

The more you search, the more you find: Cryptic diversity and admixture within the Anatolian rock lizards (*Squamata*, *Darevskia*)

Catarina Rato¹  | Manos Stratakis^{2,3} | Diana Sousa-Guedes⁴  | Neftali Sillero⁴  |
Claudia Corti⁵  | Susana Freitas⁶ | D. James Harris^{1,7} | Miguel A. Carretero^{1,7} 

¹CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Vila do Conde, Portugal

²Department of Biology, School of Sciences and Engineering, University of Crete, Greece

³Natural History Museum of Crete, School of Sciences and Engineering, University of Crete, Irakleio, Greece

⁴Centro de Investigação em Ciências Geo-Espaciais (CICGE), Faculdade de Ciências da Universidade do Porto, Vila Nova de Gaia, Portugal

⁵Museo di Storia Naturale dell'Università di Firenze, Firenze, Italy

⁶Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

⁷Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Correspondence

Catarina Rato, CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas n°7, Vairão 4485 - 661, Vila do Conde, Portugal.
Email: catarina.rato@cibio.up.pt

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Abstract

Anatolia is characterized by a complex topological structure and a wide diversity of climatic regions. This geological context has been crucial during the evolution of the rock lizard genus *Darevskia*, promoting the origin of several species within this group of lizards. Our study focuses on the evolutionary history of the *rudis* species complex, comprised by *Darevskia rudis*, *D. valentini* and *D. portschinskii*, distributed across Anatolia and the Caucasus, with overlapping ranges. Previous studies have suggested the existence of ancient gene flow between these taxa, which, allied with poor sampling, led to weakly resolved phylogenies and mismatching topologies. Here, we employ two mitochondrial (cytb and ND4) and two nuclear (MC1R and C-mos) DNA fragments, coupled with ecological niche modelling and a significant representation of the species' wide distribution range to further assess relationships. The specimens identified either as *Darevskia rudis* or as *D. valentini* appear as paraphyletic and substructured into four clades, which started to differentiate during the lower Pleistocene (around 1.3 Mya). We also differentiate genetically the recently described *Darevskia bithynica* and identify the Kizil River valley as a strong vicariant candidate to explain the origin of this species by allopatric speciation. Moreover, the eastern Black Sea Mountains seem to have played an important role as glacial refugium for several populations of *D. rudis*. Overall, the present study confirms the circum-Black Sea as a potential Pleistocene refuge and supports

the refugia-within-refugia hypothesis with the finding of several geographically coherent evolutionary units in the region.

KEYWORDS

Anatolia, *Darevskia rudis* species complex, ecological niche models, mtDNA, nDNA, speciation

1 | INTRODUCTION

A 'refugia-within-refugia' pattern during Pleistocene climatic oscillations has been proposed for reptiles in Anatolia, similarly to the southern European refugia (Gómez & Lunt, 2007), with several studies describing secondary contacts and hybridization events (Fritz et al., 2009; Guicking et al., 2009; Kindler et al., 2013; Stöck et al., 2012; Vamberger et al., 2013). However, the Irano-Anatolian region has a relatively low reptile richness (e.g., Bellati et al., 2015; Ficetola et al., 2013; Fritz et al., 2009; Jablonski et al., 2019; Kapli et al., 2013; Kornilios et al., 2012; Mashkaryan et al., 2014), compared with other hotspots (Mittermeier et al., 2004). Although in recent years several studies are countering this regional bias (e.g., Ahmadzadeh, Carretero, et al., 2013; Ahmadzadeh, Flecks, Carretero, Mozaffari, et al., 2013; Arribas et al., 2013; Avcı et al., 2015; Bellati et al., 2015; Freitas, Rocha, et al., 2016; Freitas, Vavakou, et al., 2016; Ilgaz et al., 2005; Sindaco et al., 2000; Tarkhnishvili et al., 2013; Vamberger et al., 2013; Yıldız et al., 2012), some areas and taxa remain poorly assessed.

Anatolia is a predominantly mountainous region with complex geomorphology and a high variety of climatic regions and vegetation types (Sindaco et al., 2000). Furthermore, several mountain ranges have played an important role in reptile speciation and in the definition of different biogeographical sub-regions (Sindaco et al., 2000 and references therein). Further, through geological time Anatolia has been intermittently linked with the Balkans, the Caucasus and the Middle East; these connections and reconnections have further shaped the flora and fauna of the region (Dufresnes et al., 2016; Seddon et al., 2002; Veith et al., 2003; Wielstra et al., 2010).

The rock lizards genus *Darevskia* Arribas 1997 belonging to the family Lacertidae constitutes the reptile group with the highest species richness in the Caucasus region, currently comprising 34 described species (Ahmadzadeh, Flecks, Carretero, Mozaffari, et al., 2013; Arnold et al., 2007; Tarkhnishvili et al., 2013; Uetz et al., 2019) distributed from West-Central Asia to the Balkans. Attempts to accurately distinguish some members of the genus have been hindered either due to evolutionary conservativeness in overall morphology or to convergence associated with repeated adaptation to similar environments, coupled with comparatively high morphological variation within populations (Ahmadzadeh, Flecks,

Carretero, Mozaffari, et al., 2013; Darevsky, 1967; Gabelaia et al., 2017; Tarkhnishvili et al., 2013; Tuniyev et al., 2011). This makes their morphological variation complex, often lacking simple diagnostic traits, and consequently misleading evolutionary reconstructions, as happens in other lizard groups (e.g. *Podarcis* lizards; Kaliontzopoulou et al., 2012). Delimitation of *Darevskia* species is further complicated by their frequent overlapping ranges and their weak habitat segregation (Ahmadzadeh, Flecks, Carretero, Mozaffari, et al., 2013; Darevsky, 1967; Freitas, Rocha, et al., 2016; Petrosyan et al., 2019). Also, evidence of recurrent hybridization (past and present) was found across the genus, which might blur phylogenetic reconstructions (Danielyan et al., 2008). Some of these hybridization events have produced parthenogenetic species, combining genomes of unrelated parental species with which they sometimes backcross locally (Danielyan et al., 2008; Freitas et al., 2019).

The first phylogenetic analyses of *Darevskia*, using mitochondrial DNA sequences and/or allozyme data (Fu et al., 1997; Murphy et al., 1996, 2000), placed all sexual rock lizard species into three major monophyletic clades: *caucasica*, *saxicola* and *rudis*. The latter clade comprised the species *D. rudis* (Bedriaga, 1886), *D. portschinskii* (Kessler, 1878), *D. valentini* (Boettger, 1892) and *D. parvula* (Lantz & Cyren, 1913). However, later assessments based on a single mitochondrial DNA phylogeny (Tarkhnishvili, 2012) proposed the removal of *D. parvula* from the *rudis* clade, with *D. rudis* and *D. valentini* as sister taxa, and *D. portschinskii* as sister to both.

The same topology is also supported by Tarkhnishvili et al. (2013), who detected the existence of ongoing gene flow from *D. rudis* to *D. portschinskii*, but not to *D. valentini*. Hence, although both studies suggest that divergent evolution within the *rudis* clade started in the middle or late Pleistocene, none of the lineages seem to have achieved the stage of complete lineage sorting. Detection of hybridization within the *rudis* clade has also been assessed using genomic data, with apparent gene flow between *D. rudis* and both *D. valentini* and *D. portschinskii* (Freitas, 2017). Moreover, a study based solely on the external morphology and osteology of specimens from northwestern Anatolia (Arribas et al., 2013) proposed the elevation of *D. rudis bithynica* to the species rank as *D. bithynica* (Méhely, 1909) with two subspecies: *D. b. bithynica* and *D. b. tristis* (Lantz & Cyren, 1936). More recently, genetic analysis suggested a polytomy

between samples identified as *D. bithynica* and *D. rudis* sensu stricto but limited sampling and markers prevented further conclusions from being made (Koç et al., 2017).

It is important to highlight that in all previously cited studies performed on the *rudis* clade, estimates of phylogeny were based on a single mitochondrial marker (Cytochrome-*b*) and sampling was geographically very limited. Given the large geographic distributions of *D. rudis* and *D. valentini*, in such a heterogeneous and complex habitat, the low sampling of the above-mentioned studies is very likely responsible for under-representing intraspecific genetic structure. As a result, depending on the markers used and geographic locations sampled, *D. rudis* can appear as a sister species of *D. valentini* (Freitas, Rocha, et al., 2016), paraphyletic (Freitas, Vavakou, et al., 2016), sister species with *D. portschinskii* (Freitas, Rocha, et al., 2016) or even a sister clade to the *D. portschinskii* and *D. valentini* sister pair (Freitas, 2017). However, these trees only included a very reduced number of *D. rudis* individuals, and one of the markers used (Cytochrome-*b*) has been shown to be an unreliable marker in several *Darevskia* studies (Freitas, Vavakou, et al., 2016; Saberi-Pirooz et al., 2018), as well as in other lacertid genera (e.g. *Timon*; Miraldo et al., 2012; *Podarcis*; Podnar et al., 2007) due to the existence of nuclear copies. Very recently, Murtskhvaladze et al. (2020) analysed the phylogeny of *Darevskia* rock lizards using mitogenomes, concluding that *D. portschinskii* and *D. valentini* are sister taxa, with *D. rudis* as their sister clade. The estimated divergence time among these groups was 1 Mya. Nevertheless, the sampling from this study was restricted to Georgia, one genomic compartment, and to one individual per species. On the other hand, the recent phylogenomic study of Garcia-Porta et al. (2019), based on a data set from 262 lacertid species, suggests that *D. portschinskii* is the sister taxon of *D. rudis* and *D. valentini*, and estimated cladogenesis is 1.34 Mya.

Clearly, there are still uncertainties about the way each *rudis* taxon is phylogenetically related, and how their biogeographical history and evolutionary niche shifts across Anatolia. This lack of clarity is mainly due to limited sampling of individuals across the region, and in some cases the use of a single marker.

To overcome these limitations, in this study, we combine a multilocus approach with ecological niche models using a significant sampling effort across the distribution of *D. rudis* and *D. valentini*. We aim to assess the genetic structure and phylogenetic relationships among the taxa, contrasting the genetic information with their historical and forecasted shifts in habitat suitability. We use two mitochondrial and two nuclear DNA markers coupled with geographic information associated with those genetically assessed records, in order to: 1) build a calibrated species tree; 2) analyse the effective population size changes through time of each taxon; and to 3) infer the most likely phylogeographic shifts that might explain diversification events.

2 | MATERIAL AND METHODS

2.1 | Sampling and study area

We collected and used a total of 151 *Darevskia* specimens, with species assignment performed in the field based on external morphological traits (Arakelyan et al., 2011; Arribas et al., 2013; Doronin, 2017). These included 114 *D. rudis*, 24 *D. valentini*, six *D. portschinskii*, two *D. clarkorum*, one *D. parvula* and four *D. praticola* (information about each specimen is given in Table S1). The sampling was performed across the northern coast of Turkey, Southwest Georgia, Western Armenia and Nagorno-Karabakh (Figure 1).

2.2 | DNA extraction, amplification and sequencing

Genomic DNA was extracted from muscle tail tissue previously preserved in 96% alcohol, using a standard high-salt protocol (Sambrook et al., 1989). Polymerase chain reaction (PCR) was used to amplify two mitochondrial (mtDNA; Cytochrome-*b* and ND4) and two nuclear DNA (nDNA) fragments (MC1R and C-mos). Amplification and sequencing of Cytochrome-*b* (*cytb*) was performed using the primers Gludg (Palumbi et al., 2002) and Peil (Ptacek et al., 1994). A fragment including the terminal portion of the ND4 gene and the tRNAs for Histamine, Serine and Leucine was amplified using the primers ND4 and Leu (Arévalo et al., 1994). Regarding the MC1R and the oocyte maturation factor (C-mos) fragments, the primers MC1R_F and MC1R_R (Pinho et al., 2010), and LSC1 and LSC2 (Godinho et al., 2006) were used for both amplification and sequencing, respectively.

