Original Research Article

Mid-Pleistocene Transition as a trigger for diversification in the Irano-Anatolian region: Evidence revealed by phylogeography and distribution pattern of the eastern three-lined lizard

Somayeh Ghane-Ameleh [a], Milad Khosravi [a,b], Reihaneh Saberi-Pirooz [a], Elham Ebrahimi [a], Marzieh Asadi Aghbolaghi [a], Faraham Ahmadzadeh [a,*]

[a] *Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C., Evin, Tehran, Iran*
[b] *Department of Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Evin, Tehran, Iran*

ABSTRACT

Past climatic events are one of the major drivers for divergence of species that could shape the present biodiversity and species dispersal patterns. Irano-Anatolian region is one of the biodiversity hotspots in the world. This region is predicted to serve various refugia during Quaternary glaciations. The eastern three-lined lizard (*Lacerta media media*) is a medium-sized lacertid species, which is originated from central Anatolian and is currently distributed almost across Irano-Anatolian region. In this study, the phylogeography, demographic history of the subspecies using mtDNA genes (cyt *b*, 12S and 16S) along with the species distribution models (SDMs) under past-present-future scenarios have been investigated. The results revealed that four distinct clades are well-established within the *L. m. media* which may intensely diverge during Pleistocene climatic fluctuations since the Mid-Pleistocene Transition (MPT). The past climate oscillations may isolate the population in various refugia throughout the Irano-Anatolian region which has been led to this divergence. The results of SDMs also indicates several localities with suitable climate conditions for eastern three-lined lizard in the region since LGM (21 thousand years ago) until the current date. This suitability can be expanded under future climate scenarios considering the existence of various low and high altitude refugia. Some conservation implications arise from the results that emphasize the importance of the region and its multiple refugia in preserving biodiversity. It can be suggested that, future conservation practices should be focused on the obtained biogeographical units such as central Anatolia, west and northwestern Iran, which encompass high levels of diversity including distinct clades within the eastern three-lined lizard.

1. Introduction

The Quaternary period can be characterized by its periodic intense climate oscillations (2.5 million years ago (Mya) to present; Lisiecki and Raymo, 2005; Hansen et al., 2013) which shaped biogeographic patterns and genetic structures of many species (Hewitt,

* Corresponding author.
 E-mail address: f_ahmadzade@sbu.ac.ir (F. Ahmadzadeh).

2004). A series of these remarkable oscillations has commenced since the Mid-Pleistocene Transition (MPT; Also called the Mid-Pleistocene Revolution or MPR; Maslin and Ridgwell, 2005) which was started around ~1.25 and completed by ~0.7 Mya. This transition is characterized by alternation in the intervals of glacial-interglacial cycles (from 41 Ka (thousand years) to 100 Ka) with higher amplitudes (Willeit et al., 2019). The new situation followed by post-MPT colder climate conditions and more intense fluctuations (Clark et al., 2006; Willeit et al., 2019). Throughout the fluctuations, species restricted and expanded their distribution ranges. In glacial cycles, they were restricted to refugia with mild and temperate climates, and in the interglacial periods, species recolonization reversed direction toward cool regions (Bennett and Provan, 2008; Provan and Bennett, 2008). During these climatic fluctuations, the lower latitudes such as regions in the Middle East and North Africa, which currently are categorized as arid and semiarid regions, have experienced a variety of dry and humid climate conditions (Schuster et al., 2006; Wanner et al., 2008; Ahmadzadeh et al., 2013a). Moreover, the higher altitudes have borne drier and colder climates than the lower altitudes (Hofreiter and Stewart, 2009; Kehl, 2009). Since the Last Glacial Maximum (LGM), the climate has substantially shifted from cooler and arid to moderate conditions in the Holocene with extending steppe habitats in lower altitudes of the Middle East (Kuhle, 2008; Djamali et al., 2008).

The Irano-Anatolian region is a global biodiversity hotspot (Mittermeier et al., 2005; Noroozi et al., 2018). The landmass has specific geomorphology including various lowlands and highlands, particularly in the Anatolia and the Zagros Mountains ranges. Due to complex topography and positioning, it is assumed that the area contains several refugia during the past temperature fluctuations in the Quaternary period. This would be a possible explanation of the rich diversity in the region (Mittermeier et al., 2005; Kehl, 2009; Bilgin, 2011; Giokas et al., 2011; Noroozi et al., 2018; Malekoutian et al., 2020). So, according to its relatively complex geological structure, unique climatic environment and complicated evolutionary history of its species, understanding the historical aspect of many species distribution in the region has been the focus of several studies (Ahmadzadeh et al., 2013b, 2016; Shahabi et al., 2017).

The region plays a vital role in small vertebrate diversification (Torki et al., 2011; Ahmadzadeh et al., 2012; Hendrix et al., 2014; Aghbolaghi et al., 2019). In recent years, several studies indicated that the genetic structures and distribution patterns of many species are related to past climate change in the Irano-Anatolian region (Farasat et al., 2016; Afroosheh et al., 2019; Aghbolaghi et al., 2020). Despite this, the phylogeography, speciation and demography history of many small vertebrates were poorly understood. Small vertebrates like most amphibians and reptiles are generally known as species with critical temperature and humidity requirements (Agustí et al., 2009; Kafash and Yousefi, 2017; Kafash et al., 2020).

Reptiles are exothermic species, so they are sensitive to climatic fluctuations that cause drastic changes in their distribution patterns. Lacertid lizards are perfect animal models to study the influence of paleo and present climate conditions on their genetic structure and distribution (Ahmadzadeh et al., 2013b). The green lizards of the genus *Lacerta* are medium-sized members of Lacertidae, including several species groups with a high level of morphological and habitat diversities. Of the which, *Lacerta trilineata* group comprises three species, i.e., *Lacerta trilineata, Lacerta pamphylica, Lacerta media* (Arnold, 1973; Arnold et al., 2007). Originated in the Anatolia, *Lacerta media* is widely distributed in the eastern coasts of the Mediterranean Sea throughout Anatolia to the west of Iran (Ahmadzadeh et al., 2013c; ŠMÍD et al., 2014). *Lacerta media* forms a complex species with five subspecies. The nominate one, *Lacerta media media* (eastern three-lined lizard) is distributed from the center and east of Turkey to western Iran (Ahmadzadeh et al., 2013b,c). The eastern three-lined lizard has not been properly investigated in terms of genetic diversity and geographical structure, as well as the impact of climate fluctuations on the subspecies, has not been well recognized (Ahmadzadeh et al., 2013c).

