; SD (Hd)	2N	Haplo freq	S	Π ; SD (Π)	Hd; SD (Hd)
Dovžanova soteska (A)	46.385601	14.330936	5	H5 (2); H6 (1); H7 (1); H8 (1)	9	0.00924; 0.00210	0.900; 0.161	8	N1 (7); N10 (1)	1	0.00040; 0.00029	0.250; 0.180
Iški vintgar (B)	45.9069	14.494	4	H5 (4)	0	0.00000; 0.00000	0.000; 0.000	-	-	-	-	-
Planinska jama (C)	45.8216	14.2471	3	H5 (3)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (1); N3 (1)	1	0.00160; 0.00080	1.000; 0.500
Trnovski gozd Predmeja Goljaki (D)	45.95709	13.89497	5	H5 (2); H19 (1); H20 (1); H21 (1)	12	0.01134; 0.00482	0.900; 0.161	6	N1 (4); N7 (1); N8 (1)	2	0.00138; 0.00054	0.600; 0.215
Lusevera (E)	46.274911	13.269927	3	H36 (2)	0	0.00000; 0.00000	0.000; 0.000	4	N1 (4)	0	0.00000; 0.00000	0.000; 0.000
Malborghetto (F)	46.505699	13.440583	2	H36 (3)	0	0.00000; 0.00000	0.000; 0.000	-	-	-	-	-
Bilpa (G)	45.512	14.9625	5	H2 (5)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Črni vrh (H)	45.671318	14.722323	1	H3 (1)	n.c	n.c	n.c	2	N1 (1); N5 (1)	1	0.00160; 0.00080	1.000; 0.500
Jasenak (I)	45.1773	14.9777	1	H5 (1)	n.c	n.c	n.c	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Kameni zid (J)	45.6127	14.7346	5	H3 (7)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Klek (K)	45.2571	15.1443	11	H1 (1); H9 (1); H10 (1); H11 (3); H12 (1); H13 (1); H14 (1); H15 (1); H16 (1)	11	0.00588; 0.00175	0.945; 0.066	18	N1 (17); N11 (1)	1	0.00018; 0.00015	0.111; 0.096
Kuželjska stena (L)	45.4839	14.8228	3	H3 (3)	0	0.00000; 0.00000	0.000; 0.000	-	-	-	-	-
Male Bele stene (M)	45.6909	14.6827	5	H3 (5)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Medvejci (N)	45.4698	14.5297	5	H5 (3); H17 (1); H18 (1)	4	0.00378; 0.00168	0.700; 0.218	6	N1 (5); N12 (1)	1	0.00053; 0.00034	0.333; 0.215
Orlovica (O)	45.5232	14.436	1	H5 (1)	n.c	n.c	n.c	2	N1 (1); N4 (1)	1	0.00160; 0.00080	1.000; 0.500
Risnjak (P)	45.4248	14.6246	8	H5 (6); H21 (1); H22 (1)	2	0.00143; 0.00066	0.464; 0.200	6	N1 (6)	0	0.00000; 0.00000	0.000; 0.000
Učka (Q)	45.2947	14.2046	5	H5 (5)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Velike Bele stene (R)	45.6753	14.7082	11	H3 (11)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Žurgarska stena (S)	45.583359	14.69992	2	H3 (2)	0	0.00000; 0.00000	0.000; 0.000	2	N1 (2)	0	0.00000; 0.00000	0.000; 0.000
Tot Northern group			85	Tot N. Haplotypes: 22	24	0.00459; 0.00076	0.797; 0.031	68	Tot N. Haplotypes: 9	6	0.00052; 0.00022	0.264; 0.103
Dinara (T)	44.06775	16.37442	3	H4 (3)	0	0.00000; 0.00000	0.000; 0.000	4	N1 (2); N2 (2)	1	0.00106; 0.00033	0.667; 0.204

Table 2 (continued)

Locality	Latitude	Longitude	CYTB		MC1R				Hd; SD (Hd)			
			N	Haplo freq	S	Π ; SD (Π)	Hd; SD (Hd)	2N		Haplo freq	S	Π ; SD (Π)
Poštrak (U)	44.25486	16.10411	1	H4 (1)	n.c	n.c	n.c	2	N2 (2)	0	0.00000; 0.00000	0.000; 0.000
Vaganski vrh (V)	44.367543	15.498687	7	H23 (3); H29 (1); H31 (1); H32 (1); H33 (1)	4	0.00320; 0.00087	0.857; 0.137	10	N2 (7); N6 (2); N9 (1)	0	0.00000; 0.00000	0.000; 0.000
Zavižan Velebit (W)	44.81402	14.9751	4	H34 (3); H35 (1)	3	0.00315; 0.00167	0.500; 0.265	6	N1 (4); N2 (2)	1	0.00085; 0.00027	0.533; 0.172
Struge pod Vaganskim vrhom (X)	44.376576	15.466281	10	H23 (2); H24 (1); H25 (1); H26 (1); H27 (1); H28 (1); H30 (1); H33 (2)	14	0.00915; 0.00204	0.956; 0.059	16	N2 (9); N6 (6); N9 (1)	2	0.00100; 0.00019	0.575; 0.080
Tot Southern group			25	Tot N. Haplo-types: 14	20	0.00800; 0.00126	0.927; 0.031	38	Tot N. Haplo-types: 4	4	0.00115; 0.00017	0.609; 0.068
TOT			110		35	0.00725; 0.00076	0.875; 0.022			10	0.00123; 0.001	0.568; 0.0023

the proportion of total genetic variance due to differences between groups of populations allowing the integration of geographical coordinates with the genetic data (SAMOVA, Spatial Analysis of MOlecular VARIance). In this way, it also recognizes genetic barriers between the identified groups. We performed this analysis setting the number of groups (K) from two to ten and selecting the best clustering option as the one returning the highest and significant value of F_{CT} (i.e. the among-group variance component) (Fig. 4a). Four populations, consisting of single individuals, were excluded from this analysis (Črni vrh, Jasenak, Orlovica and Poštak; Table 2). To verify the consistency of the results among runs, we replicated the analysis five times for each K value with 1000 independent annealing processes.