Amplifications were performed in 25 μ L of 5x reaction buffer, 3.2 mM MgCl₂, 0.16 mM dNTP mixture, 0.16 mM of each primer, 1U of Promega GoTaq DNA polymerase and 10–25 ng of template DNA. PCRs consisted of an initial denaturation at 94°C for 2 min, followed by 40 cycles that included a denaturation step at 92°C for 30 s, annealing at 57–65°C for 30 s and extension at 72°C for 1 min. A final extension was conducted for 5 min. Minor adjustments to conditions were required in some reactions. All PCR products were visualized on 2% agarose gels stained with GelRed nucleic acid stain (BIOTIUM), and successful amplifications were sent to Beckman Coulter Genomics (UK) for purification and Sanger sequencing.

GenBank DNA sequences for all genetic markers used in our study belonging to *Darevskia chlorogaster*, *D. defilippii* and *Iranolacerta brandtii* were added to our data set (Ahmadzadeh, Flecks, Carretero, Mozaffari, et al., 2013; Mendes et al., 2016; Pavlicev & Mayer, 2009), as well as sequences identified as *D. valentini* and *D. rudis* from previous studies (Freitas et al., 2019; Freitas, Rocha, et al., 2016; Saberi-Pirooz et al., 2018) (see Table S1 for accession numbers).

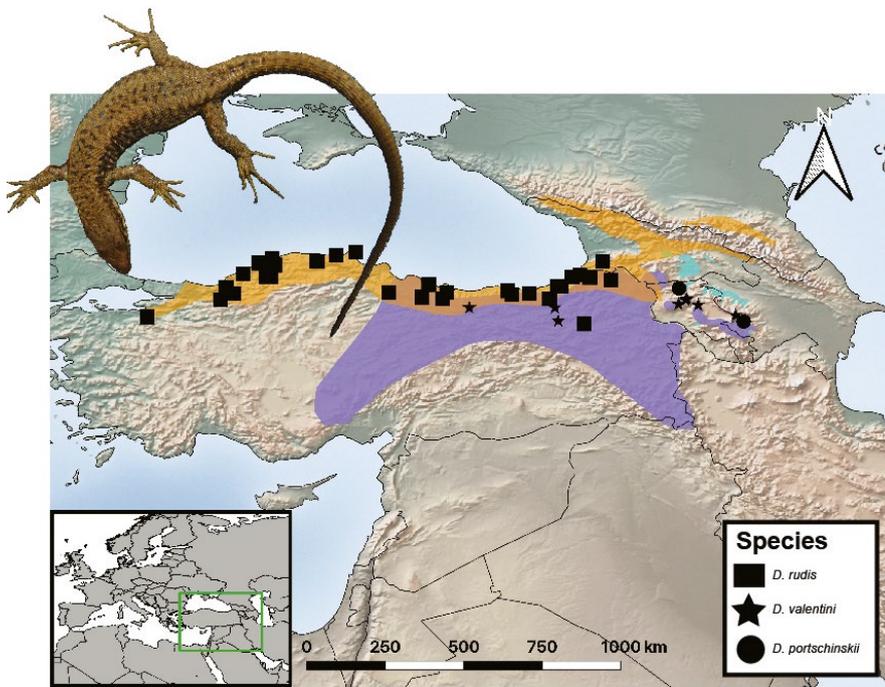


FIGURE 1 Map representing the sampling localities and IUCN geographic range of the species comprising the *D. rudis* group: the polygon in orange represents the known distribution of *D. rudis*, *D. valentini* in purple and *D. portschinskii* in light blue (Tok et al., 2009a, 2009b; Tuniyev et al., 2009) [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Phylogenetic analysis

We used Geneious Pro (Version 5.5.9; Drummond et al., 2010) to assemble the contigs and MAFFT (Version 7.0.17; Katoh & Standley, 2013) to align each set of sequences, using the default parameters. Phase reconstruction of the nDNA data set was performed using the program PHASE (Version 2.1.1; Stephens & Donnelly, 2003; Stephens et al., 2001), considering a threshold of 60% ($p = q = 0.6$), since this has been demonstrated to reduce the number of genotype uncertainties with little or no increase in false positives (Garrick et al., 2010). PHASE input files were created in SeqPHASE (Flot, 2010), and all polymorphic sites with a probability of < 0.6 were coded in both alleles with the appropriate IUPAC ambiguity code.

Phylogenetic analyses on the concatenated mitochondrial data were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods using *Iranolacerta brandtii* as an outgroup (Garcia-Porta et al., 2019). Only individuals sequenced for both the *cytb* and the *ND4* were used in this concatenated analysis.

In order to determine the best fitting nucleotide model and partition scheme for each phylogenetic analysis, we used the software PartitionFinder (Version 1.1; Lanfear et al., 2012). We tested in PartitionFinder schemes including codon partitions for ML analyses, whereas for BI analyses we selected among schemes with genes unpartitioned (to avoid over-parameterized models). Parameters used were *branchlengths = linked* and *model_selection = BIC*. We implemented partition schemes and models as described in Table S2. Before running the ML inference, identical haplotypes were collapsed using the software ALTER

(Glez-Peña et al., 2010). The ML analysis was conducted with the software GARLI (Version 2.0; Zwickl, 2006), where a tree search was performed using between 1,000 and 3,000 generations (parameter *genthreshfortopoterm*), and considering a stochastic algorithm, each resulting in a single best tree. The resulting likelihood values were compared, and the best value was obtained for 2,000 generations. Bootstrap support was calculated from 1,000 bootstrap (BP) pseudo-replicates (Felsenstein, 1985), using *genthreshfortopoterm* set to 2,000 and the best tree as a starting tree. A majority rule consensus tree was generated using the software Phyutility (Version 2.2; Smith & Dunn, 2008).

The software BEAST (Version 1.8.4; Drummond & Rambaut, 2007) was used for BI analyses. Clock models were unlinked, and trees linked as mitochondrial markers are actually linked in the genome, and a strict clock was implemented. A coalescent constant size prior was selected for the tree. Two individual runs of 30×10^6 generations were performed with a sampling frequency of 3,000. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer (Version 1.7; Rambaut et al., 2018) after obtaining an effective sample size (ESS) > 200 . Runs were combined using LogCombiner (discarding 10% of the initial runs), and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using FigTree (Version 1.4.0; Rambaut, 2009).

We inferred the phylogenetic relationships among haplotypes for each mtDNA locus, using a Median Joining network (Bandelt et al., 1999). To construct these haplotype

networks, we used the software PopART (Version 1.7; Leigh & Bryant, 2015), with the parameter epsilon set to 0. For each of the nuclear markers, we also used the parsimony connection criterion implemented in the software TCS (Version 1.21; Clement et al., 2000), and the layout was improved using tcsBU (Santos et al., 2016).

MEGA (Version 7.0.26; Kumar et al., 2016) was used to estimate uncorrected p -distances between lineages for each mtDNA marker, and DnaSP (Version 5; Rozas et al., 2003) to calculate several genetic diversity parameters.

2.4 | Species tree and divergence time estimation

We implemented a multispecies model with *BEAST, not only to determine the age of the most recent common ancestor (MRCA), but also to assess the relationships between the main clades recovered in the mtDNA phylogenetic analyses. This was performed using both mitochondrial and phased nuclear loci, and best partition and nucleotide models inferred using PartitionFinder (see Table S2). All markers were run with unlinked trees, sites and clock models so that each locus and respective priors used would not constrain the calculation of the parameters for the other marker, such as mutation rate, tree topology or branch length. A strict molecular clock was assumed, using the mutation rate for ND4 estimated for the lacertid genus *Podarcis* Wagler, 1830 (Pinho et al., 2007) and co-estimated for cytb. The *clock.rate* prior parameter for ND4 was set as a normal distribution with a mean of 0.0226 and a standard deviation of 0.0031, so that mutation rate varied between 0.0278 and 0.0174 mutation/site/million years. A uniform Yule prior was selected for the tree, with a random starting one. Two individual runs of 2×10^8 generations were performed with a sampling frequency of 10,000. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer (Version 1.7; Rambaut et al., 2018) after obtaining an effective sample size (ESS) > 200. Runs were combined using LogCombiner (discarding 10% of the initial runs), and maximum credibility trees with divergence time means and 95% highest probability densities (HPDs) were produced using Tree Annotator (both part of the BEAST package). Trees were visualized using FigTree (Version 1.4.0; Rambaut, 2009).

2.5 | Bayesian phylogeography

We inferred the diffusion of mitochondrial lineages through time for the whole *D. rudis* complex (including *D. bithynica* and *D. valentini*) using a continuous Bayesian phylogeography approach (Lemey et al., 2010), under a Gamma Relaxed Random Walk model (RRW). This method estimates

population size changes through time and also ancestral population locations. A random 'jitter' was added to each tip (window size 0.05) to aid in inference of individuals with identical GPS coordinates, and the same clock rate for ND4 from Pinho et al. (2007) was applied, as explained previously.

We used marginal likelihood estimation (MLE) and Bayes factors (BF) to select between a strict and relaxed clock model for the data. We conducted marginal likelihood estimation using Path Sampling (PS) and Stepping Stone (SS) analyses in BEAST (Version 1.8.4; Baele et al., 2012). Each clock model was first tested with analyses running for 10×10^6 generations, with sampling every 100 generations. MLE analyses were run for 50 path steps for 10^6 generations within each step and sampling every 1,000 generation. Bayes factors were calculated as two times the difference in marginal likelihood estimates between competing models and significance was determined if the BF value was >10 (Kass & Raftery, 1995). For all analyses, two independent analyses were pooled using LogCombiner. The relaxed clock was favoured over the strict (Table S3). Hence, the final Gamma RRW analysis was performed with the TN93 + I+G DNA substitution model for each mtDNA partition (as in the previous Bayesian coalescent analysis; see Table S2), with linked trees, and considering a relaxed clock. Two independent analyses were run for 25×10^6 generations, sampling every 2,500 generations, and then combined using LogCombiner. A maximum credibility tree was produced using Tree Annotator. We used the program Spread (Version 1.0.6; Bielejec et al., 2011), to produce KML files showing the 80% HPD regions of the diffusion of lineages through time on the landscape, and the TimeSlicer function to look at specific time windows.

2.6 | Population size changes

We estimated changes through time in effective population size of each mtDNA lineage using the Extended Bayesian Skyline Plot (EBS; Heled & Drummond, 2008), implemented in BEAST. We used an uncorrelated relaxed clock prior, linked trees and the ND4 clock rate after Pinho et al. (2007). The prior for the root height of the tree was given a wide uniform distribution over the interval [1, 1000] and an uninformative gamma prior (shape = 1; scale = 500) for the parameter *Demographic.popMean*. The first prior is meant to accommodate the large variation in coalescent times that can be expected under the coalescent process. The MCMC was run for a total of 2×10^8 generations (sampling every 2×10^5 steps and excluding the first 10% as burn-in). We assessed convergence by examining parameter trends between two separate runs using Tracer and built the plots using the Python scripts described in the software's tutorial.

2.7 | Environmental data

Climatic variables were obtained from WorldClim online data (www.worldclim.org; Hijmans et al., 2005). From the initial set of 19 bioclimatic variables available, we excluded BIO3, BIO14 and BIO15, as they are biased when projected to past scenarios (Bedia et al., 2013; Varela et al., 2015). From the remaining variables, we then selected seven with a Pearson correlation lower than 0.6 (Table S4). Three past climate scenarios were used: one for the Last Interglacial (LIG: ~130–116 kyr years BP; Otto-Bliesner et al., 2006) and two (CCSM and MIROC) for the Last Glacial Maximum (LGM: ~22 kyr years BP; Hijmans et al., 2005). The spatial resolution of climate variables for current and LIG past climate scenario was of 30 arc-seconds (approximately 1 km²), and for the LGM past scenarios was of 2.5 arc-minutes (approximately 5 km²).