Integrating the multidisciplinary approaches, including molecular phylogeography and species distribution modeling (SDMs), help researchers to investigate how such paleo-environmental conditions shaped the current species diversity and distribution. With the aid of molecular techniques and appropriate markers, it is possible to understand the geographical genetic variations of organisms. In addition, using the SDMs will provide valuable data regarding the influences of environmental factors on the distribution patterns of species (Guisan and Thuiller, 2005; Jeschke and Strayer, 2008; Ahmadzadeh et al., 2013a). In the present study, *L. m. media* has been examined using multidisciplinary approaches including molecular genetics and species distribution models (SDMs). Therefore, mtDNA genes (12S, 16S and cyt *b*) were used to find genetic structures within the subspecies, and SDMs following the modern niche concept (Soberón, 2007). SDMs were applied to reconstruct the past distribution range, predict the current situation and forecast the future climatic condition under different scenarios. The main aim of the present study is to determine various biogeographic processes that shaped the genetic structures of *L. m. media* in its distribution range and also evaluate the effects of climate oscillations on the structures.

2. Materials and methods

2.1. Sampling, DNA extraction and sequencing

The eastern three-lined lizard has been collected and investigated across its geographical distribution range (Fig. 1; Table S1). A small portion of distal tip muscle tissue of the tail was removed and stored in 96% ethanol and −20°C for long-term maintenance.
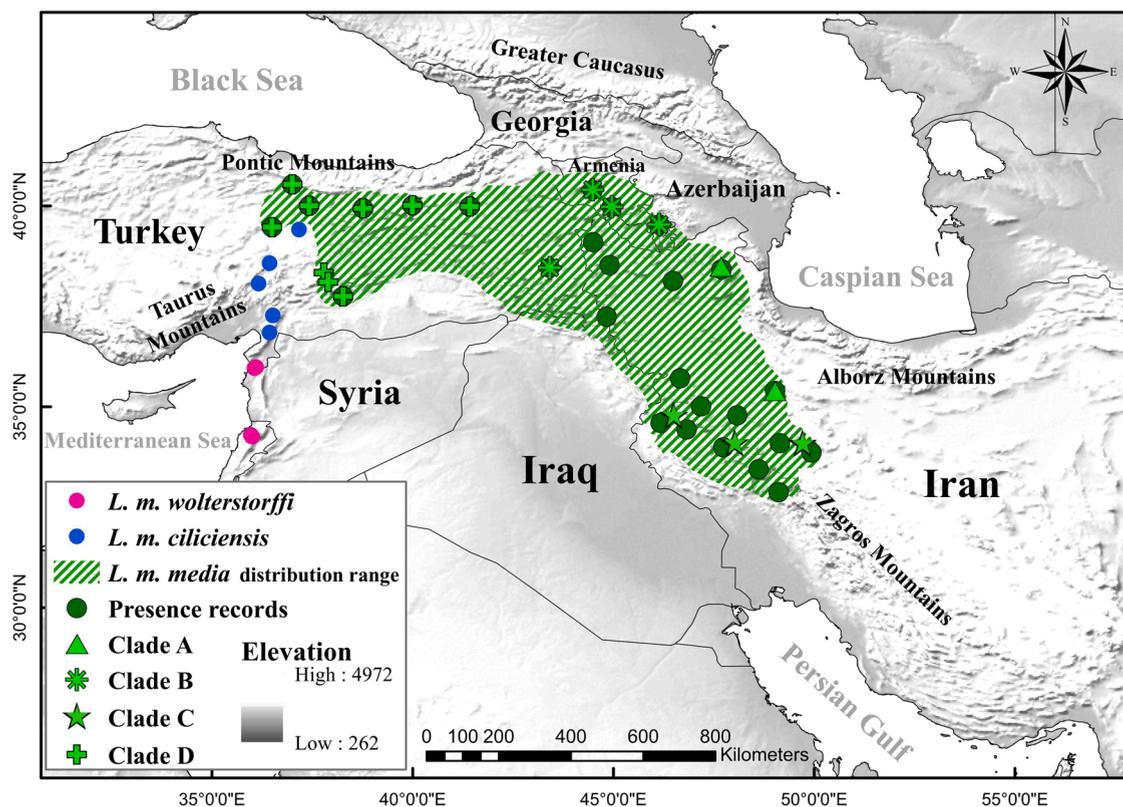
Fig. 1. The distribution range of *Lacerta trilineata* group in the Irano-Anatolian region. The distribution of *Lacerta media media* is marked with a green crosshatch. The pink, blue and dark green circles represent the distribution points of *L. m. wolterstroffi*, *L. m. ciciliensis* and the presence records of *L. m. media* (used for SDM analysis), respectively. The location of samples used for the molecular study for *L. m. media* are shown with signs including triangle (Clade A), asterisk (Clade B), star (Clade C) and plus (Clade D). The mentioned Low and High elevation values are specific to the depicted distribution range (green crosshatch).

DNA extraction was performed using the high-salt method (Sambrook et al., 1989). Partial sequences of cytochrome *b* (cyt *b*), 12S ribosomal RNA (12S) and 16S ribosomal RNA (16S) genes were amplified using the primers GluDg/Peil (Palumbi, 1991; Engström et al., 2007), 12Sa/12Sb (Kocher et al., 1989) and 16Sa/16Sb (Palumbi, 1991), respectively. The Polymerase chain reactions (PCRs) were carried out on an MJ Mini™ thermocycler (Bio-Rad) and the reactions mix were prepared in a total volume of 25 μl containing 12.5 μl of Master Mix Red (Ampliqon, Copenhagen, Denmark), 0.5 μl of each primer, 10.5 μl double distilled water (ddH$_2$O) and 1 μl of template DNA (50–100 ng). PCRs were carried out separately for each gene under the conditions mentioned in Godinho et al. (2005) and Ahmadzadeh et al. (2013c). The final products were examined with agarose gel 1% stained with Safe-Red™. The suitable amplicons were sent to Niagene Noor Inc. (Niagene, Iran) for purification and sequencing. Sequences were edited using Geneious Prime® V. 2021.0.0 program (Biomatters, https://www.geneious.com/). The generated sequences were submitted to the GenBank database.

2.2. Phylogenetic analysis

Phylogenetic relationships of the *L. trilineata* group (*Lacerta pamphylica*, *L. trilineata*, *L. media*) and also three subspecies of *L. media* (*L. m. media*, *L. m. ciciliensis* and *L. m. wolterstroffi*) were re-examined using three mtDNA genes (cyt *b*, 12S and 16S). Additional sequences from species of Lacertidae retrieved from the NCBI (www.ncbi.nlm.nih.gov) were added to our dataset (see Table S1). Two samples of *Timon princeps* were used as outgroups for the analysis. Each gene was aligned with MAFFT v.6 (Katoh et al., 2017) and then was combined, containing in 1359 base pairs (bp) alignment (cyt *b*: 646 bp, 12S: 322 bp and 16S: 391 bp). The best-fitting nucleotide substitution model was chosen for each gene separately under Akaike's Information Criterion (Akaike, 1974) using MrModeltest v.2.3 (Nylander, 2004). The best-fitting model for cyt *b* was HKY+I+G (G = 2.20 and p-inv = 0.57). GTR+G (G = 0.11) and GTR+G+I (G = 1.11 and p-inv = 0.56) were selected as the best-fitting model for 12S and 16S, respectively. A Maximum Likelihood (ML) tree was constructed using RAxML v.7.2 (Stamatakis, 2006) under the GTRGAMMA model with 1000 pseudoreplicates to assess the confidence of branches. Bayesian Inference (BI) analysis was carried out by MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001) with 5×10^7 generations and four chains, and subsampling parameters and trees every 100 generations. Finally, 10% of the posterior samples were discarded as burn-in. Subsequently, convergence to the stationary distribution was evaluated using Tracer v.1.6 (Rambaut and Drummond, 2009).