Anticipating the results of the SAMOVA analysis based on the Cytb mtDNA marker, we used migrate-n v.4 (Beerli and Palczewski 2010) to estimate gene flow between the two identified groups. We ran eight different models of migration and the best model was the one with the highest value of likelihood (detailed information on the models are on Online Resource 6). Acceptable effective MCMC sample size was reached when the models run for 100,000 generations, applying a burn-in value of 1000.

SAMOVA and migrate-n analyses for the Cytb fragment were complemented with a test to identify the most likely historical barriers that negatively affected the gene flow between populations. Using BARRIER, v. 2.2 (Manni et al. 2004) the Monmonier’s maximum difference algorithm (Monmonier 1973) built a Delaunay network between localities and compared it to a matrix of pairwise genetic distances between sequences. This method identifies the largest pairwise genetic distance between pairs of samples and then locates on the Delaunay network the edge connecting this pair, which is assumed to represent a barrier to gene flow (Fig. 2; Online Resource 7).

The variation of population sizes over time determines the distribution of pairwise nucleotide differences observed between populations (Rogers and Harpending 1992). We estimated the parameters of spatial expansion for the Cytb fragment using the software Arlequin v.3.5.2.2 (Excoffier and Lischer 2010) for the two groups identified with the SAMOVA analyses (Southern group, N = 25; Northern group, N = 85) and past demographic changes were visualized as the empirical mismatch distributions in the software DnaSP. The mismatch distribution, the observed distribution of nucleotide differences between haplotype pairs, is compared with the mismatch distribution expected under both a demographic expansion model (Rogers and Harpending 1992) and a sudden spatial expansion model (Excoffier 2004) (Fig. 4b). The sum of squared deviations between estimated and observed mismatch distributions was used as goodness-of-fit statistics, and its significance was assessed using 1000 bootstrap replicates.

To test for past population expansions, we used Tajima’s D (Tajima 1989) and Fu’s FS (Fu 1997) statistical tests. Demographic changes were also examined by calculating Harpending’s raggedness index (Harpending et al. 1993) and the sum of squared deviations (SSD) (Schneider and Excoffier 1999). Neutrality tests were calculated in Arlequin version 3.5 (Excoffier and Lischer 2010) under a demographic expansion model and 1000 simulations.

We used PartitionFinder v.2 under a greedy search (Lanfear et al. 2017) to choose the optimal partitioning scheme and the best-fitting model of evolution for the 2 Cytb alignments (the Northern group, n = 85; the Southern group, n = 25; Fig. 4c). PartitionFinder v.2 selected two partitions (1 + 2nd and 3rd codon position) in both cases. We specified a strict clock for the Cytb data in BEAST v1.8.2 (Drummond et al. 2012; <http://beast.bio.ed.ac.uk>). We inferred changes in effective population size through time by fixing the mutation rate in the Cytb gene fragment to 1.15×10^{-8} substitutions/site/year following Arribas and Carranza (2004), and selected a diffuse gamma distribution and unlinked parameters for each codon partition. We inferred changes in effective population size through time using a Bayesian Skyline Plot (BSP) model (Drummond et al. 2005). We ran two independent MCMC chains, each with 30 million states and sampling every 3000th state. Independent runs were evaluated for convergence and mixing by observing and comparing traces of each statistic and parameter in Tracer v1.6 (Rambaut and Drummond 2007; <http://beast.bio.ed.ac.uk/tracer>). We considered effective sampling size (ESS) values > 200 to be good indicators of parameter mixing. The first 10% of each run were discarded as burn-in, and samples were merged using LogCombiner v1.8.2 (Drummond et al. 2012; Fig. 4c).

Molecular phylogenetic analyses

To identify the ESUs in our dataset we conducted Bayesian inference searches based on the Cytb fragment (476 bp; Online Resource 8). PartitionFinder v.2 (Lanfear et al. 2017) was used to determine the optimal partitioning scheme and the best-fitting substitution model. Bayesian Inference (BI) analyses were conducted in MrBayes v. 3.2.6 (Ronquist et al. 2012) on CIPRES Science Gateway v. 3.3 (Miller et al. 2010). The Markov chain Monte Carlo sampling included two runs of 50 million generations (starting with random trees) and four chains (three heated, one cold) sampled every 1000 generations. The first 25% of the sampled generations were discarded as burn-in, and 37.5 million trees were retained post burn-in and summed to generate a 50% majority rule consensus tree (Online Resource 8).

Results

Updated species distribution

Published and new observation data confirmed that *Iberolacerta horvathi* occurs in at least four countries (Fig. 1a): Italy, Austria, Slovenia and Croatia. Several new distribution records from the border between Croatia and Bosnia and Herzegovina (Fig. 1a) suggest that the species probably also occurs in the latter. Within its range, the species exhibits a relatively compact distribution and seems to be present from 200 to 2000 m a.s.l. (Speybroeck et al. 2016).