2.8 | Ecological niche models

We modelled the realized niches (sensu Sillero, 2011) of the main clades (see below) at the present. Then, we projected the current models to the past climate scenarios. Ecological niche models were calculated using the Maximum Entropy algorithm implemented in Maxent (Version 3.4.1; Phillips et al., 2006, 2017). This algorithm does not predict the observed distribution of the species, but the suitable habitats for each clade in the study area, independently of whether they are occupied or not (Sillero, 2011). Maximum Entropy is a general-purpose machine learning method that uses presence and background data (Phillips et al., 2006, 2017). Background records are randomly selected and provide a spectrum of available conditions for the species in the whole area of study (Phillips et al., 2009). Maxent is particularly well suited to noisy or sparse information and is capable of simultaneously dealing with continuous and categorical variables (Phillips et al., 2006, 2017).

Maxent runs were performed with linear and quadratic features. Due to the limited number of presence points within each identified clade (see below), we performed the runs with all the points as training data for the clades, but with a 'leave-one-out' procedure to assess model performance (Olden & Jackson, 2000; Shcheglovitova & Anderson, 2013). For each clade, we iteratively removed one presence point from the data set and built a model with the remaining points (Pearson et al., 2007; Vale et al., 2016). We created models with different regularization multiplier values (from 0 to 2) and selected the value that maximized the model performance (Merow et al., 2013; Shcheglovitova & Anderson, 2013). A regularization multiplier lower than one produces a more restricted prediction, while a higher value results in a broader prediction (Phillips et al., 2006;

Shcheglovitova & Anderson, 2013). We calculated the arithmetic mean and the standard deviation of 10 replicates for each main clade, as machine learning method has a probabilistic component (Sillero & Carretero, 2013). We ran Maxent in clog-log format (Phillips et al., 2017) and duplicated records (i.e. records inside the same pixel) were removed.

Model performance was analysed with the area under the curve (AUC) of the receiver-operating characteristics (ROC) plot (Liu et al., 2005) and the true skill statistics (TSS; Allouche et al., 2006). Random models have an AUC equal to 0.5; the closer an AUC is to 1, the more discriminant the model is. TSS ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Additionally, we calculated a set of 100 null models following the methodology outlined by Raes and Ter Steege (2007). For this, we created 100 different data sets with the same number of random points as the species presences following a Poisson distribution. We ran these data sets and obtained the AUC values of the ROC plots. We compared the AUC species models values with the ones calculated for a null model using the Kruskal–Wallis test. Null models were calculated in R (Version 3.4.4; R Core Team, 2018) using the 'dismo' package (Hijmans et al., 2017).

The importance of each environmental variable was determined by the average percentage of contribution and permutation importance of each variable to the models (Phillips et al., 2006) through factor analysis: (a) a jackknife analysis of the average AUC using training and test data; and (b) a calculation of the average percentage contribution of each variable to the models. For this purpose, the variables were excluded in turn and a model was created with the remaining variables. A model was then created using each variable. Finally, the models were averaged so that each clade gets one habitat suitability map for the present and two for the past, using Quantum GIS (Version 3.4; Qgis Development Team, 2020).

3 | RESULTS

3.1 | Phylogenetic analysis and molecular dating

We found a striking lack of monophyly in the mitochondrial phylogenetic analysis among the *Darevskia rudis* and *D. valentini* individuals (Figure 2c). Both Bayesian and Maximum Likelihood phylogenetic analyses using cytb (963 bp) and ND4 (840 bp) mitochondrial markers recovered four distinct geographically coherent clades, although their phylogenetic relationships were slightly different between the two methodologies (Figure 2c and Figure S1). One clade comprises the individuals from western Anatolia, fully coinciding with

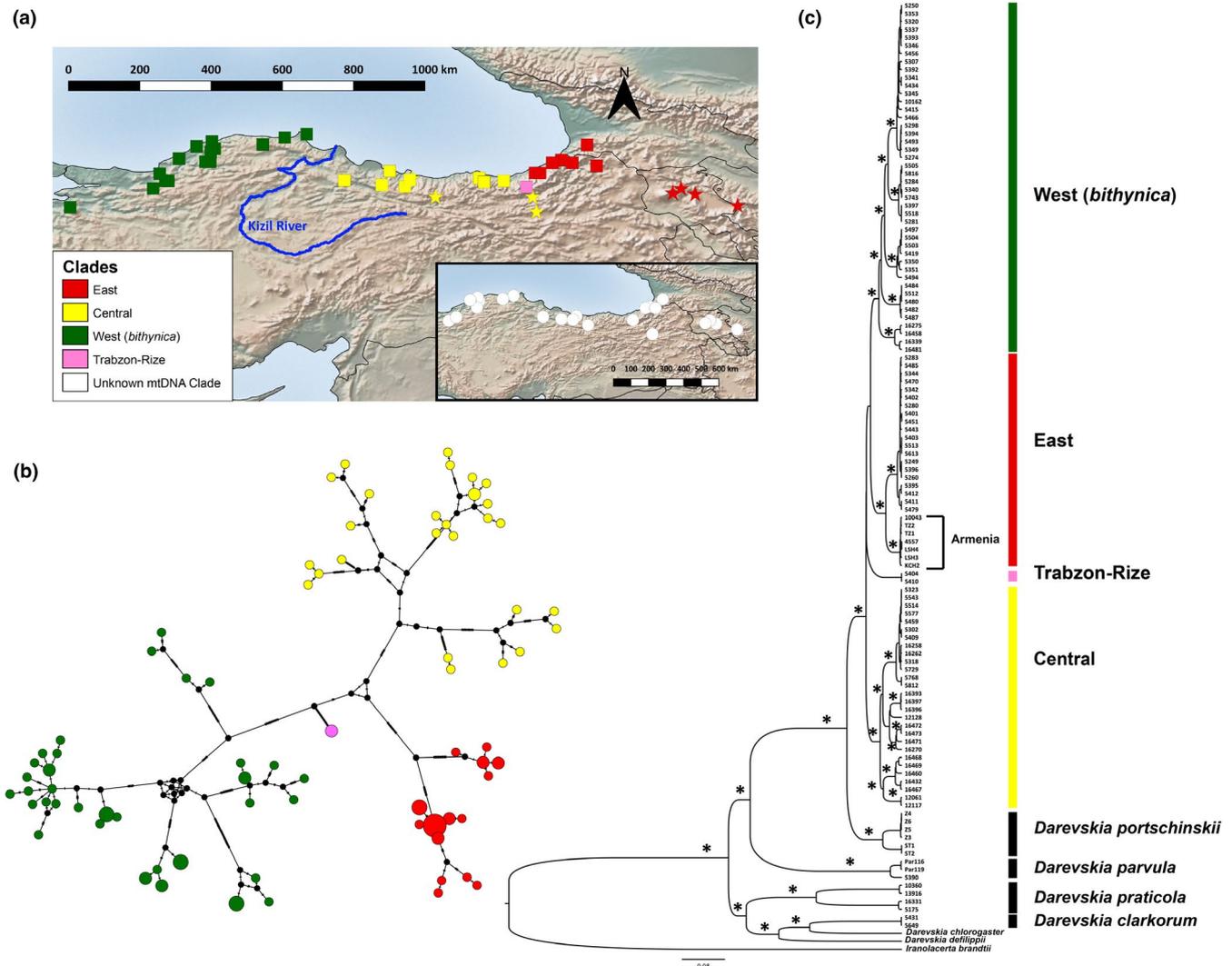


FIGURE 2 Map representing the geographic distribution of each clade (a), the median joining haplotype network (b) and Bayesian phylogenetic tree (c) estimated for the concatenated mtDNA data set (cytb + ND4). In (a), the squares represent individuals assigned as *D. rudis* and the stars as *D. valentini*. Inset map depicts the geographic location of individuals not assigned to any mitochondrial clade. The colours in the haplotype network match with the groups obtained in the phylogenetic tree. The sizes of the circles are proportional to the frequency of each haplotype. Black circles represent hypothetical haplotypes, and the mutations are shown as lines in the branches. Asterisks next to the nodes represent the posterior probabilities $\geq 90\%$. Detailed information about each specimen is given in Table S1 [Colour figure can be viewed at wileyonlinelibrary.com]

the geographic distribution reported for *Darevskia bithynica* (Figure 2a, in green) (Arribas et al., 2013). Its sister clade includes the individuals distributed across north-eastern Anatolia and southern Armenia, while the individuals occurring in north-central Anatolia represent another clade (Central Clade). Finally, two individuals from the Trabzon-Rize region (eastern Anatolia) cluster together to form a differentiated mitochondrial clade. Although with very low support (51.9%), the ML analysis (Figure S1) rendered *D. rudis*/*D. valentini* paraphyletic due to the phylogenetic position of *D. portschinskii*, while according to the BI topology (Figure 2c), the group remained as a highly supported monophyletic.

The uncorrected genetic *p*-distances between clades obtained ranged from 3.8% (Trabzon-Rize and East) to 4.8%

(*bithynica* and Central clades) for the cytb and from 3.4% (*bithynica* and East) to 4.1% (*bithynica* and Trabzon-Rize) for the ND4 marker (Table 1). The *bithynica* and Central clades were those presenting the highest values of mitochondrial genetic diversity, which is also evidenced by the haplotype networks (Figure 2b; Figure S2). The Trabzon-Rize clade was represented solely by two individuals which were identical for both mtDNA markers.

The parsimony haplotype networks obtained for each nuclear marker showed considerable haplotype sharing among mitochondrial clades, especially for the MC1R. Nevertheless, the C-mos gene fragment shows the existence of exclusive haplotypes for the West clade, and other haplotypes from the East clade endemic to Armenia (Figure 3).

TABLE 1 Genetic distances between clades for each mitochondrial locus (cytb and ND4), and genetic diversity within clade

ND4 Cytb	Genetic <i>p</i> -distance				Genetic Diversity Cytb (ND4)		
	West (<i>bithynica</i>)	East	Central	Trabzon- Rize	Na ^a	Hd ^b	π^c
West (<i>bithynica</i>)		0.03431	0.03868	0.04098	29 (25)	0.973 (0.961)	0.02157 (0.01864)
East	0.04137		0.03457	0.03945	9 (11)	0.781 (0.840)	0.00942 (0.00977)
Central	0.04780	0.03825		0.03941	19 (25)	0.966 (0.989)	0.02214 (0.02002)
Trabzon-Rize	0.04218	0.03808	0.03945		1 (1)	0.000 (0.000)	0.000 (0.000)

^aNumber of haplotypes.^bHaplotype diversity.^cNucleotide diversity.

3.2 | Species tree estimation

The topology obtained with the multilocus species tree (Figure 4) is similar to that from the mitochondrial BI phylogeny except regarding the position of *D. parvula*, for which only one individual was used. The most important result from this analysis was the paraphyly of the *D. rudis*/*D. valentini* group, contrary to what was attained in other studies (Freitas, Rocha, et al., 2016; Tarkhnishvili et al., 2013) phylogenetic position of the Trabzon-Rize clade is highly supported (92%), but the other relationships are not. The diversification of the group started around 1.3 Mya (during the lower Pleistocene), with the Trabzon-Rize clade having a basal position. The remaining clades diversified afterwards, with the West and East taxa separating last, around 0.97 Mya.

3.3 | Bayesian phylogeography

According to the obtained gamma relaxed diffusion model, the diversification of the *rudis* group's MRCA started approximately 1.3 Mya in the Ordu province, Turkey (Figure 5). During this period, the geographic distribution of the species was estimated to be subdivided into a West and an East region, which has been maintained across time. Around 1.16 Mya, the West group started to expand eastwards and the East group regressed. Nearly 1 Mya the geographic distribution of

the species was very similar to what is observed 1.3 Mya. Finally, nearly 0.5 Mya all the extant taxa had already diversified and were distributed across a West, Central and East Anatolian geographic areas, hence, in a very similar way to what is observed today (Figure 2a).