Uncorrected genetic distances (*p*-distances) within clades of *L. m. media* were estimated with PAUP v.4.0a10 (Swofford, 2003) for cyt *b* gene.

2.3. Estimating divergence time

The divergence times were estimated with BEAST v.1.7.2 (Drummond and Rambaut, 2007) using the assembled datasets. To calibrate the molecular clock, a secondary calibration approach was taken based on the age estimated for the *L. trilineata* group (4.0 Mya; Ahmadzadeh et al., 2013c). This calibration point was applied at the *L. trilineata* group node (lognormal distribution, Mean: 1.4, Standard deviation: 0.085). A lognormal relaxed clock model was used to account for lineage-specific rate heterogeneity; A Yule model of speciation was used for the tree prior. The analysis was run for 5×10^7 generations, subsampling parameters and trees every 100 generations. Tracer v.1.6 was used to further evaluate convergence to the stationary distribution.

Lineage Through Time plotting (LTT) for *L. trilineata* group was performed using Tracer v.1.6.

2.4. Demographic analysis

To explore the demographic history, slow evolving rRNA genes (12S and 16S) were excluded and mitochondrial protein-coding gene (PCG; cyt *b*) was used only (Araya-Anchetta et al., 2015).

Molecular diversity indices, including the number of haplotypes (H), haplotype diversity (*h*), and nucleotide diversity (π) were evaluated within *L. m. media* using Arlequin v.3.5 (Excoffier and Lischer, 2010).

The demographic expansion was investigated with the mismatch analysis and neutrality tests. To explore the demographic history of *L. m. media* , neutrality test analysis i.e., Tajima's *D* (Tajima, 1989), and Fu's *Fs* (Fu, 1997) indices were estimated with Arlequin v.3.5. Mismatch Distribution (MMD, frequency of pairwise nucleotide-site differences between sequences) was performed using the Arlequin v.3.5, with the statement of sudden expansion with spatial parameters.

To investigate variations in the effective population size (*Ne*) against time for *L. m. media* , the Bayesian skyline plot (BSP; Drummond et al., 2005) was performed. The analysis was obtained with BEAST v1.7.2 under the strict clock at the rate of 1.47×10^{-2} per site per Mya (previously calculated by Saberi-Pirooz et al., 2021). The analysis was run for 5×10^6 generations with subsampling trees and parameters were saved every 100 iterations.

2.5. Biogeographic analysis

To reconstruct the possible ancestral range of lineages within the *L. m. media* , the statistical dispersal-vicariance analysis (S-DIVA) and Bayesian binary MCMC (BBM) analysis done using RASP 2.1 beta (Yu et al., 2015). The analyses were run using cyt *b* sequences because of a higher mutation rate compared to the other genes in the study. Four different areas (including the Hamedan-Ardebil or Northwestern Iran, Van-Armenia, the Zagros Mountains and central Anatolia regions) within *L. m. media* distribution range were considered for this investigation. These areas are related to the main subclades within the subspecies (see results). To consider phylogenetic uncertainty, 20,000 trees generated from the BI tree were set as the input file for S-DIVA. The BBM analysis was run for 5×10^6 generations under ten MCMC and the sampling frequency was every 100 generations. The fixed Jukes-Cantor model with equal among-site rate variation was used for the BBM analysis.

The parsimony haplotype network was implemented using TCS v.1.21 (Clement et al., 2000) for the cyt *b* marker under the 95% limit of parsimony.

2.6. Species distribution modeling (past-present-future)

2.6.1. Presence records

Georeferenced localities information of the subspecies *L. m. media* was compiled during the fieldwork and from available literature sources. In total, 73 records were available for model building covering the whole known geographic range of the subspecies, mapped in ArcGIS to spot potential errors. Duplicate records (39 points) were deleted in ENM tools (34 points were used after; Fig. 1; Warren et al., 2010). To prevent spatial autocorrelation and reduce the sampling error of presence points, 'Global Moran's I' function has been used in ArcGIS 10.8.

2.6.2. Climate data and Variable selection

The 19 so-called BIOCLIM variables with a spatial resolution of 2.5 arc.min were downloaded from the second version of the WorldClim database (Fick and Hijmans, 2017; Hijmans et al., 2005; http://www.worldclim.org/bioclim). In order to reduce the Hijmans multi-collinearity of predictors, the variance inflation factor (VIF) utilized to detect collinearity. The VIF step function in the usdm package in R was used, with predictor variables failing the test removed from the analysis for that iteration (Naimi et al., 2014). The final set of variables were BIO8 = Mean Temperature of Wettest Quarter, BIO11 = Mean Temperature of Driest Quarter, BIO13 = Precipitation of Wettest Month, BIO18 = Precipitation of Warmest Quarter and BIO19 = Precipitation of Coldest Quarter.

The past data included averages of Mid-Holocene (~6000 years Before Present (BP)) and Last Glacial Maximum (LGM; ~21000 BP) Global Climate Models (GCMs) simulations following the r1i1p1 ensemble, which were obtained and downscaled using PMIP3 database (Paleoclimate Modeling Intercomparison Project Phase III, https://pmip3.lsce.ipsl.fr/; Bartlein et al., 2011; Braconnot et al., 2012). A total of 11 scenarios were available as estimates of mid-Holocene climate (BCC-CSM1–1, CCSM4, CNRM-CM5, CSIROMk3–0, CSIRO-Mk3 l-1–2, FGOALS-g2, GISS-E2-R, IPSL-CM5A-LR, MIROC-ESM, MPIESM-P, and MRICGCM3) and seven scenarios were

available for the LGM (CCSM4, CNRMCM5, FGOALS-g2, IPSL-CM5A-LR, MIROC-ESM, MPI-ESM-P, MRI-CGCM3).

Also, averages of 8 GCMs (BCC-CSM2-MR, CNRM-CM6–1, CNRMESM2–1, CanESM5, IPSL-CM6A-LR, MIROC-ES2L, MIROC6, and MRI-ESM2–0) applied for the future climate projections in four emission scenarios of the newest Intergovernmental Panel on Climate Change (IPCC) report version 6 (https://www.ipcc.ch/assessment-report/ar6/). These emission scenarios or Shared Socio-economic Pathways (SSPs), which include SSPs 126, 245, 370 and 585 cause an atmospheric temperature increase to 2.1, 3.3, 4.5, and 5.5 ℃ on average, respectively (Eyring et al., 2016).