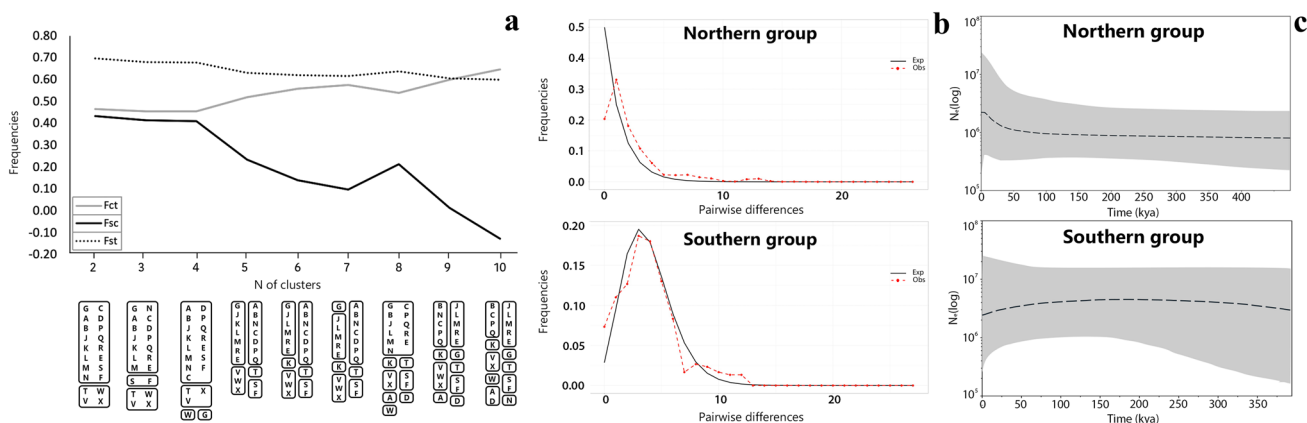


Fig. 4 **a** Geographical structure of genetic variation of *Iberolacerta horvathi* using the Spatial Analysis of MOlecular VAriance implemented in SAMOVA 2.0. At the bottom of the graph are the groups of localities as identified in the SAMOVA analyses for K ranging

from 2 to 10. **b** Mismatch distribution for the Northern and Southern groups as identified in the SAMOVA analysis. **c** Bayesian Skyline plots of the Northern (n = 85) and Southern (n = 25) groups

Realised niche model and environmental characteristics of occurrence area

The average Maxent model obtained very high average training and test AUC values (0.953 ± 0.003 and 0.939 ± 0.004 , respectively). In all cases, null models performed worse than Maxent species models (ANOVA test: $F_{1,109} = 1479$, $p < 0.0001$; mean \pm SD of null models: 0.68 ± 0.02). Three variables each contributed at least 15% and in total 84.5% to the Maxent model (Table 1): precipitation of wettest quarter of the year (39.4%), altitude (30.1%), and mean temperature of driest quarter of the year (15.0%). The suitable area extends over 114,653 km² and follows the mountainous ranges from the Karawanks, Julian and Kamnik-Savinja Alps, along the north-western portion of the Dinaric Mountains of Slovenia and Croatia into Bosnia and Herzegovina mountains (Fig. 1b).

Extracted values for environmental variables contributing the most to the model (Table 1) showed that the area with predicted species occurrences (Fig. 1b) was mostly related to the precipitation in the wettest quarter of the year with the mean of 407 ± 18 mm (min–max range: 361–497 mm), the mean altitude of 1101 ± 354 m a.s.l. (min–max range: 265–3076 m a.s.l.) and the mean temperature of the driest quarter of the year of -0.1 ± 4.8 °C (min–max range: -7.9 to 17.8 °C). Compared to available environmental conditions in the study area, *I. horvathi* selects for relatively high elevation, high rainy/snowy conditions in winters and low temperatures in summer.

Genetic diversity

The Cytb alignment (476 bp) had 35 polymorphic sites (7.3%) of which 25 were parsimony-informative. After phasing the alignment of the MC1R (626 bp), we identified ten variable sites (1.6%; seven of which were due to heterozygous sites), with five substitutions being parsimony-informative (Table 2).

The mitochondrial data recovered 36 haplotypes. The overall nucleotide and haplotype diversity were 0.00725 (Π) and 0.875 (Hd), with the highest value of Π found in the population of Trnovski gozd Predmeja Goljaki (0.01134), in the north-west, and the highest value of Hd in Struge pod Vaganskim vrhom (0.956), in the southern part of the species' range, where eight haplotypes were identified (Table 2).

The nuclear alignment recovered 12 haplotypes. Overall nucleotide and haplotype diversity were 0.00123 (Π) and 0.568 (Hd). The highest values of Π and Hd were found for Planinska jama, Črni vrh and Orlovica (0.00160 Π ; 1 Hd).

When the two main groups identified with the SAMOVA analysis were analyzed, we recovered a total of 22 and 9 haplotypes for the mitochondrial and nuclear gene fragment for the Northern group and 14 and 4 haplotypes for the Southern

group (Table 2). The observed nucleotide and haplotype diversity for the Cytb gene fragment is 0.00459 (Π) and 0.797 (Hd) for the Northern group and 0.0080 (Π) and 0.927 (Hd) for the Southern group (Table 2). In the nuclear marker the observed nucleotide and haplotype diversity is 0.00052 (Π) and 0.264 (Hd) for the Northern group and 0.00115 (Π) and 0.609 (Hd) for the Southern group (Table 2).