3.4 | Population size changes

Because the Trabzon-Rize clade is composed of only two individuals, the EBSP was not performed for this group (Figure 6). This analysis shows that all other clades have maintained a stable population size across time. This is supported by the lack of star-shape haplotypes in the mtDNA networks (Figure 2b; Figure S2) and a RRW model not indicating any great population geographic expansion (Figure 5). The West clade is the one depicting a higher ancestral population effective size and the East clade the lowest, which is still observed today by their current genetic diversity (Table 1).

3.5 | Ecological niche models

The Maxent models were not performed for the Trabzon-Rize clade, due to the low number of presence points (only two). For the three other main clades, the models were built on 15 presence points for the West clade, 11

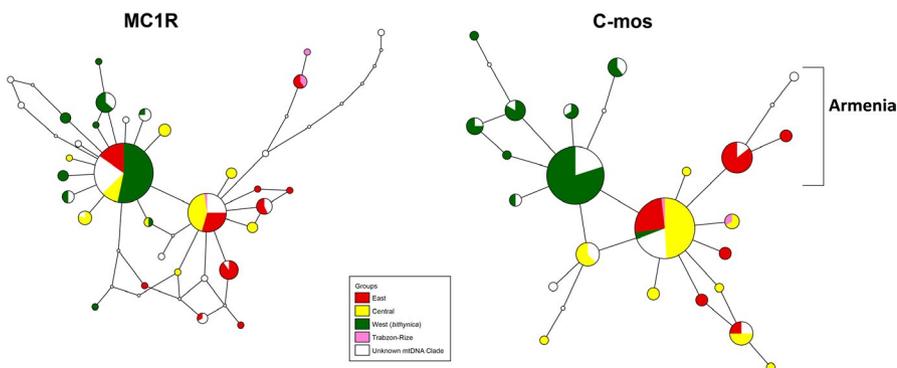


FIGURE 3 Haplotype networks estimated under the parsimony connection criterion for each nuclear marker (MC1R and C-mos). The colours match with the groups obtained in the phylogenetic tree from Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

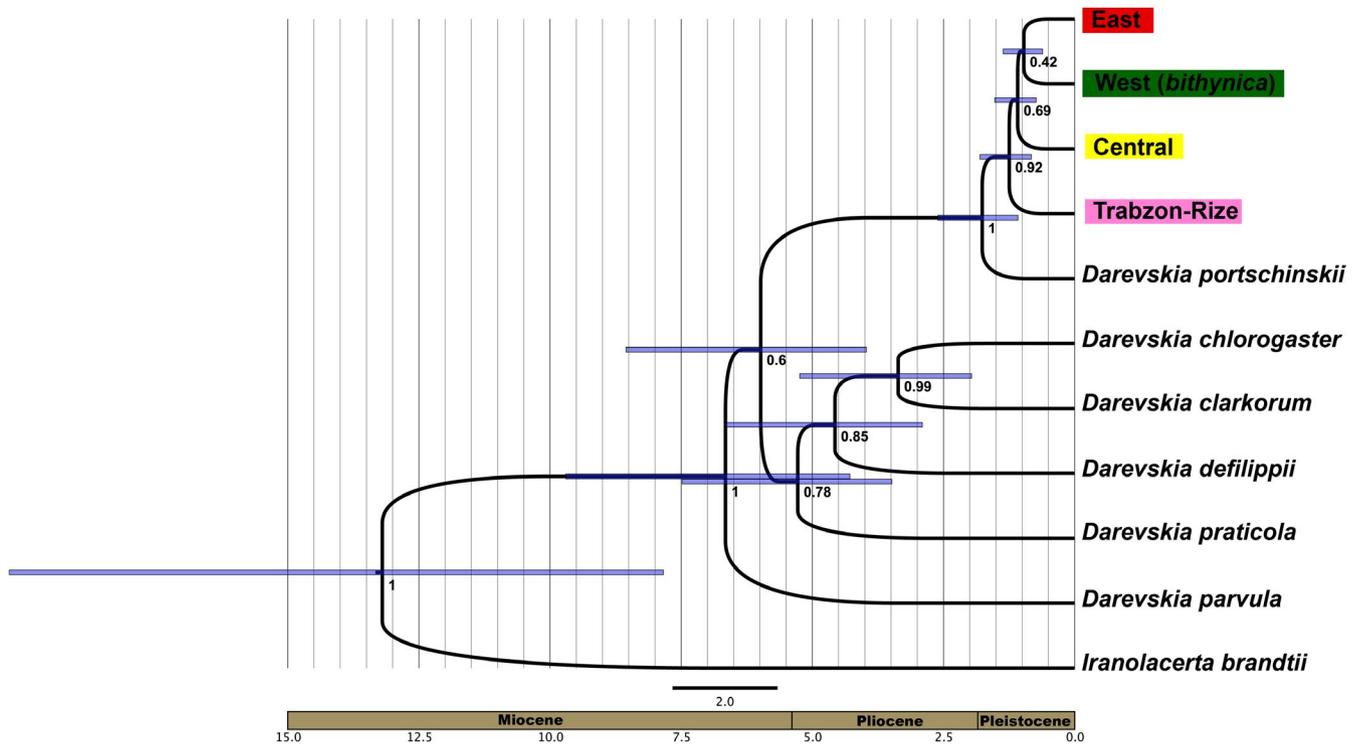


FIGURE 4 Species tree depicting phylogenetic relationships between the main lineages of the *D. rudis*/*D. valentini* species group, inferred with *BEAST using mitochondrial (cytb and ND4) and nuclear (MC1R and C-mos) DNA sequence data. Posterior Probability values of each node are reported next to the branches, and the blue bars indicate 95% HPDs of estimated divergence times. Below the tree is represented the timeline and duration of each geological time [Colour figure can be viewed at wileyonlinelibrary.com]

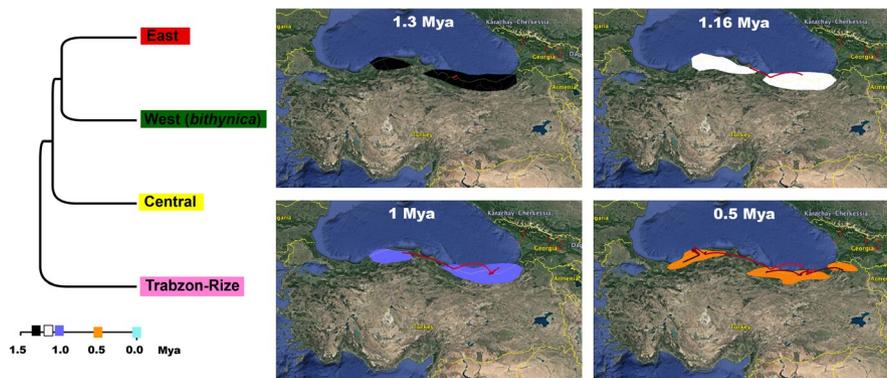


FIGURE 5 Continuous diffusion phylogeographic reconstruction for the *D. rudis*/*D. valentini* species group at three time slices (1.3 Mya, 1.16 Mya, 1 Mya and 0.5 Mya), as represented in the species tree on the left. The MCC gene tree is denoted with a red-black colour gradient corresponding to the relative age of dispersal. The polygons in the map represent the 80% HPD intervals for the location of each node of the sampled genealogies, a measure of the uncertainty of the estimated location. The colours of the polygons at each time slice are the same as the ones represented in the species tree time scale. The red dot in the 1.3 Mya time slice corresponds to the estimated location of the MRCA [Colour figure can be viewed at wileyonlinelibrary.com]

for the East clade and 12 for the Central clade. All models attained mean AUC values higher than 0.9 (West: $\bar{x} = 0.962$, $SD = 0.004$; East: $\bar{x} = 0.956$, $SD = 0.006$; Central: $\bar{x} = 0.942$, $SD = 0.010$). The clade models were significantly better than the null models (Kruskal–Wallis; West: $\chi^2 = 38.768$, $df = 1$, $p = 4.7E-10$; East: $\chi^2 = 29.465$, $df = 1$, $p = 5.69E-08$; Central: $\chi^2 = 31.859$, $df = 1$, $p = 1.66E-08$). The TSS values showed also good model

performance (West: $\bar{x} = 0.804$, $SD = 0.044$; \bar{x} = East: 0.777 , $SD = 0.059$; Central: $\bar{x} = 0.667$, $SD = 0.066$).

The variables that more strongly contributed to the western and Central models were temperature seasonality, mean temperature of the wettest quarter and mean diurnal range (Table S4). For the eastern clade model, annual precipitation, maximum temperature of the warmest month and mean

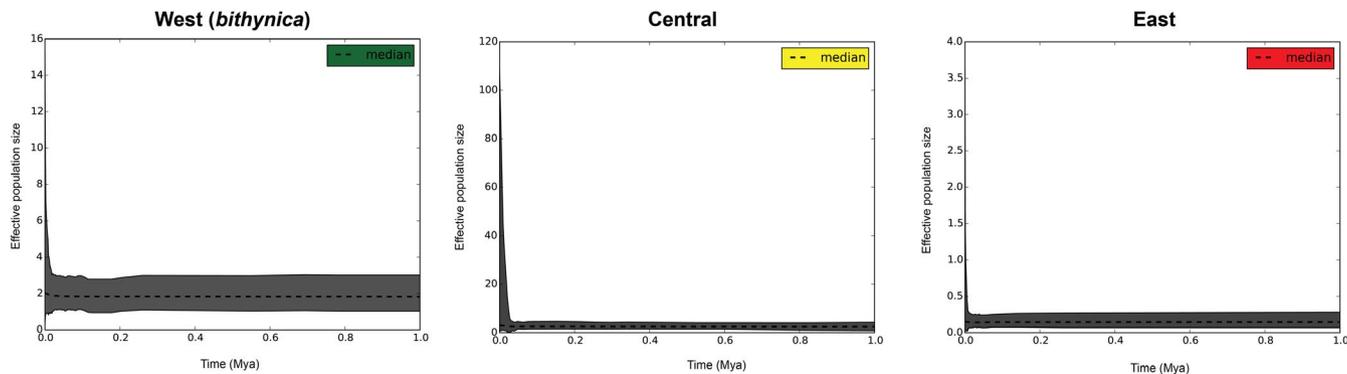


FIGURE 6 Extended Bayesian Skyline Plot (EBS), representing the effective population size through time for the West, East and Central mtDNA clades of the *D. rudis/D. valentini* group. The median population size (dotted line) is represented together with the lower and higher bounds of its 95% HPD (grey area). The x-axis represents the temporal scale in millions of years (Mya). Population size is represented in the y-axis (units = population size \times generation time) [Colour figure can be viewed at wileyonlinelibrary.com]

temperature of the wettest quarter were the ones that affected the model the most.

The current suitable area of the western clade was mostly located along the west coast of Anatolia (Figure 7). On the contrary, the current suitable habitat for the eastern clade was mostly at the east coast of Anatolia, Georgia, and slightly in Armenia and southern Russia. The current suitable area for the Central clade is restricted to a small area at the Pontus and Paphlagonia regions.

When projecting to the past scenarios, there was almost no suitable area for the *bithynica* and the Eastern clades during the LIG, but larger areas during the LGM than at present. The opposite happens with the Central clade, with a wide suitable area during the LIG and almost none during the LGM.

4 | DISCUSSION

Due to its geographic position and complex orogeny, the Anatolian Peninsula has repeatedly acted as both a barrier and a bridge for biodiversity between Asia and Europe (Bilgin, 2011). In particular, the Pontic Mountains in northern Anatolia and immediately south of the Black Sea clearly separate the Pontic region from the Anatolian Plateau. Within the Pontic region, the rivers Kizil and Yezil dissect the Pontic Mountains into western and eastern sections (Sindaco et al., 2000). During glacial stages, high mountains acted as barriers to species dispersal, while during interglacial phases, individuals dispersing from their refugia will often meet, promoting secondary contacts and hybridization among partially distinct lineages. Indeed, such orographic and climatic patterns disrupting gene flow and promoting isolation have been invoked to explain the great cryptic genetic diversity observed in multiple Anatolian herpetofaunal taxa including amphibians (Akin et al., 2010; Gvoždík et al., 2015; Stöck et al., 2012; Veith & Steinfartz, 2004), chelonians (Fritz et al., 2008, 2009; Mashkaryan et al., 2014; Vamberger

et al., 2013), lizards (Bellati et al., 2015; Kapli et al., 2013; Kornilios et al., 2019), worm lizards (Sindaco et al., 2014) and snakes (Guicking et al., 2009; Jablonski et al., 2019; Kindler et al., 2013; Kornilios et al., 2011).