2.6.3. SDM analysis

Given that the use of individual models increases the likelihood of inaccurate results (Fechter and Storch, 2014), one solution is to use an ensemble approach in which the results of several models are combined to form a single result more accurately (Araújo and New, 2007). In this study, the *sdm* package used in R ver. 4.0 (Naimi and Araújo, 2016). The ensemble model was created by combining nine SDMs including generalized linear models (GLM; McCullagh and Nelder, 1989), generalized additive models (GAM; Hastie and Tibshirani, 1986), boosted regression trees (BRT; Friedman, 2001), support vector machine (SVM; Vapnik, 1995), multiple adaptive regression splines (MARS; Friedman, 1991), classification and regression trees (CART; (Brieman et al., 1984), random forest (RF; Breiman, 2001), maximum entropy (MaxEnt, Phillips et al., 2006), and flexible discriminant analysis (FDA; Hastie et al., 1994).

The models' performance was evaluated based upon the both threshold-dependent and threshold-independent criteria including the True Skill Statistic (TSS, from −1 to 1), Deviance (from 1 to 0), Correlation (COR, from 0 to 1), and Area under the ROC Curve (AUC, from 0 to 1) (Allouche et al., 2006; Giovanelli et al., 2010; Morán-Ordóñez et al., 2017). Percentage contribution of all layers with model-based approach was calculated.

Environmental conditions exceeding the training range of the SDM were quantified using multivariate environmental similarity surfaces (MESS). Also based on the SDM projections under current, mid-Holocene, and LGM conditions, a Habitat suitable consistency overtime (Stability through time) map was prepared in Arc GIS 10.8. So that, habitats which were jointly favorable for the species under current, mid-Holocene, and LGM conditions were identified.

3. Results

3.1. Phylogenetic analysis

The BI consensus tree showed a similar topology to the ML tree. The results (Fig. S1) indicated that three subspecies of *L. media* (*L. m. media*, *L. m. wolterstorffi* and *L. m. ciliciensis*), were separated from each other with high support values (BS = 100, PP = 1.00). Based on phylogenetic analysis, four reciprocally monophyletic clades were recognized within *L. m. media* , Clade A (Hamedan-Ardebil; Northwestern Iran), Clade B (Van-Armenia), Clade C (Zagros Mountains) and Clade D (central Anatolia). Clade A formed a sister clade to other clades within *L. m. media* . At the intraspecific level, clades of the subspecies were well-supported (BS = 100, PP = 1.00; Clade D: BS = 80.3; Fig. S1).

The uncorrected genetic distances for cyt *b* among clades are displayed in Table 1. The genetic distances between Clade A and the rest of the clades are approximately 2%. Clade D had a lower distance to Clade B (1.02%).

3.2. Estimating divergence time

Based on the dated tree, *L. m. media* separated from *L. m. ciliciensis* 2.44 Mya (95% Highest Posterior Density (HPD): 1.62–3.37 Mya, Fig. 2). These subspecies diverged from *L. m. wolterstorffi* around 3.46 Mya (95% HPD: 2.57 – 4.28 Mya). The subspecific divergence within *L. m. media* happened about 1.11 Mya (95% HPD: 0.72–1.64 Mya). The result indicated Clade A diverged from the ancestor of other clades around 1.11 Mya (95% HPD: 0.72–1.64 Mya). Clade B, 0.87 Mya (95% HPD: 0.53–1.32 Mya) separated from Clade C/D and Clade C and Clade D separated from each other about 0.71 Mya (95% HPD: 0.43–1.05 Mya; Fig. 2).

The LTT plot revealed a recent increase in the number of lineages over time (nearly 3.5 Mya) for *L. trilineata* group (Fig. S2).

3.3. Genetic structure and demographic analysis

The molecular diversity indices were demonstrated in Table 2 for *L. m. media* and its clades with cyt *b* marker. The diagrams of mismatch distribution (MMD) for the gene showed multimodal distribution, usually associated with constant population size (Fig. S3A). Tajima's *D* (Tajima's $D = 0.469$, $p > 0.05$), and Fu's *Fs* (Fu's Fs $= -0.0973$, $p > 0.05$) were non-significant for cyt *b*.

The BSP of *L. m. media* (for cyt *b*) showed a nearly stable in *Ne* from approximately 400 Kya (thousand years ago), with a slight decline in *Ne* from 50 Kya and then a mild increase (Fig. S3B).

Table 1
Uncorrected genetic distances (*p*-distances) between four clades of *Lacerta media media* based on cyt *b* marker.

	Clade A	Clade B	Clade C	Clade D
Clade A				
Clade B	0.0210			
Clade C	0.0235	0.0145		
Clade D	0.0203	0.0102	0.0135	
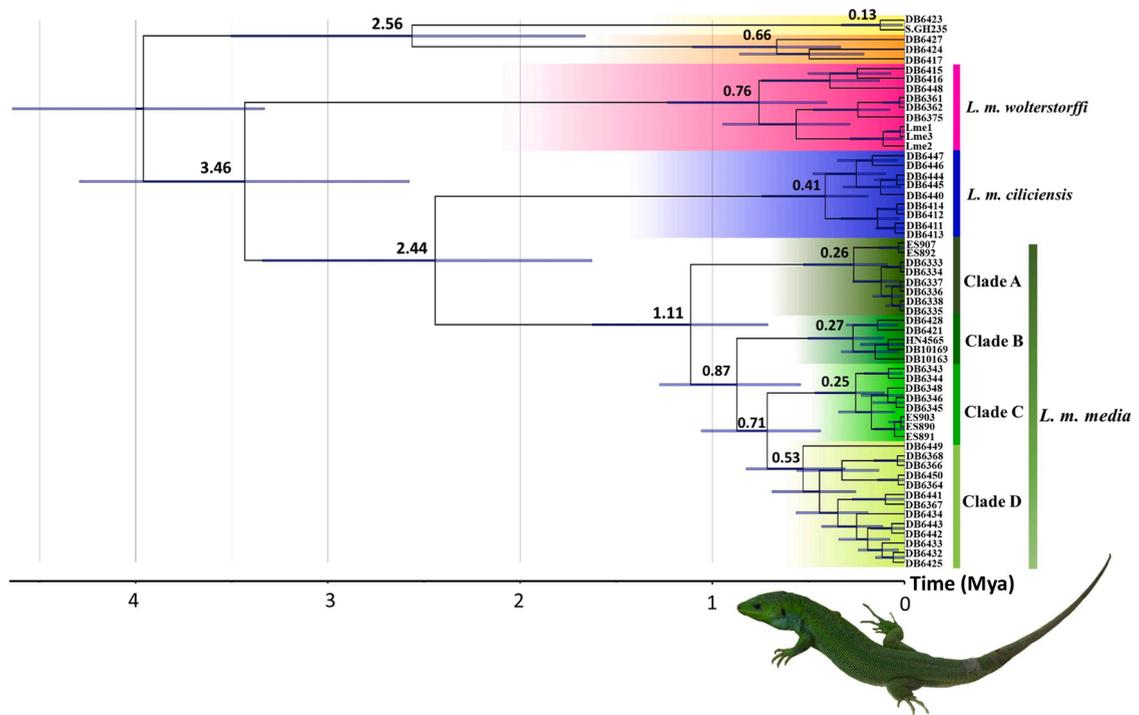
Fig. 2. Time-calibrated phylogenetic trees using the combined dataset (cyt *b*, 16S and 12S). Blue bars show 95% highest posterior density intervals of estimated node ages; numbers the above branches are mean node ages (Million years ago, Mya). The three-lined lizard photo copyright by ©Faraham Ahmadzadeh.