Haplotype network

Analyzing the mitochondrial marker, we found that haplotypes H3 and H5 were the most abundant, with the latter being the most widespread (Fig. 2). Haplotype H3 was recovered in six proximal localities, while haplotype H5 was recovered in nine central populations (Fig. 2). Haplotype H36 was found only in the two northernmost populations (Lusevera and Malborghetto; Fig. 2), while haplotype H4 was identified in the two southernmost populations (Dinara and Poštak; Fig. 2). We found two shared unique haplotypes in the southern populations of Vaganski vrh and Struge pod Vaganskim vrhom (H23 and H33), whereas haplotype H21 was found in two central populations (Trnovski gozd Predmeja Goljaki and Risnjak) (Fig. 2). Interestingly, the population of Klek was characterized by only private haplotypes and did not share any allele with other localities. On the overall, 29 non-shared haplotypes were recovered (Fig. 2).

Haplotype (N1) was the most widespread MC1R haplotype, shared by most populations, except for some of the southernmost localities (Fig. 3). In contrast, haplotype N2 was shared exclusively by the five southernmost populations, while haplotypes N6 and N9 were identified only in two relatively isolated southern populations: Vaganski vrh and Struge pod Vaganskim vrhom (Fig. 3). Eight non-shared haplotypes were found in seven localities from the central area (Fig. 3).

Genetic structure

The analysis of the Cytb fragment identified one putative barrier to gene flow between the Klek and Zavižan Velebit populations (Fig. 2). The best partitioning of the genetic diversity (as suggested by the SAMOVA analyses) was obtained when populations were grouped in two clusters (Fig. 4a). Increasing the number of K to three retrieved similar (although slightly lower) values, and with $K > 3$ the new groups that are suggested contain only one single population, resulting in a non-informative-clustering. For $K = 2$ ($p < 0.001$) SAMOVA explained 46% of the overall molecular variance (Fig. 4a). The population of Poštak was not included in the SAMOVA analyses because DNA of only one individual was successfully amplified. Since it shared the same haplotype of Dinara (H4), we assigned it to the Southern group.

The model selected by migrate-n analyses was model 6, which suggests that the Northern group split from the Southern group and that migration events occurred from South to North after this split. However, model 3 received a very similar Log (mL) and this model suggests for an independent origin for the two groups, with migration events from the Southern group towards the Northern group (see Online Resource 6 for more details). Model 4, which consider the two groups as belonging to one panmictic population has been rejected as the model receiving the least support among the one tested here (Online Resource 6).

The suggested barrier to gene flow identified using the software BARRIER coincided with the two geographic groups (North and South) as identified in the SAMOVA analyses (Online Resource 7).

Historical demography

Negative neutrality test (Fu's F_s and Tajima's D) values observed in both groups identified by the SAMOVA analyses ($K=2$), provide evidence of past population expansions, especially in the Northern group. Non-significance of square deviations (SSD) and raggedness index further support such observations (Fig. 4b; Online Resource 9). Non-significant values for SSD suggest that the data do not deviate from that expected under the model of expansion. Non-significant raggedness values also indicate population expansion. Non-significant values in goodness-of-fit distribution for all populations suggest that population expansions are likely recent (Rogers 1995) (Online Resource 9).

Partition finder recovered a partition scheme of two partitions according to codon positions (1 + 2, 3) and the following models: Northern group, SYM + I (1st and 2nd codon) and HKY + X (3rd codon); Southern group, TRNEF + I (1st and 2nd codon) and HKY + X (3rd codon). Because these models cannot be directly implemented in BEAST 1.8.4 we used the HKY + G model taking into account the partition scheme (1 + 2, 3) and unlinking substitution rate parameters, rate heterogeneity and base frequencies across codon positions. The runs showed high Effective Sample Size (> 200), indicating adequate sampling of the posterior distribution. Bayesian skyline plots inferred from the Southern group ($n=25$) indicated a stable population throughout the Riss glaciation (~350–130 kya, Marine isotope stages 6, 8, 10) followed by moderate change in the population demographics, with a population decrease at the onset of the Eemian interglacial (130–117 kya), which progresses throughout the whole duration of the Würm glaciation (117 kya–Holocene) (Fig. 4c). In contrast, the Bayesian skyline plots of the Northern group ($n=85$) suggested population stasis throughout the Riss glaciation with a population expansion towards the Late Pleistocene, initiating circa 50 kya (Fig. 4c).

Molecular phylogenetic analyses

The best partitioning scheme for phylogenetic analysis included two partitions: codon positions (1 + 2, 3). We used the SYM + I + G model for the 1st and 2nd codon position partition and the GTR + I model for 3rd codon position partition.

Partitioned Bayesian inference searches identify three main ESUs: analyzed individuals of all northern populations formed a monophyletic clade (ESU3 in Online Resource 8) sister of the clade that contains the individual of the southern populations of Vaganski vrh, Struge pod Vaganskim vrhom and Zavižan Velebit (ESU2 in Online Resource 8). The ESU3 + ESU2 clade is sister of the clade that contains the individuals of the southern populations from Dinara and Poštak (ESU1 in Online Resource 8).

Discussion

Ecological specialization and climatic oscillations have both left their footprint in the distribution and genetic structure of this lizard species. While current environmental conditions might account for the present restricted geographic range, the phylogeographical reconstruction allowed the interpretation of the displayed genetic diversity.