According to our results, the individuals identified either as *Darevskia rudis* or as *D. valentini* appear as paraphyletic and substructured into four mitochondrial clades. These started to differentiate during the lower Pleistocene (around 1.3 Mya, Figure 4), soon after the separation of *D. portschinskii* from their common ancestor. The estimated timing of divergence obtained here is slightly older than that reported in previous studies (Mid-Pleistocene in Tarkhnishvili, 2012; Tarkhnishvili et al., 2013), but similar to the estimates in Murtskhvaladze et al. (2020) (1 Mya) and Garcia-Porta et al. (2019) (1.34 Mya). However, the proposed estimates of relationships among these taxa seem to be as many as the studies investigating them. For instance, in some studies, *D. portschinskii* and *D. rudis* appear as sister taxa, with *D. valentini* in a basal position (Freitas, Rocha, et al., 2016; Freitas, Vavakou, et al., 2016), while in others *D. portschinskii* clusters with *D. valentini*, and *D. rudis* is their sister taxon (Freitas, 2017; Murtskhvaladze et al., 2020). Although the support of *D. portschinskii* as sister taxon to *D. rudis* and *D. valentini* is strong in our study, the same cannot be said concerning the remaining clades (Figure 4). This newly obtained phylogenetic pattern showing a lack of differentiation between *D. rudis* and *D. valentini* specimens is essentially the consequence of our improved sampling in comparison to the previous studies. Indeed, in every prior study (Freitas, Rocha, et al., 2016; Freitas, Vavakou, et al., 2016; Tarkhnishvili, 2012; Tarkhnishvili et al., 2013), all sequenced individuals of *D. valentini* were from Armenia, while sampling of *D. rudis* was limited to eastern Anatolia. Essentially, the phylogenetic relationships obtained from these studies are a subset of our results; from the eastern clade, previous studies only included Armenian *D. valentini*, a few specimens of *D. rudis* from the Central clade, and none from western

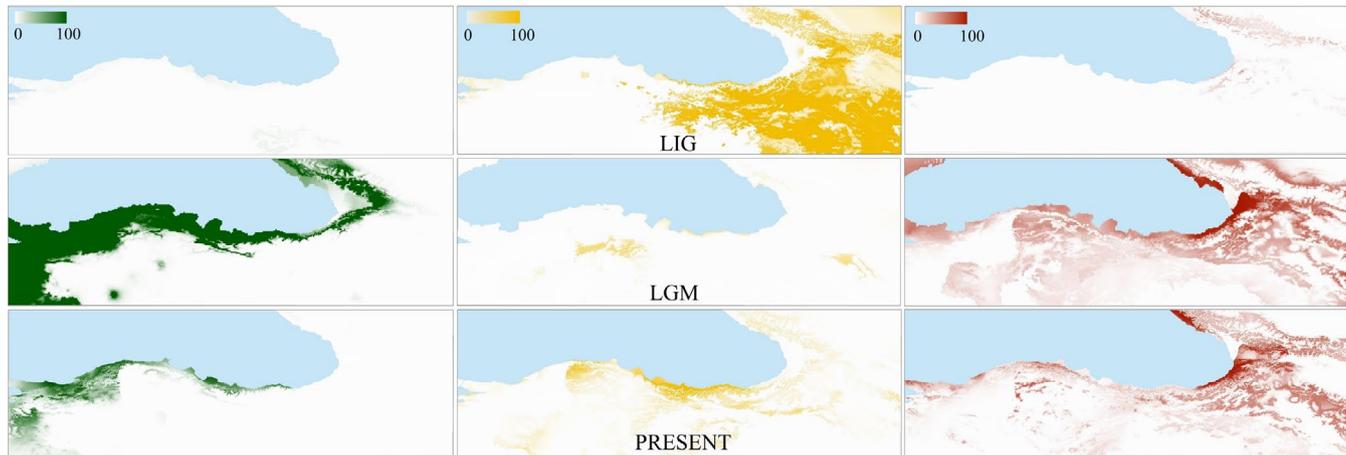


FIGURE 7 Models of the present distribution of the West (green), Central (yellow) and East (red) clades and projections to the past scenarios (LIG and LGM) [Colour figure can be viewed at wileyonlinelibrary.com]

Anatolia or the Trabzon-Rize region. Hence, unsurprisingly, their results supported *D. rudis* and *D. valentini* as two monophyletic taxa.

Furthermore, after a sampling weighted towards the Caucasus region, Tarkhnishvili et al. (2013) concluded that the common ancestor of *D. valentini* and *D. rudis* came probably from the Lesser Caucasus and outside the immediate Black Sea area. In contrast, our results from the Bayesian phylogeography analysis (Figure 5) suggest that the diversification of this group took place most likely in the Ordu area (Anatolia), followed by the establishment of two distinct geographic clusters separated by the Kizil River valley, a well-recognized barrier dividing the Pontic Mountains into western and eastern sections (Sindaco et al., 2000). Remarkably, along with the diversification of *D. rudis* and *D. valentini*, the Kizil River has remained a permanent geographic barrier and a strong vicariant candidate to explain the origin of *D. bithynica* by allopatric speciation. Indeed, the Pontic Region has already been identified as a possible refugium and the origin for radiation in the Anatolian mountain frogs (Veith et al., 2003), and an important dispersal route for many Euro-Siberian reptiles, including the lizard *Lacerta viridis* (Mashkaryan et al., 2014) and the snakes *Coronella austriaca*, *Zamenis longissimus* and *Vipera trauscaucasiana* (Billing et al., 1990). Interestingly, Arribas et al. (2013) had already suggested that the Pontic mountains had played an important role in the speciation and differentiation of *D. bithynica*, but in this study we were able to pinpoint the most likely geographic barrier for the species, promoting its isolation and diversification from the remaining members of the *rudis* complex.

Additionally, beyond the preliminary genetic analysis by Koç et al. (2017), our study is also the first one corroborating *D. bithynica* as a full species, with supporting evidence from both mitochondrial and nuclear DNA markers.

However, the recognition of this species together with the clade structure and evidence of gene flow between the other clades strongly questions the validity of the taxonomic arrangement with *D. rudis* and *D. valentini* as two independent taxa.

The hypothesis of vicariance as the speciation process generating *D. bithynica* is also supported by their present potential ecological niche models (Figure 7). Between the western (*bithynica*) and central clades, there is clear ecological niche overlap, both sharing the same bioclimatic variables contributing to their distribution models (Table S4). The eastern and western clades also share part of their potential distribution range. During allopatric speciation, the preservation of ecological similarity among populations over time (niche conservatism; Peterson, 2011; Wiens & Graham, 2005) is common. Limited adaptation to the ecological conditions at the geographic barrier can promote genetic isolation and differentiation between vicariant populations (Ahmadzadeh, Flecks, Carretero, Böhme, et al., 2013; Ahmadzadeh, Flecks, Carretero, Mozaffari, et al., 2013; Cadena et al., 2012; Hua & Wiens, 2013; Kozak & Wiens, 2007; Rato et al., 2015; Wiens, 2004). This is the most likely scenario responsible for *D. bithynica*'s speciation from the remaining clades of the *rudis* group.

In general, the Bayesian phylogeography model (Figure 5) does not show notable geographic expansion events during the differentiation of the *rudis* complex. This is corroborated by the EBSP analysis, which showed there were no changes in the effective population size through time (Figure 6). Accordingly, neither mtDNA nor nDNA haplotype networks (Figures 2b and 3; Figure S2) display a star-shaped configuration, which would be typical of a population expansion.

According to the projections of the ecological niche models to the past, during the Last Interglacial (~120–40 kyr years BP) the environment was unsuitable for both the western and

eastern clades, while favourable for the central clade with a massive niche shift compared to their present distribution. However, during the Last Glacial Maximum (~22 kyr years BP), the distribution of both the western and eastern clades increased considerably, and the opposite occurred for the central clade. Currently, the suitable areas for both western and eastern clades are smaller when compared to the ones during the LGM and slightly larger regarding the central clade. Despite these inferred range fluctuations over time, the molecular markers used in this study did not detect any population size differences. Presumably, the decrease in the range from the LGM to the present erased any signs of ancient population expansion, since it seems unlikely that a past niche shift would have had no populational effect.

Surprisingly, aridification of the habitat and colder temperatures during glacial periods seem to have provided favourable environmental conditions for both *D. bithynica* and the eastern clade, contrary to a wetter LIG environment. During glacial periods, they were likely pushed to the Pontic mountain tops where they were able to thrive. As far as we know, Late Pleistocene Anatolian glaciers developed solely in high mountains, such as Taurus (in south-eastern Anatolia) and the eastern Black Sea (Sarıkaya & Ciner, 2015). Consequently, at least around the western and central regions of the Black Sea, the populations were probably not organized in isolated ‘pockets’, but instead connected and able to spread. On the other hand, the eastern Black Sea Mountains might have acted as a refugium for the Trabzon-Rize clade. Although we could not assess the ecological niche models for this clade due to its small sample size, these mountains are a candidate for the origin and point of diversification for the Trabzon-Rize clade during Pleistocene's climatic oscillations. Indeed, several studies have demonstrated the hypothesis of a circum-Black Sea Pleistocene refugia for populations of distinct herpetofaunal groups (e.g. Fritz et al., 2009; Guicking et al., 2009; Gvoždík et al., 2015; Stöck et al., 2012), as seems to be the case in the *rudis* species complex.

When present and/or past gene flow occurs among taxa, evolutionary biologists are sometimes challenged with poorly resolved phylogenetic relationships and a lack of monophyly inferred from individual genealogies. Initial studies (Tarkhishvili et al., 2013) detected little current gene flow between *D. rudis* and *D. valentini*, in contrast to the high level of recombinant gene flow from *D. rudis* to *D. portschinskii*. However, more recently, the existence of past gene flow between *D. rudis* and both *D. valentini* and *D. portschinskii* has been described using genomic data (Freitas, 2017). In fact, while both the species tree and BI analysis show that the most basal split among these three species separates *D. portschinskii* of the *D. rudis*/*D. valentini* pair (as in Garcia-Porta et al., 2019), we cannot ignore that the ML analysis (Figure S1) produced an alternative topology, although

without strong support. According to the ML phylogeny, *D. portschinskii* is sister to the Trabzon-Rize clade, both having a basal position, and rendering the *rudis* group as paraphyletic. We interpret this as further evidence that the *rudis* group is comprised of recently separated species, making it difficult to obtain fully resolved phylogenies.

Although we cannot ascertain if gene flow is currently taking place among all species of the *rudis* group, both mt- and nDNA results clearly indicate past hybridization at different time scales between *D. rudis* and *D. valentini*; mitochondrial phylogenies show a lack of monophyly, and nDNA haplotype networks display a clear pattern of incomplete lineage sorting with several shared haplotypes. The only exception is *D. bithynica*, which has exclusive haplotypes for C-mos, corroborating its recognition as an independent taxon.

In fact, hybridization among *Darevskia* species is well-known and the base for the creation of parthenogenetic forms in this lizard group. These events took place during the Pleistocene climate cycles (Freitas, Rocha, et al., 2016), when subsequent expansion–contraction of populations would have allowed the secondary contact among sexual *Darevskia* lineages in incomplete stages of reproductive isolation (Vrijenhoek, 1989). Most likely, these hybridization episodes occurred several times and in separate geographic areas. In some cases, such episodes originated parthenogenetic hybrids with limited backcrossing with the parentals (Freitas et al., 2019; Freitas, Rocha, et al., 2016), while in cases such as the one reported here, it seems to have resulted in massive introgression and incomplete gene sorting.