Table 2
Molecular diversity indices based on cyt *b* for *Lacerta media media* and its clades including the sample size (N), the number of haplotypes (H), haplotype diversity (*h*), and nucleotide diversity (π).

	N	H	*h*	π
L. m. media	34	15	0.92	0.0135
Clade A	8	2	0.42	0.0006
Clade B	5	3	0.70	0.0012
Clade C	8	3	0.46	0.0016
Clade D	13	7	0.91	0.0052

3.4. Biogeographic analysis

Based on S-DIVA analysis, two dispersal and three vicariance events occurred in *L. m. media* . Node 77 and 86 showed dispersal events, these nodes referred to the most recent common ancestor (henceforth MRCA) of Clade B/D and Clade (B-D)/A, respectively. Nodes 72, 85 and 86 demonstrated vicariance events. Node 85 is assigned to the MRCA of Clade B and D/C (Fig. 3A). The BBM analysis also showed dispersal and vicariance events in 72, 85 and 86 nodes (Fig. 3B).

The parsimony haplotype network showed one super-haplogroup, with 15 haplotypes based on cyt *b* (Fig. 4). Each clade in the analysis formed a sub-haplogroup, therefore, four sub-haplogroups were indicated for *L. m. media* . The result signified that A and C sub-haplogroups were separated by several step-mutations from others. The B and D sub-haplogroups were located near each other with three step-mutations. The sub-haplogroup D contains more haplotypes (seven) rather than other sub-haplogroups which comprise two or three haplotypes.

3.5. Species distribution modeling (past-present-future)

3.5.1. Model evaluation and variable contribution

Although the predictive performance was different between the algorithms for all four evaluated criteria, the resulting SDMs often showed relatively good model performances (Fig. S4A). The RF and MAXENT models gave the best performance for AUC and SVM and MAXENT models gave the best performance for COR criteria as threshold-independent performance measures (Fig. S4A). In the case of threshold-dependent criteria, the MAXENT demonstrated the best performance for both Deviance and TSS measures, followed by RF and FDA (Fig. S4A).
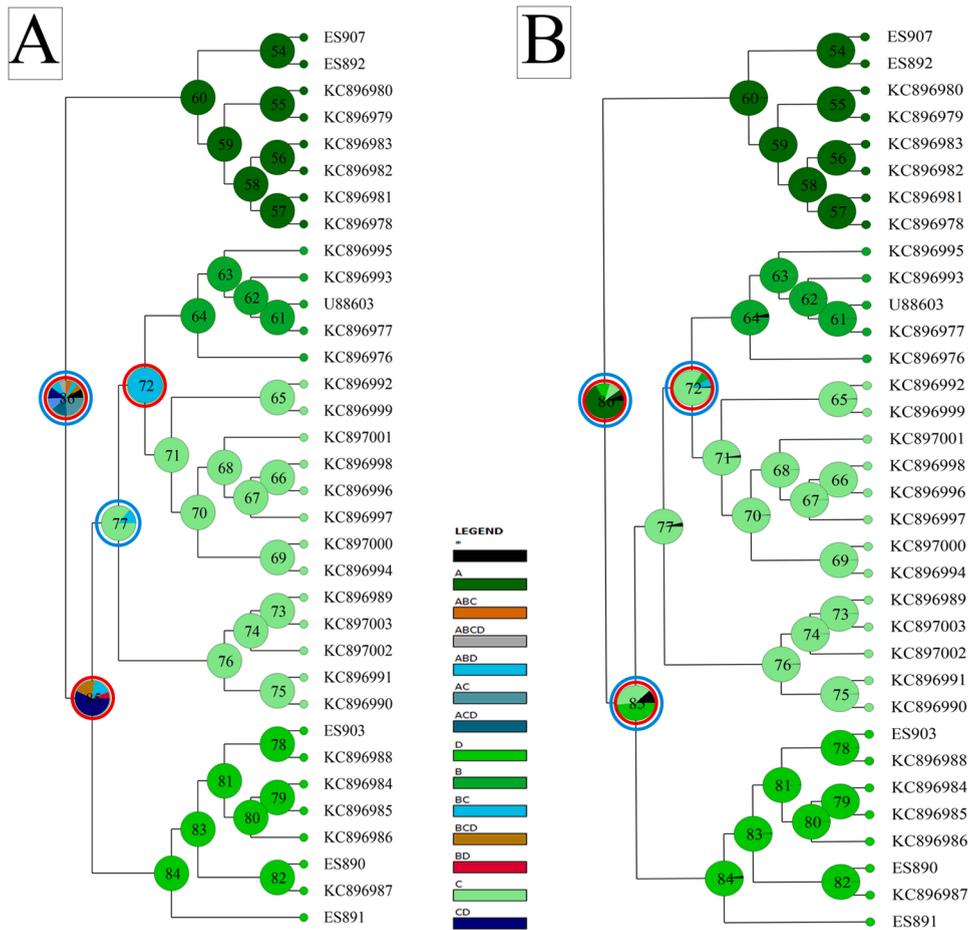
Fig. 3. The biogeographic analysis of *Lacerta media media* using S-DIVA (A) and BBM (B) based on cyt *b*. The blue and red circles around the nodes show dispersal and vicariance events, respectively. The node numbers have been put within the circles.

The Mean Temperature of Wettest Quarter (Bio 8 = 37.8%) and Mean Temperature of Coldest Quarter (Bio 11 = 21.2%) have been found which had the strongest effect on the *L. m. media* distribution (Table 3; Fig. S4B).

3.5.2. Change of the distribution area since the Last Glacial Maximum

Based on Fig. 5, the areas with the highest suitability include central Turkey, western and northwestern Iran and the Zagros Mountains which continues as a separate patch in western Afghanistan (Fig. 5). Climate suitability in the mid-Holocene (~6k BP) showed that the range of species distribution was north of the Zagros Mountains and southeastern Turkey, but in the current climatic conditions, it covers a large part of the Zagros Mountains. The suitable climate of *L. m. media* in LGM showed three detached habitat patches, one in the central part of the Zagros Mountains in Iran and the two others in central Turkey and western Afghanistan. According to the results of the stability map through time in this study, the Zagros Mountains, northwestern Iran, southeastern and central Turkey as well as western Afghanistan acted as climate refugia for the *L. m. media* during climate change (Fig. 5).

3.5.3. Prediction of the future potential distribution

The ensemble models projected into the future showed that the four climate change scenarios (SSPs 126, 245, 370, and 585) for 2040, 2060, 2080, and 2100 can change the distribution potential of the *L. m. media* in Iran and Turkey and from northern Afghanistan to the border of Afghanistan with Turkmenistan and south Turkmenistan. The results showed that the suitable climate area increases in SSPs 245, 370, and 585 for the year 2100, while it will decrease, more or less, in the three-time periods. Generally, stability through time for this species suggests that the north Zagros Mountains, western Afghanistan and central Turkey could act as potential refugia (Fig. S5).