Results showed that the distribution of *Iberolacerta horvathi* is wider and more connected than formerly thought (Vogrin et al. 2009). Generally, this lizard occurs in pristine montane habitats or mountain valleys with low disturbance, while at low and mid-elevations it often comes into contact with other lacertid species. In these instances, *Podarcis muralis* seems to be the dominant competitor when sun-exposed areas are limited, while at higher altitudes *I. horvathi* is favoured by its specialized ecophysiology and behavior (Osojnik et al. 2013; Žagar et al. 2015a, b, c). Most previously known records were from the Alpine region (mostly Julian Alps) and from some parts of the Dinaric mountain range (especially Velebit) in Croatia, which coincides with the localities where there have been some survey efforts (e.g., De Luca 1989, 1992; Grillitsch et al. 2001; Lapini et al. 2004; Cabela et al. 2007; Rassati 2010; De Marchi et al. 2020) and where the species seems demographically stable. Our surveys identified 32 new records in the central part of the species distribution (both in the pre-Alpine area and in the northern Dinaric mountains of Central and Southern Slovenia, such as Notranjska, Kočevska), where the species was also found at mid (400–800 m a.s.l. in the pre-Alpine areas in central Slovenia) and low altitudes (e.g., at ca. 200 m a.s.l. at the border between Slovenia and Croatia; Žagar 2008).

Throughout *I. horvathi*'s distribution, we identified two discontinuities (Fig. 1a). From north to south, the first

discontinuity is observed in Slovenia between the high montane areas of the Alps and the pre-Alpine area along the Idrijca river (between Tolmin and Cerknò; see Fig. 1a). After this gap, the distribution records are scattered but generally homogeneous across the Dinaric Mountains, at least until Paklenica in southern Velebit, where the second distribution gap is found (see Fig. 1a). However, unlike the first distribution gap, this might be the result of limited sampling. Moreover, the South-eastern portion of the species range continues to be poorly characterized, but the presence of multiple minefields from past armed conflicts will unfortunately continue to limit the field exploration of these areas.

Ecological niche modelling provided a clear insight into the habitat preferences of the species. The most explanatory model variables were connected to topography and climate: higher altitudes, mid-to high precipitation in the wettest quarter of the year, and low mean temperatures in the driest quarter of the year. This suggests that the species is a mountain specialist that occupies harsh mountainous environments with long snowfall periods, high seasonal differences between dry and relatively cold summers and wet and cold winters. Multiple ecophysiological studies have shown that *I. horvathi* has advantageous traits to cope with thermally more restrictive environments such as those at higher altitudes (Osojnik et al. 2013; Žagar et al. 2015c). Our realized niche model results support the previous findings of a fundamental niche, based on ecophysiology and behaviour data. Interestingly, the habitat suitability model predicts a large gap of unsuitable habitat between the northern Dinaric Mountains and Velebit (see Fig. 1b). This area overlaps with the most likely barrier to gene flow identified with BARRIER and corresponds to the proposed geographic separation between the Northern and Southern phylogeographic groups as defined here.

Although mitochondrial markers are expected to reveal greater structure, likely overestimating intraspecific diversity, and MC1R evolution might be under selection (Harding et al. 2000; not tested here), it seems that *Iberolacerta horvathi* has a considerably high genetic variability if compared to other species with equally restricted distribution. For example, the wall lizard *Podarcis bocagei* is currently distributed in the North-western Iberian Peninsula. The pattern of its genetic variability (with higher genetic diversity in southern populations and the lack of haplotypes occurring only in the northern portion of the species distribution) suggests that during the Pleistocene the species was confined to a single glacial refugium most probably in the South-eastern part of its current range, expanding from there during the interglacials (Pinho et al. 2007, 2011). In the Balkan Peninsula, the lizards *Dalmatolacerta oxycephala* and *Dinarolacerta mosorensis* show higher genetic diversity indices in the southern parts of their current distributional ranges, and it is likely that both species survived in multiple microrefugia

during the Pleistocene climatic oscillations (Podnar et al. 2014). In contrast, the genetic evidence for *I. horvathi* reveal high diversity (in terms of nucleotide and haplotype diversities and in number of unique haplotypes) both in the Northern and Southern groups, with no haplotype shared between these groups. This suggests that the Southern group made only limited contributions to the re-colonization of the species in the North, showing signs of long-term isolation and a moderate demographic decrease starting at the onset of the Eemian interglacial. In contrast, the populations of the Northern group display signatures of persistence followed by rapid re-colonization and demographic expansion during the last interglacial (Fig. 4b, c). Hence, the species likely survived in at least two main refugia, one in the South and one in the central/northern portion of the species distribution. Our combined results also suggest that the Northern group might have separated (with northwards migrations) from the Southern group during interglacial periods (Online Resource 6), and that these migrations events likely reduced in frequency with the formation of geographical barriers or gaps in habitat suitability.

Conclusions and conservation implications

Our study contributes to a better understanding of the distribution, genetic structure and habitat preferences of an endemic and poorly known lizard species. Phylogeographic analyses identified two main groups, one in the north and one in the south. This structure probably reflects the specialized ecology of the species: the gap of currently unsuitable habitat between the two groups was likely maintained during the previous interglacials and could have played a key role in the evolution of this geographically-restricted species. Due to lower spatial connectivity, the peripheral populations of the Southern group might be more threatened if the extent of suitable habitats decreases. We also recommend investing more effort in understanding the potential effects of predicted climate change on their long term survival. In contrast, the northern populations have a higher degree of connectivity and seem to be less threatened provided that pristine habitats are maintained. However, it is in the north, where the lowland populations are more isolated, harbor unique haplotypes, and suffer competitive interactions from other lacertid species and live under suboptimal conditions (Cabela et al. 2007). As such, we recommend that the three ESUs and the lowland populations of the Northern group should receive most attention in the conservation efforts, to guarantee the preservation of the genetic diversity of this species.