Overall, this is yet another study endorsing the complexity within *Darevskia* rock lizards regarding both speciation/differentiation and biogeography. Beyond the karyological traits shaping reproductive compatibility between members of the genus in other areas (Kupriyanova, 2010; Murphy et al., 2000), the biogeographical context and in particular the current and past topographic and climatic characteristics of the region played a fundamental role in the diversification of North Anatolian *Darevskia*.

To conclude, our research highlights the importance of extensive sampling across a group's distribution range, in order to obtain a more accurate phylogenetic/phylogeographic pattern. By including a more representative data set of the total distribution range, we were able to uncover the complexity of this lizard group inferring its evolutionary dynamics in space and time. Besides the focus on the group of species, this study also shows how the biogeographical complexity of a region will be reflected in a compound evolutionary history of its current biodiversity. However, further sampling is still of paramount importance to fully assess the evolutionary history of the *rudis* species complex; specifically, more specimens identified as *D. valentini* from south-western Georgia and south of the Pontic Mountains are needed, covering the entire range of this taxon. Our

study also raises questions concerning the validity of both *D. rudis* and *D. valentini* as two independent species, highlighting the need for further taxonomic changes within the *rudis* group.

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ORCID

Catarina Rato  <https://orcid.org/0000-0002-8292-2433>

Diana Sousa-Guedes  <https://orcid.org/0000-0002-4496-6283>

Neftali Sillero  <https://orcid.org/0000-0002-3490-3780>

Miguel A. Carretero  <https://orcid.org/0000-0002-2335-7198>

Claudia Corti  <https://orcid.org/0000-0001-6015-0092>

REFERENCES

- Ahmadzadeh, F., Carretero, M. A., Rödder, D., Harris, D. J., Freitas, S. N., Perera, A., & Böhme, W. (2013). Inferring the effects of past climate fluctuations on the distribution pattern of *Iranolacerta* (Reptilia, Lacertidae): Evidence from mitochondrial DNA and species distribution models. *Zoologischer Anzeiger*, 252, 141–148. <https://doi.org/10.1016/j.jcz.2012.05.002>
- Ahmadzadeh, F., Flecks, M., Carretero, M. A., Böhme, W., Ilgaz, C., Engler, J. O., James Harris, D., Üzüüm, N., & Rödder, D. (2013). Rapid lizard radiation lacking niche conservatism: Ecological diversification within a complex landscape. *Journal of Biogeography*, 40(9), 1807–1818. <https://doi.org/10.1111/jbi.12121>
- Ahmadzadeh, F., Flecks, M., Carretero, M. A., Mozaffari, O., Böhme, W., Harris, D. J., Freitas, S., & Rödder, D. (2013). Cryptic speciation patterns in Iranian rock lizards uncovered by integrative taxonomy. *PLoS One*, 8(12), e80563. <https://doi.org/10.1371/journal.pone.0080563>
- Akın, Ç., Can Bilgin, C., Beerli, P., Westaway, R., Ohst, T., Litvinchuk, S. N., Uzzell, T., Bilgin, M., Hotz, H., Guex, G.-D., & Plötner, J. (2010). Phylogeographic patterns of genetic diversity in eastern Mediterranean water frogs were determined by geological processes and climate change in the Late Cenozoic. *Journal of Biogeography*, 37, 2111–2124. <https://doi.org/10.1111/j.1365-2699.2010.02368.x>
- Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, 43, 1223–1232. <https://doi.org/10.1111/j.1365-2664.2006.01214.x>
- Arakelyan, M., Danielyan, F. D., Corti, C., Sindaco, R., & Levinton, A. E. (2011). *Herpetofauna of Armenia and Nagorno Karabakh*. Society for the Study of Amphibians and Reptiles.
- Arévalo, E., Davis, S. K., & Sites, J. W. Jr (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology*, 43, 387–418.
- Arnold, E. N., Arribas, O., & Carranza, A. S. (2007). Systematics of the palaearctic and oriental lizard tribe Lacertini (Squamata: Lacertidae: Lacertinae), with description of eight new genera. *Zootaxa*, 1430, 1–86.
- Arribas, O., Ilgaz, Ç., Kumlutaş, Y., Durmuş, S. H., Avci, A., & Üzüüm, N. (2013). External morphology and osteology of *Darevskia rudis* (Bedriaga, 1886), with a taxonomic revision of the Pontic and Small-Caucasus populations (Squamata: Lacertidae). *Zootaxa*, 3626(4), 401–428.
- Avci, A., Ilgaz, Ç., Bozkurt, E., Üzüüm, N., & Olgun, K. (2015). A new record of *Iranolacerta brandtii* (de Filippi, 1863) (Sauria: Lacertidae) in eastern Anatolia, Turkey. *Russian Journal of Herpetology*, 22(1), 68–74.
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. V. (2012). Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, 29, 2157–2167. <https://doi.org/10.1093/molbev/mss084>
- Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16, 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Bedia, J., Herrera, S., & Gutiérrez, J. M. (2013). Dangers of using global bioclimatic datasets for ecological niche modeling. Limitations for future climate projections. *Global and Planetary Change*, 107, 1–12. <https://doi.org/10.1016/j.gloplacha.2013.04.005>
- Bellati, A., Carranza, S., Garcia-Porta, J., Fasola, M., & Sindaco, R. (2015). Cryptic diversity within the *Anatololacerta* species complex (Squamata: Lacertidae) in the Anatolian Peninsula: Evidence from a multi-locus approach. *Molecular Phylogenetics and Evolution*, 82, 219–233. <https://doi.org/10.1016/j.ympev.2014.10.003>
- Bielejec, F., Rambaut, A., Suchard, M. A., & Lemey, P. (2011). SPREAD: Spatial Phylogenetic Reconstruction of Evolutionary Dynamics [Computer software]. *Bioinformatics*, 27(20), 2910–2912. <https://doi.org/10.1093/bioinformatics/btr481>
- Bilgin, R. (2011). Back to the suture: The distribution of intraspecific genetic diversity in and around Anatolia. *International Journal of Molecular Sciences*, 12, 4080–4103. <https://doi.org/10.3390/ijms12064080>
- Billing, H., Nilson, G., & Sattler, U. (1990). *Vipera pontica* sp.n. a new viper species in the *kaznakovi* group (Reptilia, Viperidae) from northeastern Turkey and adjacent Transcaucasia. *Zoologica Scripta*, 19(2), 227–231.
- Cadena, C. D., Kozak, K. H., Gómez, J. P., Parra, J. L., McCain, C. M., Bowie, R. C. K., Carnaval, A. C., Moritz, C., Rahbek, C., Roberts, T. E., Sanders, N. J., Schneider, C. J., VanDerWal, J., Zamudio, K. R., & Graham, C. H. (2012). Latitude, elevational climatic zonation and speciation in the New World vertebrates. *Proceedings of the Royal Society B*, 279(1726), 194–201.
- Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies [Computer Software]. *Molecular Ecology*, 9(10), 1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>

- Danielyan, F., Arakelyan, M., & Stepanyan, I. (2008). Hybrids of *Darevskia valentini*, *D. armeniaca* and *D. unisexualis* from a sympatric population in Armenia. *Amphibia Reptilia*, 29(4), 487–504. <https://doi.org/10.1163/156853808786230424>
- Darevsky, I. S. (1967). *Rock lizards of the Caucasus: systematics, ecology and phylogeny of the polymorphic groups of Caucasian rock lizards of the subgenus Archaeolacerta*. Nauka.
- Doronin, I. V. (2017). Review of type specimens of the Rock Lizards of *Darevskia (rudis)* complex (Sauria: Lacertidae). *Proceedings of the Zoological Institute of the Russian Academy of Sciences*, 321, 339–361.
- Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Field, M., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., & Wilson, A. (2010). *Geneious [Computer software]*. <http://www.geneious.com/>
- Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees [Computer software]. *BMC Evolutionary Biology*, 7, 214. <https://doi.org/10.1186/1471-2148-7-214>
- Dufresnes, C., Litvinchuk, S. N., Leuenberger, J., Ghali, K., Zinenko, O., Stöck, M., & Perrin, N. (2016). Evolutionary melting pots: A biodiversity hotspot shaped by ring diversifications around the Black Sea in the Eastern tree frog (*Hyla orientalis*). *Molecular Ecology*, 25, 4285–4300. <https://doi.org/10.1111/mec.13706>
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39(4), 783. <https://doi.org/10.2307/2408678>
- Ficetola, G. F., Bonardi, A., Sindaco, R., & Padoa-Schioppa, E. (2013). Estimating patterns of reptile biodiversity in remote regions. *Journal of Biogeography*, 40(6), 1202–1211. <https://doi.org/10.1111/jbi.12060>
- Flot, J. F. (2010). SeqPHASE: A web tool for interconverting PHASE input/output files and FASTA sequence alignments [Computer software]. *Molecular Ecology Resources*, 10, 162–166. <https://doi.org/10.1111/j.1755-0998.2009.02732.x>
- Freitas, S. (2017). *Why sex? Darevskia answers*. PhD, University of Porto, Portugal.
- Freitas, S., Harris, D. J., Sillero, N., Arakelyan, M., Butlin, R. K., & Carretero, M. A. (2019). The role of hybridisation in the origin and evolutionary persistence of vertebrate parthenogens: A case study of *Darevskia* lizards. *Heredity*, 123(6), 795–808. <https://doi.org/10.1038/s41437-41019-40256-41435>
- Freitas, S., Rocha, S., Campos, J., Ahmadzadeh, F., Corti, C., Sillero, N., Ilgaz, Ç., Kumlutaş, Y., Arakelyan, M., Harris, D. J., & Carretero, M. A. (2016). Parthenogenesis through the ice ages: A biogeographic analysis of Caucasian rock lizards (genus *Darevskia*). *Molecular Phylogenetics and Evolution*, 102, 117–127. <https://doi.org/10.1016/j.ympev.2016.05.035>
- Freitas, S., Vavakou, A., Arakelyan, M., Drovetski, S. V., Crnobrnja-isailović, J., Kidov, A. A., Cogălniceanu, D., Corti, C., Lymberakis, P., Harris, D. J., & Carretero, M. A. (2016). Cryptic diversity and unexpected evolutionary patterns in the meadow lizard, *Darevskia praticola* (Eversmann, 1834). *Systematics and Biodiversity*, 14(2), 184–197.
- Fritz, U., Ayaz, D., Buschbom, J., Kami, H. G., Mazanaeva, L. F., Aloufi, A. A., Auer, M., Rifai, L., Šilic, T., & Hundsdoerfer, A. K. (2008). Go east: Phylogeographies of *Mauremys caspica* and *M. rivulata* - discordance of morphology, mitochondrial and nuclear genomic markers and rare hybridization. *Journal of Evolutionary Biology*, 21(2), 527–540.
- Fritz, U., Ayaz, D., Hundsdoerfer, A. K., Kotenko, T., Guicking, D., Wink, M., Tok, C. V., Çiçek, K., & Buschbom, J. (2009). Mitochondrial diversity of European pond turtles (*Emys orbicularis*) in Anatolia and the Ponto-Caspian Region: Multiple old refuges, hotspot of extant diversification and critically endangered endemics. *Organisms Diversity and Evolution*, 9, 100–114. <https://doi.org/10.1016/j.ode.2009.02.002>
- Fu, J., Murphy, R. W., & Darevsky, I. S. (1997). Toward the phylogeny of caucasian rock lizards: Implications from mitochondrial DNA gene sequences (Reptilia: Lacertidae). *Zoological Journal of the Linnean Society*, 120(4), 463–477. <https://doi.org/10.1111/j.1096-3642.1997.tb01283.x>
- Gabelaia, M., Adriaens, D., & Tarkhishvili, D. N. (2017). Phylogenetic signals in scale shape in Caucasian rock lizards (*Darevskia* species). *Zoologischer Anzeiger*, 268, 32–40. <https://doi.org/10.1016/j.jcz.2017.04.004>
- García-Porta, J., Irisarri, I., Kirchner, M., Rodríguez, A., Kirchhof, S., Brown, J. L., MacLeod, A., Turner, A. P., Ahmadzadeh, F., Albaladejo, G., Crnobrnja-Isailovic, J., De la Riva, I., Fawzi, A., Galán, P., Göçmen, B., Harris, D. J., Jiménez-Robles, O., Joger, U., Jovanović Glavaš, O., ... Wollenberg Valero, K. C. (2019). Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature Communications*, 10(1), 4077. <https://doi.org/10.1038/s41467-019-11943-x>
- Garrick, R. C., Sunnucks, P., & Dyer, R. J. (2010). Nuclear gene phylogeography using PHASE: Dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. *BMC Evolutionary Biology*, 10, 118. <https://doi.org/10.1186/1471-2148-1110-1118>
- Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., & Posada, D. (2010). ALTER: Program-oriented format conversion of DNA and protein alignments [Computer software]. *Nucleic Acids Research*, 38(Suppl 2), W14–W18.
- Godinho, R., Domingues, V., Crespo, E. G., & Ferrand, N. (2006). Extensive intraspecific polymorphism detected by SSCP at the nuclear C-mos gene in the endemic Iberian lizard *Lacerta schreiberi*. *Molecular Ecology*, 15, 731–738. <https://doi.org/10.1111/j.1365-294X.2006.02813.x>
- Gómez, A., & Lunt, D. H. (2007). In S. Weiss, & N. Ferrand (Eds.), *Refugia within refugia: Patterns of phylogeographic concordance in the Iberian Peninsula* (pp. 155–188). Kluwer Academic Publishers.
- Guicking, D., Joger, U., & Wink, M. (2009). Cryptic diversity in a Eurasian water snake (*Natrix tessellata*, Serpentes: Colubridae): Evidence from mitochondrial sequence data and nuclear ISSR-PCR fingerprinting. *Organisms Diversity and Evolution*, 9(3), 201–214. <https://doi.org/10.1016/j.ode.2009.03.001>
- Gvozdík, V., Canestrelli, D., García-París, M., Moravec, J., Nascetti, G., Recuero, E., Teixeira, J., & Kotlík, P. (2015). Speciation history and widespread introgression in the European short-call tree frogs (*Hyla arborea* sensu lato, *H. intermedia* and *H. sarda*). *Molecular Phylogenetics and Evolution*, 83, 143–155. <https://doi.org/10.1016/j.ympev.2014.11.012>
- Heled, J., & Drummond, A. J. (2008). Bayesian inference of population size history from multiple loci. *BMC Evolutionary Biology*, 8, 289. <https://doi.org/10.1186/1471-2148-8-289>
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978. <https://doi.org/10.1002/joc.1276>
- Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2017). *Package 'dismo' - Species Distribution Modeling*. CRAN Repository.