4. Discussion

In the present study, we examined the phylogenetic relationship, demographic history, and habitat suitability of the eastern three-lined lizard using genetic and SDMs approaches. We also investigated the historical phylogeography of the eastern three-lined lizard to understand the impact of the Pleistocene climatic oscillations on its genetic structure.
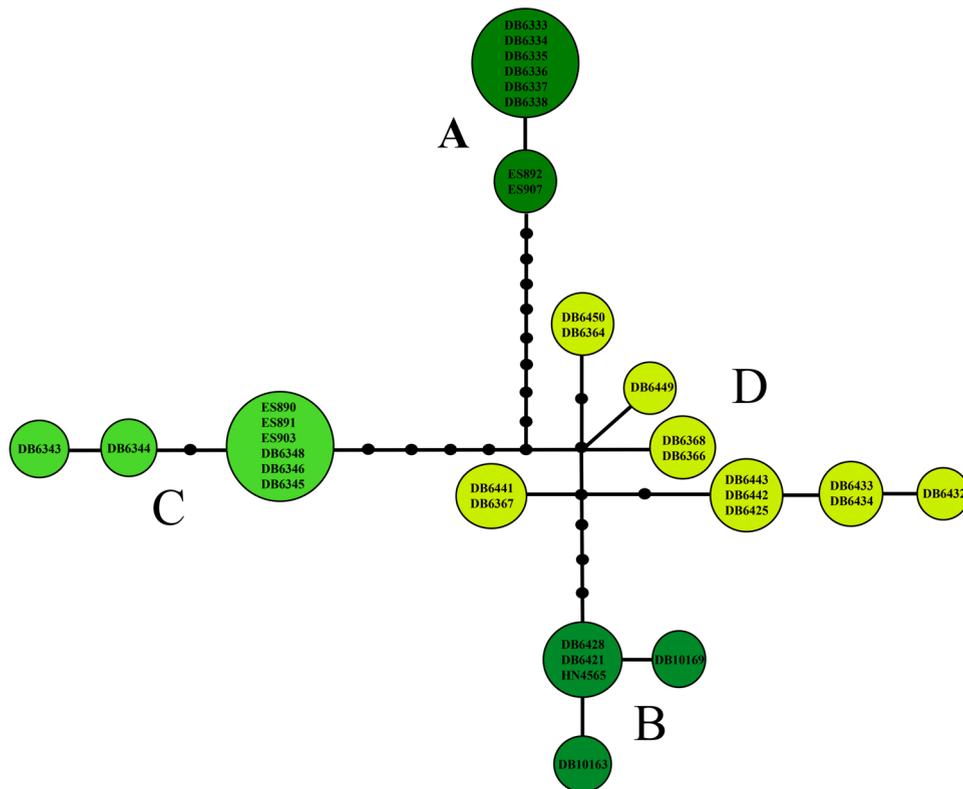
Fig. 4. Parsimony haplotype network of the *Lacerta media media* using cyt *b* gene. The individuals of each subclade are color-coded with the gradient of green (A: dark green, B: forest green, C: bright green, and D: lime green), respectively (see Fig. 2). Step-mutations (missing or gap values) are shown with dark cycles in the line.

Table 3
The contribution of the evaluated layers on the distribution of *Lacerta media media.*

Variable	Percentage contribution
BIO 8	37.8%
BIO 11	21.2%
BIO 13	16.8%
BIO 18	14.12%
BIO 19	10.08%

The results of the phylogenetic study using the three mitochondrial genes (cyt *b*, 12S and 16S) revealed that the *L. media* creates a well-supported monophyletic clade within the *L. trilineata* group (Fig. S1). In addition, the results indicated four highly supported clades within the eastern three-lined lizard (A-D); Clade A was recovered as sister to the rest of clades within the subspecies. The findings are in line with the results of Ahmadzadeh et al. (2013c), although the study included limited specimens from a sole location in Iran.

Based on the time-calibrated tree, the divergence of *L. trilineata* group (*L. media* and *L. trilineata – L. pamphylica*) happened around 4 Mya (95% HPD: 3.33–4.64) in the Pliocene epoch (Fig. 2). Moreover, the LTT plot showed that the diversification within *L. trilineata* group occurred about 3.5 Mya with a rapid divergence (Fig. S2). The time and rapid diversification probably related to geological and environmental changes in the Anatolian plateau, such as the emergence of the Taurus and the Amanos Mountain ranges in the south, the Pontic Mountains in the north, and the Anatolian Diagonal in the east (Davis, 1971; Kosswig, 1955; Bilgin, 2011; Ahmadzadeh et al., 2013c). The intraspecific divergence of *L. media* can be explained mainly through past climatic events during Pliocene and Pleistocene epochs. The subspecies of *L. media* originated from Anatolia, dispersed through multiple separate routes southward to the Mediterranean region and eastward to Armenia, northwestern Iran and the Zagros Mountains (Ahmadzadeh et al., 2013c). Based on our findings, the diversification of *L. m. media* happened about 1.11 Mya (95% HPD: 0.72 – 1.64 Mya). It can be suggested that, climatic fluctuations in the Pleistocene epoch and in particular, the Mid-Pleistocene Transition (MPT; approximately 0.7 – 1.25 Mya; Clark et al., 2006; Willeit et al., 2019) had a crucial influence on initiating the divergence within the *L. m. media* . During MPT, a change occurred in the glacial and interglacial cycles both in the terms of duration and amplitude (i.e., temperature change). The periods have shifted from the pre-MPT cycles with a dominant length of 41,000 years and low-amplitude to the prolonged post-MPT cycles having
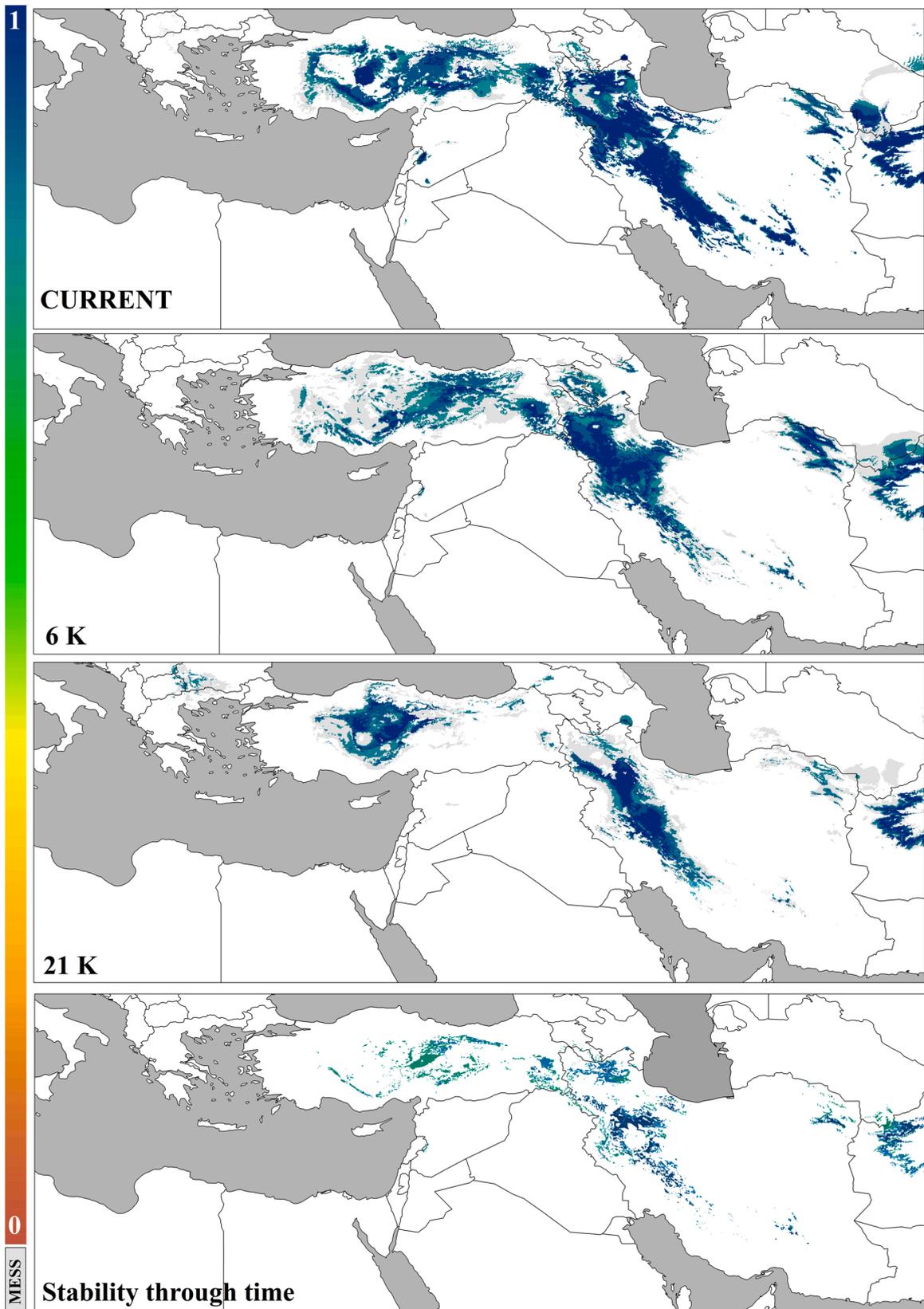
(caption on next page)