The conservation status of a species can more easily be assessed if its distribution, genetic variability across space, and life history are well understood. Currently, *I. horvathi* is classified as Near Threatened under IUCN Red listing

criteria (Vogrin et al. 2009), mostly based on its small Area of Occupancy and on the apparent fragmentation of its range. Our results demonstrate that the distribution of *I. horvathi* is less fragmented than previously thought. The Area of Occupancy proposed in the IUCN assessment currently accounts for only 11.6% of the suitable area for this species predicted by our Maxent model, although presence-only algorithms are not able to predict occurrence probability but only habitat quality. Therefore, the species likely does not occupy all suitable habitats predicted by the model. Still, these results provide a fairly good approximation of the species habitat needs. Using the Maxent model we identified areas of high suitability that still lack supporting distributional data and should be the target of future field efforts. While the present paper was under final review, De Marchi et al. (2020) published a new study on a distributional model of *I. horvathi* in the Alps, that also contributed with new locality records expanding the distribution of the species westward. Our study, together with this recent one, clearly demonstrates that habitat models provide robust information to guide targeted field searches.

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Author contributions WC and AŽ have contributed equally to the development of this study and the preparation of the manuscript. AŽ, MK, ML, MP, NT, MAC and AC contributed to distributional data and tissue collection. WC, AŽ, NS, MJJ and AC contributed to the data generation and data analyses. AŽ, MAC and AC conceived and designed the study. WC, AŽ and AC wrote the manuscript and all authors contributed to the writing and revision.

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Data availability All molecular data are available on public database GenBank, with accession numbers: MN096384–MN096546.

Declarations

Conflict of interest The authors declare no conflict of interest and we confirm we all abide to the ethical guidelines of the journal.

References

- Abellán P, Svenning J-C (2014) Climatic stability and biodiversity. *Biol J Linn Soc Lond* 113:13–28. <https://doi.org/10.1111/bij.12309>
- Anderson RP, Raza A (2010) The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: preliminary tests with montane rodents (genus *Nephelomys*) in Venezuela. *J Biogeogr* 37(7):1378–1393
- Arnold EN (1987) Resource partition among lacertid lizards in southern Europe. *J Zool* 1:739–782
- Arnold EN, Ovenden D (2004) A field guide to the reptiles and amphibians of Britain and Europe. Collins, London
- Arnold EN, Arribas O, Carranza S (2007) Systematics of the Palaearctic and Oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new genera. *Zootaxa* 1430:86
- Arribas OJ (1999) Phylogeny and relationships of the mountain lizards of Europe and Near East (*Archaeolacerta* Mertens, 1921, sensu lato) and their relationships among the Eurasian lacertid radiation. *Russ J Herpetol* 6:1–22
- Arribas O, Carranza S (2004) Morphological and genetic evidence of the full species status of *Iberolacerta cyreni martinezricae* (Arribas, 1996). *Zootaxa* 634:1–24
- Avise J (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge
- Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* 185:313–326
- Bruford MW, Hanotte O, Brookfield JFY, Burke T (1992) Multi and single locus DNA fingerprinting. In: Hoelzel AR (ed) Molecular analysis of populations: a practical approach. IRL Press, Oxford, pp 225–269
- Buades JM, Rodríguez V, Terrasa B, Pérez-Mellado V, Brown RP et al (2013) Variability of the mc1r gene in melanistic and non-melanistic *Podarcis lilfordi* and *Podarcis pityusensis* from the Balearic Archipelago. *PLOS ONE* 8(1):e53088. <https://doi.org/10.1371/journal.pone.0053088>
- Cabela A, Grillitsch H, Tiedemann F (2004) *Lacerta horvathi* (MÉHELY, 1904) in the Tyrol south of the Central Alps. *Herpetozoa* 16:175–176
- Cabela A, Grillitsch H, Tiedemann F (2007) Habitatpräferenzen von *Podarcis muralis* (Laurenti, 1768) und *Iberolacerta horvathi* (Mehely, 1904) bei gemeinsamem Vorkommen. *Herpetozoa* 19:149–160
- Canestrelli D, Cimmaruta R, Nascetti G (2007) Phylogeography and historical demography of the Italian treefrog *Hyla intermedia* reveals multiple refugia, population expansions and secondary contacts within peninsular Italy. *Mol Ecol* 16:4808–4821