- Hua, X., & Wiens, J. J. (2013). How does climate influence speciation? *American Naturalist*, *182*, 1–12. <https://doi.org/10.1086/670690>
- Ilgaz, Ç., Baran, I., Kumlutaş, Y., & Avci, A. (2005). A new record of *Mesalina brevirostris* (Reptilia: Sauria: Lacertidae) from Southeastern Anatolia. *Russian Journal of Herpetology*, *12*, 230–236.
- Jablonski, D., Nagy, Z. T., Avci, A., Olgun, K., Kukushkin, O. V., Safaei-Mahroo, B., & Jandzik, D. (2019). Cryptic diversity in the smooth snake (*Coronella austriaca*). *Amphibia Reptilia*, *40*, 179–192. <https://doi.org/10.1163/15685381-20181025>
- Kalioztzopoulou, A., Carretero, M. A., & Llorente, G. A. (2012). Morphology of the *Podarcis* wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: Patterns of variation in a putative cryptic species complex. *Zoological Journal of the Linnean Society*, *164*, 173–193. <https://doi.org/10.1111/j.1096-3642.2011.00760.x>
- Kapli, P., Botoni, D., Ilgaz, Ç., Kumlutaş, Y., Avci, A., Rastegar-Pouyani, N., Fathinia, B., Lymberakis, P., Ahmadzadeh, F., & Poulakakis, N. (2013). Molecular phylogeny and historical biogeography of the Anatolian lizard *Apathya* (Squamata, Lacertidae). *Molecular Phylogenetics and Evolution*, *66*, 992–1001. <https://doi.org/10.1016/j.ympev.2012.12.002>
- Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, *90*, 773–795. <https://doi.org/10.1080/01621459.1995.10476572>
- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability [Computer software]. *Molecular Biology and Evolution*, *30*, 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kindler, C., Böhme, W., Corti, C., Gvozdífk, V., Jablonski, D., Jandzik, D., Metallinou, M., Široký, P., & Fritz, U. (2013). Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megaloccephala*). *Zoologica Scripta*, *42*(5), 458–472. <https://doi.org/10.1111/zsc.12018>
- Koç, H., Kutrup, B., Eroğlu, O., Bülbül, U., Kurnaz, M., Afan, F., & Eroğlu, A. İ. (2017). Phylogenetic relationships of *D. rudis* (Bedriaga, 1886) and *D. bithynica* (Mehely, 1909) based on microsatellite and mitochondrial DNA in Turkey. *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, *28*(6), 814–825. <https://doi.org/10.1080/24701394.2016.1197215>
- Kornilios, P., Ilgaz, Ç., Kumlutaş, Y., Giokas, S., Fragedakis-Tsolis, S., & Chondropoulos, B. (2011). The role of Anatolian refugia in herpetofaunal diversity: An mtDNA analysis of *Typhlops vermicularis* Merrem, 1820 (Squamata, Typhlopidae). *Amphibia-Reptilia*, *32*, 351–363. <https://doi.org/10.1163/017353711X579858>
- Kornilios, P., Ilgaz, Ç., Kumlutaş, Y., Lymberakis, P., Moravec, J., Sindaco, R., Rastegar-Pouyani, N., Afroosheh, M., Giokas, S., Fragedakis-Tsolis, S., & Chondropoulos, B. (2012). Neogene climatic oscillations shape the biogeography and evolutionary history of the Eurasian blindsnake. *Molecular Phylogenetics and Evolution*, *62*, 856–873. <https://doi.org/10.1016/j.ympev.2011.11.035>
- Kornilios, P., Thanou, E., Lymberakis, P., Ilgaz, Ç., Kumlutaş, Y., & Leaché, A. (2019). Genome-wide markers untangle the green-lizard radiation in the Aegean Sea and support a rare biogeographical pattern. *Journal of Biogeography*, *46*, 552–567. <https://doi.org/10.1111/jbi.13524>
- Kozak, K. H., & Wiens, J. J. (2007). Climatic zonation drives latitudinal variation in speciation mechanisms. *Proceedings of the Royal Society B*, *274*, 2995–3003. <https://doi.org/10.1098/rspb.2007.1106>
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets [Computer Software]. *Molecular Biology and Evolution*, *33*, 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kupriyanova, L. (2010). Cytogenetic and genetic trends in the evolution of unisexual lizards. *Cytogenetic and Genome Research*, *127*, 273–279. <https://doi.org/10.1159/000303325>
- Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses [Computer software]. *Molecular Biology and Evolution*, *29*(6), 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction [Computer software]. *Methods in Ecology and Evolution*, *6*(9), 1110–1116.
- Lemey, P., Rambaut, A., Welch, J. J., & Suchard, M. A. (2010). Phylogeography takes a relaxed random walk in continuous space and time. *Molecular Biology and Evolution*, *27*(8), 1877–1885. <https://doi.org/10.1093/molbev/msq067>
- Liu, C., Berry, P. M., Dawson, T. P., & Pearson, R. G. (2005). Selecting thresholds of occurrence in the prediction of species distributions. *Ecography*, *28*, 385–393. <https://doi.org/10.1111/j.0906-7590.2005.03957.x>
- Mashkaryan, V., Vamberger, M., Arakelyan, M., Hezaveh, N., Carretero, M. A., Corti, C., Harris, D. J., & Fritz, U. (2014). Gene flow among deeply divergent mtDNA lineages of *Testudo graeca* (Linnaeus, 1758) in Transcaucasia. *Amphibia Reptilia*, *34*, 337–351. <https://doi.org/10.1163/15685381-00002895>
- Mendes, J., Harris, D. J., Carranza, S., & Salvi, D. (2016). Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenation approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees. *Molecular Phylogenetics and Evolution*, *100*, 254–267.
- Merow, C., Smith, M. J., & Silander, J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography*, *36*(10), 1058–1069. <https://doi.org/10.1111/j.1600-0587.2013.07872.x>
- Miraldo, A., Hewitt, G. M., Dear, P. H., Paulo, O. S., & Emerson, B. C. (2012). Numts help to reconstruct the demographic history of the ocellated lizard (*Lacerta lepida*) in a secondary contact zone. *Molecular Ecology*, *21*, 1005–1018. <https://doi.org/10.1111/j.1365-294X.2011.05422.x>
- Mittermeier, R. A., Gil, P. R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C. G., Lamoreux, J., & Da Fonseca, G. A. B. (2004). *Hotspots revisited: Earth's biologically richest and most threatened terrestrial ecoregions*. University of Chicago Press.
- Murphy, R. W., Darevsky, I. S., Macculloch, R. D., Fu, J., & Kupriyanova, L. A. (1996). Evolution of the bisexual species of caucasian rock lizards: A phylogenetic evaluation of allozyme data. *Russian Journal of Herpetology*, *3*(1), 18–31.
- Murphy, R. W., Fu, J., Macculloch, R. D., Darevsky, I. S., & Kupriyanova, L. A. (2000). A fine line between sex and unisexuality: The phylogenetic constraints on parthenogenesis in lacertid lizards. *Zoological Journal of the Linnean Society*, *130*, 527–549. <https://doi.org/10.1111/j.1096-3642.2000.tb02200.x>
- Murtskhvaladze, M., Tarkhishvili, D., Anderson, C. L., & Kotorashvili, A. (2020). Phylogeny of caucasian rock lizards (*Darevskia*) and other true lizards based on mitogenome analysis: Optimisation of the algorithms and gene selection. *PLoS One*, *15*(6), e0233680. <https://doi.org/10.1371/journal.pone.0233680>