Fig. 5. The potential distribution range of the *Lacerta media media* under current climatic conditions, mid-Holocene (6 K), last glacial maximum, LGM (21 K). Stability through time may indicate potential refugia in Irano-Anatolian region (including central Anatolia, NW Iran and the central Zagros Mountains).

an average length of approximately 100,000 years with higher amplitude (i.e., both colder glacial and warmer interglacial in comparison with pre-MPT cycles, specifically the Eemian interglacial before LGM; Maslin and Brierley, 2015; Willeit et al., 2019). These intense fluctuations (i.e., extremely cold glacial and warm interglacial periods) may have induced periodic restrictions and expansions between various high- and low-altitude refugia in the Irano-Anatolian region and as the result, triggered the divergence within clades in the eastern three-lined lizard. The new climate conditions continued during the post-MPT periods which could probably have continued this divergence (Burke et al., 2018; Clark et al., 2006; Hansen et al., 2013; Lisiecki and Raymo, 2005; Willeit et al., 2019). Climate fluctuations during the Holocene epoch, as a complementary hypothesis, could have also affected the distribution of today's populations. The high humidity at the beginning of this period (Morellón et al., 2018) has probably dispersed the populations in the low altitudes. As the result, the connectivity of populations was easier (Ehlers and Gibbard, 2004; Kaufman et al., 2004). Nevertheless, as the climate shifted since Mid- to late Holocene, which has resulted in drier climate in the middle latitudes having weaker monsoons (Wanner et al., 2008), populations have possibly moved to the higher altitudes. The results obtained from habitat modeling in the present study (the impact of Bio 8 and Bio 11 factors on the species distribution) and also some other ecological investigations (Loos et al., 2011; Mazanaeva and Askenderov, 2016; Heidari, 2020) proved that the three-lined lizard is highly linked to the cold climates with high humidity and woodlands. According to previously mentioned, the LTT plot (Fig. S2) demonstrated a rapid diversification within *L. trilineata* group. Previous studies have stated that subfamily Lacertinae diversified rapidly and their distribution ranges are fragmented (Pavlicev and Mayer, 2009). Beside the mentioned geological and environmental events, it is believed that, this (rapid divergence and fragmented dispersal range) may have occurred, because the distribution range of Lacertids could have diminished or populations have experienced several climatic fluctuations which probably have isolated in different refugia as the result (Ahmadzadeh et al., 2013b; Saberi-Pirooz et al., 2021).

The results of SDIVA and BBM analyses (Fig. 3) revealed the role of vicariance and dispersal events in the divergence of *L. m. media* clades which may be attributed to the mentioned periodic restrictions and expansions during the climatic fluctuations. Possibly, the existence of complex topography and multiple refugia across the distribution range of the subspecies have led to the present diversity and distribution pattern of clades. The effect of this specific geomorphology and the rich biodiversity of the Irano-Anatolian region has been mentioned frequently in previous studies (e.g., Giokas et al., 2011; Noroozi et al., 2018, 2019; Malekoutian et al., 2020). Considering the findings of previous studies (e.g., Ahmadzadeh et al., 2013c; Sagonas et al., 2014), the hypothetical ancestor and origin of *L. trilineata* group and *L. m. media* as well, are assumed to be located in central Anatolia. The existence of several sub-clades and also high haplotype diversity ($h = 0.91$; see Table 2), in Clade D (from central Anatolia) could be the result of the occurrence of the clade in the origin of the diversification (Ahmadzadeh et al., 2013c; Sagonas et al., 2014). Populations in the Clade A may have isolated earlier and undergone several climatic fluctuations in northwestern Iran during MPT. Genetic differences (based on mtDNA) between the populations from northwestern Iran (Clade A) and the other populations (see Table 1) and also the earlier separation of the clade from the origin of subspecies (Fig. 2) are in line with the result of the haplotype network (distant positioning of Clade A haplotypes with several step mutations from other clades; Fig. 4).

The demographic analyses (Fig. S3) based on the cyt *b* sequences suggest that *L. m. media* demonstrated stability in the population size. The existence of several clades in the widespread distribution range of the subspecies could be also related to the presence of multiple refugia. The presence of multiple refugia (different refugium for each clade) could explain both the rich genetic diversity and prolonged demographic stability of *L. m. media* and it is probably in concordance with 'refugia-within-refugia' scenario (Gómez and Lunt, 2007; Canestrelli and Nascetti, 2008; Noroozi et al., 2018; Ahmadzadeh et al., 2020; Amiri et al., 2021).