- Canestrelli D, Cimmaruta R, Nascetti G (2008) Population genetic structure and diversity of the Apennine endemic stream frog, *Rana italica*: insights on the Pleistocene evolutionary history of the Italian peninsular biota. *Mol Ecol* 17:3856–3872
- Capula M, Luiselli L (1990) Notes on the occurrence and distribution of *Lacerta horvathi* MÉHELY, 1904 in Federal Republic of Germany. *Herpetol J* 1:535–536
- Carranza S, Arnold EN, Amat F (2004) DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? *Syst Biodivers* 2:57–77
- Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1660
- Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Understanding evolutionary process in conservation biology. *Tree* 15:290–295
- Crochet PA, Chaline O, Surget-Groba Y, Debain C, Cheylan M (2004) Speciation in mountains: phylogeography and phylogeny of the rock lizards genus *Iberolacerta* (Reptilia: Lacertidae). *Mol Phylogenet Evol* 30:860–866
- Crottini A, Andreone F, Kosuch J, Borkin LJ, Litvinchuk SN, Eggert C, Veith M (2007) Fossorial but widespread: the phylogeography of the common spadefoot toad (*Pelobates fuscus*), and the role of the Po Valley as a major source of genetic variability. *Mol Ecol* 16:2734–2754
- De Luca N (1989) Taxonomic and biogeographic characteristics of Horvath's rock lizard (*Lacerta horvathi* Mehely, 1904, Lacertidae, Reptilia) in Yugoslavia. *Scopelia* 18:1–48
- De Luca N (1992) Notes on biology and ecology of the Horvath's rock lizard (*Lacerta horvathi* Méhely, 1904, Reptilia: Lacertidae). In: Korsós Z, Kiss I (eds) Proceeding of the sixth ordinary general meeting of the Societas Europaea Herpetologica, Budapest 1991. Hungarian Natural History Museum, Budapest, pp 129–135
- De Marchi G, Bombieri G, Boz B, Lenardi F, Richard J (2020) Has the West been won? A field survey and a species distribution model of *Iberolacerta horvathi* in the Alps. *Acta Herpetol*. https://doi.org/10.13128/a_h-8448
- dos Santos AM, Cabezas MP, Tavares AI, Xavier R, Branco M (2015) tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics* 32:627–628
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 22:1185–1192
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Bio Evol* 29:1969–1973
- Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Mol Ecol* 11:2571–2581
- Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Mol Ecol* 13:853–864
- 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 Res* 10:564–567
- Flohn H, Fantechi R (1984) The climate of Europe: past, present and future. Reidel, Dordrecht, pp 1–356
- Freitas S, Rocha S, Campos J, Ahmadzadeh F, Corti C, Sillero N, Ilgaz Ç, Kumlutaş Y, Arakelyan M, Harris DJ, Carretero MA (2016) Parthenogenesis through the ice ages: A biogeographic analysis of Caucasian rock lizards (genus *Darevskia*). *Mol Phylogenet Evol* 102:117–127. <https://doi.org/10.1016/j.ympev.2016.05.035>
- Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147(2):915–25
- García-Porta J, Irisarri I, Kirchner M et al (2019) Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nat Commun* 10:4077
- Gasc JP, Cabela A, Crnobrnja-Isailovic J, Dolmen D, Grossenbacher K, Haffner P, Lescure J, Martens H, Martínez Rica JP, Maurin H (1997) Atlas of amphibians and reptiles in Europe. Societas Europaea Herpetologica and Museum National d-Histoire Naturelle (IEGB/SPN), Paris
- Gómez A, Lunt DH (2007) Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: Weiss S, Ferrand N (eds) Phylogeography of southern European refugia. Springer, Dordrecht, pp 155–188
- Grillitsch H, Cabela A, Tiedemann F (2001) *Lacerta horvathi* Méhely, 1904. Kroatische Gebirgsseidechse. In: Cabela A, Grillitsch H, Tiedemann F (eds) Atlas zur Verbreitung und Ökologie der Amphibien und Reptilien in Österreich. Umweltbundesamt-Naturhistorisches Museum Wien, Wien, pp 481–488
- Guillera-Arroita G, Lahoz-Monfort JJ, Elith J (2014) Maxent is not a presence-absence method: a comment on Thibaud et al. *Methods Ecol Evol* 5:1192–1197
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acid Sympos Ser* 41:95–98
- Harding RM, Healy E, Ray AJ, Ellis NS, Flanagan N, Todd C, Dixon C, Sajantila A, Jackson IJ, Birch-Machin MA, Rees JL (2000) Evidence for variable selective pressures at MC1R. *Am J Hum Genet* 66(4):1351–1361
- Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. *Curr Anthr* 34(4):483–496
- Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol J Linn Soc* 58:247–276
- Hewitt GM (2000) The genetic legacy of the quaternary ice ages. *Nature* 405:907
- Hewitt GM (2004) The structure of biodiversity—insights from molecular phylogeography. *Front Zool* 1:4
- Hewitt GM (2011) Mediterranean peninsulas: the evolution of hot-spots. In: Zachos FE, Habel JC (eds) Biodiversity hotspots. Springer, Heidelberg, pp 123–147
- 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
- Jelić D, Kuljerić M, Koren T, Treer D, Šalamon D, Lončar M, Podnar Lešić M, Janev Hutinec B, Bogdanović T, Mekinić S, Jelić K (2015) Red book of amphibians and reptiles of Croatia. Ministry of Environment and Nature Protection, State Institution for Nature Protection, Zagreb
- Lanfear R, Frandsen P, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol Biol Evol* 34:772–773
- Lapini L, Dall'Asta A, Luiselli L, Nardi P (2004) *Lacerta horvathi* in Italy: a review with new data on distribution, spacing strategy and territoriality (Reptilia, Lacertidae). *B Zool* 71:145–151
- Liu C, Berry PM, Dawson TP, Pearson RG (2005) Selecting thresholds of occurrence in the prediction of species distributions. *Ecography* 28:385–393
- Lobo JM, Jiménez-Valverde A, Real R (2008) AUC: a misleading measure of the performance of predictive distribution models. *Global Ecol Biogeogr* 17:145–151
- Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by “Monmonier's algorithm.” *Human Biol* 76:173–190
- Maura M, Salvi D, Bologna MA, Nascetti G, Canestrelli D (2014) Northern richness and cryptic refugia: phylogeography of the