- Olden, J. D., & Jackson, D. A. (2000). Torturing data for the sake of generality: How valid are our regression models? *Ecoscience*, 7(4), 501–510. <https://doi.org/10.1080/11956860.2000.11682622>
- Otto-Bliesner, B. L., Marshall, S. J., Overpeck, J. T., Miller, G. H., Hu, A., & Members, C. L. I. P. (2006). Simulating arctic climate warmth and icefield retreat in the Last Interglaciation. *Science*, 311(5768), 1751–1753.
- Palumbi, S. R., Martin, A. P., McMillan, W. O., Romano, S. R., Grabowski, G., & Stice, L. (2002). *The simple fool's guide to PCR, version 2*. Department of Zoology, University of Hawaii.
- Pavlicev, M., & Mayer, W. (2009). Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodological artefact? *Molecular Phylogenetics and Evolution*, 52, 727–734.
- Pearson, R. G., Raxworthy, C. J., Nakamura, M., & Townsend Peterson, A. (2007). Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography*, 34, 102–117. <https://doi.org/10.1111/j.1365-2699.2006.01594.x>
- Peterson, A. T. (2011). Ecological niche conservatism: A time-structured review of evidence. *Journal of Biogeography*, 38, 817–827. <https://doi.org/10.1111/j.1365-2699.2010.02456.x>
- Petrosyan, V., Osipov, F., Bobrov, V., Dergunova, N., Nazarenko, E., Omelchenko, A., Danielyan, F., & Arakelyan, M. (2019). Analysis of geographical distribution of the parthenogenetic rock lizard *Darevskia armeniaca* and its parental species (*D. mixta*, *D. valentini*) based on ecological modelling. *Salamandra*, 55, 173–190.
- Phillips, S. J., Anderson, R. P., Dudík, M., Schapire, R. E., & Blair, M. E. (2017). Opening the black box: An open-source release of Maxent [Computer software]. *Ecography*, 40(7), 887–893. <https://doi.org/10.1111/ecog.03049>
- Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distribution [Computer software]. *Ecological Modelling*, 190, 231–259. <https://doi.org/10.1016/j.ecolmodel.2005.03.026>
- Phillips, S. J., Dudík, M., Elith, J., Graham, C. H., Lehmann, A., Leathwick, J., & Ferrier, S. (2009). Sample selection bias and presence-only distribution models: Implications for background and pseudo-absence data. *Ecological Applications*, 19(1), 181–197. <https://doi.org/10.1890/07-2153.1>
- Pinho, C., Harris, D. J., & Ferrand, N. (2007). Contrasting patterns of population subdivision and historical demography in three western Mediterranean lizard species inferred from mitochondrial DNA variation. *Molecular Ecology*, 16, 1191–1205. <https://doi.org/10.1111/j.1365-294X.2007.03230.x>
- Pinho, C., Rocha, S., Carvalho, B. M., Lopes, S., Mourão, S., Vallinoto, M., Brunes, T. O., Haddad, C. F. B., Gonçalves, H., Sequeira, F., & Ferrand, N. (2010). New primers for the amplification and sequencing of nuclear loci in a taxonomically wide set of reptiles and amphibians. *Conservation Genetics Resources*, 2(1), 181–185. <https://doi.org/10.1007/s12686-009-9126-4>
- Podnar, M., Haring, E., Pinsker, W., & Mayer, W. (2007). Unusual origin of a nuclear pseudogene in the Italian wall lizard: Intergenomic and interspecific transfer of a large section of the mitochondrial genome in the genus *Podarcis* (Lacertidae). *Journal of Molecular Evolution*, 64, 308–320. <https://doi.org/10.1007/s00239-005-0259-0>
- Ptacek, M. B., Gerhardt, H. C., & Sage, R. D. (1994). Speciation by polyploidy in treefrogs: Multiple origins of the tetraploid. *Hyla versicolor*. *Evolution*, 48(3), 898–908.
- Qgis Development Team (2020). *QGIS Geographic Information System. Open Source Geospatial Foundation Project [Computer software]*. <http://qgis.osgeo.org>
- R Core Team (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Raes, N., & Ter Steege, H. (2007). A null-model for significance testing of presence-only species distribution models. *Ecography*, 30, 727–736. <https://doi.org/10.1111/j.2007.0906-7590.05041.x>
- Rambaut, A. (2009). *Figtree [Computer software]*. <http://tree.bio.ed.ac.uk/software/figtree/>
- Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7 [Computer software]. *Systematic Biology*, syy032. <https://doi.org/10.1093/sysbio/syy1032>
- Rato, C., Harris, D. J., Perera, A., Carvalho, S. B., Carretero, M. A., & Rödder, D. (2015). A combination of divergence and conservatism in the niche evolution of the Moorish gecko, *Tarentola mauritanica* (Gekkota: Phyllodactylidae). *PLoS One*, 10(5), e0127980. <https://doi.org/10.1371/journal.pone.0127980>
- Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods [Computer Software]. *Bioinformatics*, 19, 2496–2497. <https://doi.org/10.1093/bioinformatics/btg359>
- Saberi-Pirooz, R., Ahmadzadeh, F., Ataei, S., Taati, M., Qashqaei, A. T., & Carretero, M. A. (2018). A phylogenetic assessment of the meadow lizard *Darevskia praticola* (Eversmann, 1834) from Iran. *Zootaxa*, 4441(1), 46–58. <https://doi.org/10.11646/zootaxa.4441.1.2>
- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual*. Cold Spring Harbour Press.
- Santos, A. M., Cabezas, M. P., Tavares, A. I., Xavier, R., & Branco, M. (2016). tcsBU: A tool to extend TCS network layout and visualization [Computer Software]. *Bioinformatics*, 32(4), 627–628. <https://doi.org/10.1093/bioinformatics/btv636>
- Sarikaya, M. A., & Ciner, A. (2015). Late pleistocene glaciations and paleoclimate of Turkey. *Bulletin of the Mineral Research and Exploration*, 2015(150), 107–127. <https://doi.org/10.19111/bmre.35245>
- Seddon, J. M., Santucci, F., Reeve, N., & Hewitt, G. M. (2002). Caucasus Mountains divide postulated postglacial colonization routes in the white-breasted hedgehog, *Erinaceus concolor*. *Journal of Evolutionary Biology*, 15, 463–467. <https://doi.org/10.1046/j.1420-9101.2002.00408.x>
- Shcheglovitova, M., & Anderson, R. P. (2013). Estimating optimal complexity for ecological niche models: A jackknife approach for species with small sample sizes. *Ecological Modelling*, 269, 9–17. <https://doi.org/10.1016/j.ecolmodel.2013.08.011>
- Sillero, N. (2011). What does ecological modelling model? A proposed classification of ecological niche models based on their underlying methods. *Ecological Modelling*, 222, 1343–1346. <https://doi.org/10.1016/j.ecolmodel.2011.01.018>
- Sillero, N., & Carretero, M. A. (2013). Modelling the past and future distribution of contracting species. The Iberian lizard *Podarcis carbonelli* (Squamata: Lacertidae) as a case study. *Zoologischer Anzeiger*, 252(3), 289–298. <https://doi.org/10.1016/j.jcz.2012.08.004>
- Sindaco, R., Kornilios, P., Sacchi, R., & Lymberakis, P. (2014). Taxonomic reassessment of *Blanus strauchi* (Bedriaga, 1884) (Squamata: Amphisbaenia: Blanidae), with the description of a new species from south-east Anatolia (Turkey). *Zootaxa*, 3795, 311–326.

- Sindaco, R., Venchi, A., Carpaneto, G. M., & Bologna, M. A. (2000). The reptiles of Anatolia: A checklist and zoogeographical analysis. *Biogeographia – The Journal of Integrative Biogeography*, *21*, 441–554. <https://doi.org/10.21426/b6110017>
- Smith, S. A., & Dunn, C. W. (2008). Phyutility: A phyloinformatics tool for trees, alignments and molecular data [Computer software]. *Bioinformatics*, *24*, 715–716. <https://doi.org/10.1093/bioinformatics/btm619>
- Stephens, M., & Donnelly, P. (2003). A comparison of bayesian methods for haplotype reconstruction [Computer software]. *American Journal of Human Genetics*, *73*, 1162–1169.
- Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data [Computer Software]. *American Journal of Human Genetics*, *68*, 978–989. <https://doi.org/10.1086/319501>
- Stöck, M., Dufresnes, C., Litvinchuk, S. N., Lymberakis, P., Biollay, S., Berroneau, M., Borzée, A., Ghali, K., Ogielska, M., & Perrin, N. (2012). Cryptic diversity among Western Palearctic tree frogs: Postglacial range expansion, range limits, and secondary contacts of three European tree frog lineages (*Hyla arborea* group). *Molecular Phylogenetics and Evolution*, *65*(1), 1–9. <https://doi.org/10.1016/j.ympev.2012.05.014>
- Tarkhnishvili, D. (2012). Evolutionary history, habitats, diversification, and speciation in Caucasian rock lizards. In *Advances in Zoology Research* (Vol. 2. pp. 79–120). Hauppauge, NY: Nova Science Publishers.
- Tarkhnishvili, D., Murtskhaladze, M., & Gavashelishvili, A. (2013). Speciation in Caucasian lizards: Climatic dissimilarity of the habitats is more important than isolation time. *Biological Journal of the Linnean Society*, *109*, 876–892. <https://doi.org/10.1111/bij.12092>
- Tok, V., Ugurtas, I., Sevinç, M., Böhme, W., Crochet, P.-A., Turiyev, B., & Kaya, U. (2009a). *Darevskia rudis*. *The IUCN Red List of Threatened Species 2009: e.T164633A5913665*. <https://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T164633A5913665.en> 27 January 2020.
- Tok, V., Ugurtas, I., Sevinç, M., Böhme, W., Crochet, P.-A., Turiyev, B., & Kaya, U. (2009b). *Darevskia valentini*. *The IUCN Red List of Threatened Species 2009: e.T164707A5919336*. <https://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T164707A5919336.en> on 27 January 2020.
- Tuniyev, B., Ananjeva, N. B., Agasyan, A., Orlov, N. L., & Tuniyev, S. (2009). *Darevskia portschinskii*. *The IUCN Red List of Threatened Species 2009: e.T164654A114556728*. <https://doi.org/10.2305/IUCN.UK.2009.RLTS.T164654A5915799.en> on 27 January 2020
- Tuniyev, S. B., Doronin, I. A., Kidov, A. A., & Tuniyev, B. S. (2011). Systematic and geographical variability of meadow lizard, *Darevskia praticola* (Reptilia: Sauria) in the Caucasus. *Russian Journal of Herpetology*, *18*, 295–316.
- Uetz, P., Freed, P., & Hošek, J. (2019). *The Reptile Database*. <http://www.reptile-database.org> on January 27, 2020
- Vale, C. G., Campos, J. C., Silva, T. L., Gonçalves, D. V., Sow, A. S., Martínez-Freiría, F., Boratyński, Z., & Brito, J. C. (2016). Biogeography and conservation of mammals from the west sahra-sahel: An application of ecological niche-based models and gis. *Hystrix, the Italian Journal of Mammalogy*, *27*(1), <https://doi.org/10.4404/hystrix-27.1-11659>
- Vamberger, M., Stuckas, H., Ayaz, D., Graciá, E., Aloufi, A. A., Els, J., Mazanaeva, L. F., Kami, H. G., & Fritz, U. (2013). Conservation genetics and phylogeography of the poorly known Middle Eastern terrapin *Mauremys caspica* (Testudines: Geoemydidae). *Organisms Diversity and Evolution*, *13*(1), 77–85. <https://doi.org/10.1007/s13127-012-0102-6>
- Varela, S., Lima-Ribeiro, M. S., & Terribile, L. C. (2015). A short guide to the climatic variables of the last glacial maximum for biogeographers. *PLoS One*, *10*(6), e0129037. <https://doi.org/10.1371/journal.pone.0129037>
- Veith, M., Schmidler, J. F., Kosuch, J., Baran, I., & Seitz, A. (2003). Palaeoclimatic changes explain Anatolian mountain frog evolution: A test for alternating vicariance and dispersal events. *Molecular Ecology*, *12*, 185–199. <https://doi.org/10.1046/j.1365-294X.2003.01714.x>
- Veith, M., & Steinfartz, S. (2004). When non-monophyly results in taxonomic consequences – the case of *Mertensiella* within the Salamandridae (Amphibia: Urodela). *Salamandra*, *40*, 67–80.
- Vrijenhoek, R. C. (1989). Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. *Evolution and Ecology of Unisexual Vertebrates*, *466*, 24–31.
- Wielstra, B., Themudo, G. E., Güçlü, Ö., Olgun, K., Poyarkov, N. A., & Arntzen, J. W. (2010). Cryptic crested newt diversity at the Eurasian transition: The mitochondrial DNA phylogeography of Near Eastern *Triturus* newts. *Molecular Phylogenetics and Evolution*, *56*, 888–896. <https://doi.org/10.1016/j.ympev.2010.04.030>
- Wiens, J. J. (2004). Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. *Evolution*, *58*(1), 193–197. <https://doi.org/10.1111/j.0014-3820.2004.tb01586.x>
- Wiens, J. J., & Graham, C. H. (2005). Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 519–539. <https://doi.org/10.1146/annurev.ecolsys.36.102803.095431>
- Yıldız, M. Z., Akman, B., & Göçmen, B. (2012). New locality records of the Snake eyed Lizard, *Ophisops elegans* Ménétries, 1832 (Squamata: Lacertidae), in the western Black Sea region of Anatolia. *Turkish Journal of Zoology*, *36*, 259–265.
- Zwickl, D. J. (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [Computer software]*. PhD, The University of Arizona, Tucson.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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