- Italian smooth newt *Lissotriton vulgaris meridionalis*. Biol J Linn Soc 113:590–603
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE), New Orleans, LA, pp. 1–8.
- Monmonier MS (1973) Maximum-difference barriers: an alternative numerical regionalization method. Geogr Anal 5:245–261
- Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
- Osojnik N, Žagar A, Carretero MA, Garcia-Munoz E, Vrezec A (2013) Ecophysiological dissimilarities of two sympatric lizards. Herpetologica 69:445–454
- Pfenninger M, Posada D (2002) Phylogeographic history of the land snail *Candidula unifasciata* (Poirot 1801) (Helicellinae, Stylomatophora): fragmentation, corridor migration and secondary contact. Evolution 56:1776–1788
- 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 distributions. Ecol Model 190:231–259
- Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31:161–175
- Phillips SJ, Dudík M, Schapire RE (2004) A maximum entropy approach to species distribution modeling. In: Proceedings of the twenty-first international conference on Machine learning. ACM, New York, p. 83
- 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 et al (2010) New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. Con Gen Res 2:181–185
- Podnar M, Bruvo Mađarić B, Mayer W (2014) Non-concordant phylogeographical patterns of three widely codistributed endemic Western Balkans lacertid lizards (Reptilia, Lacertidae) shaped by specific habitat requirements and different responses to Pleistocene climatic oscillations. J Zool Sys Evol Res 52:119–129
- Posada D, Crandall KA (2001) Intraspecific phylogenetics: trees grafting into network. Trends Ecol Evol 16:37–45
- Pozzi A (1966) Geonemia e catalogo ragionato degli Anfibi e dei Rettili della Jugoslavia. Natura 57:5–55
- Raes N, ter Steege H (2007) A null-model for significance testing of presence-only species distribution models. Ecography 30:727–736
- Rambaut A, Drummond AJ (2007) Tracer v. 1.6. <http://beast.bio.ed.ac.uk/tracer>
- Rassati G (2010) Contributo alla conoscenza della distribuzione della Lucertola di Horvath *Iberolacerta horvathi* e della Lucertola dei muri *Podarcis muralis* in Friuli Venezia Giulia e in Veneto. Atti di Museo Civico di Storia Naturale Trieste 54:133–146
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Sys Biol 61:539–542
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol Biol Evol 34:3299–3302
- Rull V (2009) Microrefugia. J Biogeogr 36:481–484
- 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, Schembri PJ, Sciberras A, Harris DJ (2014) Evolutionary history of the Maltese wall lizard *Podarcis filfolensis*: insights on the ‘expansion–contraction’ model of the Pleistocene biogeography. Mol Ecol 23:1167–1187
- Schmidler H, Schmidler J (1996) Zur Reptilienfauna der Nördlichen Kalkalpen zwischen Isar und Inn (Bayern/Tirol). Mitt. Landesverband Amphibien- und Reptilien-Schutz (LARS) in Bayern. München 15:1–36
- Schneider S, Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152(3):1079–1089
- 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, Bonardi A, Corti C, Creemers R, Crochet P, Ficetola GF, Kuzmin S, Lymberakis P, Pous PD, 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
- Speybroeck J, Beukema W, Bok B, Van Der Voort J (2016) Field guide to the amphibians and reptiles of Britain and Europe. Bloomsbury Publishing, London
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Gen 68:978–989
- Stewart JR (2009) The evolutionary consequence of the individualistic response to climate change. J Evol Biol 22:2363–2375
- Szencel-Jablonka A, Mazgajski TD, Bury S, Najbar B, Rybacki M, Bogdanowicz W, Mazgajska J (2015) Smooth Snake Phylogeography and Population Structure. Biol J Linn Soc Lond 115:195–210
- Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol 7:453–464
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123(3):585–595
- Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633
- Toews DP, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol 21(16):3907–3930
- Uetz P, Freed P, Hošek J (2019) The reptile database. <http://www.reptile-database.org>. Accessed 1 Feb 2019.
- VanDerWal J, Shoo LP, Graham C, Williams SE (2009) Selecting pseudo-absence data for presence-only distribution modeling: how far should you stray from what you know? Ecol Model 220:589–594
- Vogrin M, Böhme W, Crochet P-A, Nettmann HK, Sindaco R, Romano A (2009) *Iberolacerta horvathi* (errata version published in 2016). IUCN Red List Threat Species. <https://doi.org/10.2305/IUCN.UK.2009.RLTS.T61515A12498717.en>

- Zeisset I, Beebee TJC (2008) Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity* 101:109–119
- Žagar A (2008) The lowest altitudinal record of Horvath's Rock Lizard (*Iberolacerta horvathi*) in Slovenia. *Nat Slo* 10:59–61
- Žagar A, Kos I, Vrezec A (2013) Habitat segregation patterns of reptiles in Northern Dinaric Mountains (Slovenia). *Amphibia-Reptilia* 34:263–268
- Žagar A, Bitenc K, Vrezec A, Carretero MA (2015a) Predators as mediators: differential antipredator behavior in competitive lizard species in a multi-predator environment. *Zool Anzeiger* 259:31–40
- Žagar A, Simčič T, Carretero MA, Vrezec A (2015b) The role of metabolism in understanding the altitudinal segregation pattern of two potentially interacting lizards. *Comp Biochem Physiol A* 179:1–6
- Žagar A, Carretero MA, Osojnik N, Sillero N, Vrezec A (2015c) A place in the sun: interspecific interference affects thermoregulation in coexisting lizards. *Behav Ecol Sociobiol* 69:1127–1137

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