The results of SDM analyses demonstrated habitat suitability in the central Anatolia and northern and central parts of the Zagros Mountains as well as northeastern Iran and western parts of Afghanistan during LGM, and these areas probably served suitable low-altitude refugia at the glacial periods. This suitability has expanded through earth warming till the current time (Fig. 5). The distribution of the subspecies is anticipated to expand under future climatic scenarios. This expansion from LGM towards the present and future could be explained by the presence of high-altitude habitats in the Irano-Anatolian region which was also occupied by this subspecies in the past periods, especially during the interglacial periods. The areas around northern parts of the Zagros Mountains have shown more stable conditions for *L. m. media* than central Anatolia since LGM (but this stability is not more than Western Afghanistan). The habitat suitability and possible existence of refugia as well as biodiversity hotspots in northwestern Iran and the northern Zagros Mountains have been proven in several studies (Ahmadzadeh et al., 2013b; Noroozi et al., 2019; Heidari, 2020). However, several past and present suitable habitats in western Afghanistan are not known to have been inhabited by the subspecies (there is no occurrence record).

The results obtained from molecular approaches are in line with SDM analyses and both methods emphasize the importance of the refugia in species survival during glacial periods. According to the findings, the Anatolian and Zagros Mountains were considered as refugia during past earth's temperature fluctuations in the northern hemisphere (Giokas et al., 2011; Malekoutian et al., 2020). In the case of *L. m. media*, it can be observed that the Iranian part of Irano-Anatolian region had a crucial role in preserving unique populations like the samples in Clade A. Although, for further evaluation of these implications using more samples and markers (especially nuclear markers) is recommended. The application of both mtDNA and nuclear markers (multilocus evidence) would help to infer more robust results. However, based on the previous study, the combination of protein-coding markers and rRNA genes to reconstruct the phylogenetic relationships and resolve the controversial genealogy is helpful (Rubinoff and Holland, 2005). Since the present study is a sequential investigation of the previous studies on *L. media*, the same molecular markers were used. So, lacking the nuclear DNA

genes in the study is a result of the limited use of the nuclear markers in the previous investigations.

Although the present study limits using nuclear genes and estimation of gene flows and admixture between the populations (and haplotypes), some conservation implications can be raised. Based on the obtained results, four main genetic structures have recognized within the subspecies using mtDNA. Among them, Clade D contains a rich diversity ($h = 0.91$) and its geographical distribution (i.e., central Anatolia) as the hypothetical origin that, has a high conservation value (Srinivas et al., 2021). Also, distinctness of a clade such as Clade A, has its specific conservation importance. As a peripheral clade, the populations of Clade A probably have limited contact with populations within other clades (considering higher genetic distances with other clades, Table 1). According to the previous literature, peripheral populations could have their own conservation values (Lesica and Allendorf, 1995; Channell, 2004; Cadotte and Jonathan Davies, 2010; Buerki et al., 2015; Pollock et al., 2015). The concordant results of stability through time analysis, provide further evidence and indicate that central Anatolia, NW and Western Iran and central Zagros Mountains had more stable conditions during the past climatic fluctuations (Fig. 5), which supposed to preserve well the populations within the eastern three-lined lizard. It is believed that climate stability of an area could explain genetic diversity of its biota and emphasize on conservation priority of that area (Carnaval et al., 2009; de Oliveira et al., 2015). In the broad sense, the methods utilized for the present study (molecular and SDMs), can be enlightening for more critical conservation practices at the biogeographical scales (Richardson and Whittaker, 2010.).

5. Conclusion

The specific geomorphology and climatic oscillations especially the Mid-Pleistocene Transition are major drivers for species divergence in the Irano-Anatolian region. The evolution process of the eastern three-lined lizard has started by MPT around 1 Mya in the region. This special climatic event diverged four separate clades across the distribution range of the subspecies. These clades are probably preserved by multiple refugia in both low and high altitudes. Besides, the results of SDM prove the stability of climatic conditions of mentioned regions at least since 20 Kya. Analyzing more samples from each clade, and using suitable nuclear markers to investigate the species, lineages, are suggested. Furthermore, the use of microsatellite markers for detailed population genetics is also proposed.

Declaration of Competing Interest

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2021.e01839.

References

Afroosheh, M., Rödder, D., Mikulicek, P., Akmali, V., Vaissi, S., Fleck, J., Schneider, W., Sharifi, M., 2019. Mitochondrial DNA variation and Quaternary range dynamics in the endangered yellow spotted mountain newt, *Neurergus derjugini* (Caudata, Salamandridae). J. Zool. Syst. Evol. Res 57 (3), 580–590.

Aghbolaghi, M.A., Ahmadzadeh, F., Kiabi, B., Keyghobadi, N., 2019. The permanent inhabitant of the oak trees: phylogeography and genetic structure of the Persian squirrel (*Sciurus anomalus*). Biol. J. Linn. Soc. 127 (2), 197–212.

Aghbolaghi, M.A., Ahmadzadeh, F., Kiabi, B.H., Keyghobadi, N., 2020. Evolutionary history of the Persian squirrel (*Sciurus anomalus*): it emerged on the Eurasian continent in the Miocene. Zool. Anz. 287, 17–24.

Agustí, J., Blain, H.A., Cuenca-Bescós, G., Bailon, S., 2009. Climate forcing of first hominid dispersal in Western Europe. J. Hum. Evol. 57 (6), 815–821.

Ahmadzadeh, F., Carretero, M.A., Harris, D.J., Perera, A., Böhme, W., 2012. A molecular phylogeny of the eastern group of ocellated lizard genus *Timon* (Sauria: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphib. -Reptil 33 (1), 1–10.

Ahmadzadeh, F., Carretero, M.A., Rödder, D., Harris, D.J., Freitas, S.N., Perera, A., Böhme, W., 2013a. Inferring the effects of past climate fluctuations on the distribution pattern of *Iranolacerta* (Reptilia, Lacertidae): Evidence from mitochondrial DNA and species distribution models. Zool. Anz. 252 (2), 141–148.

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Böhme, W., James Harris, D., Üzüm, N., Rödder, D., 2013b. Rapid lizard radiation lacking niche conservatism: ecological diversification within a complex landscape. J. Biogeogr. 40 (9), 1807–1818.

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Böhme, W., Ilgaz, Ç., Harris, D.J., Engler, J.O., Üzüm, N., Carretero, M.A., 2013c. Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards. Biol. J. Linn. Soc. 110 (2), 398–408.

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Böhme, W., Ihlow, F., Kapli, P., Miraldo, A., Rödder, D., 2016. Separate histories in both sides of the Mediterranean: phylogeny and niche evolution of ocellated lizards. J. Biogeogr. 43 (6), 1242–1253.

Ahmadzadeh, F., Shahrokhi, G., Saberi-Pirooz, R., Oladi, M., Taati, M., Poyarkov, N.A., Rödder, D., 2020. Alborz Heritage: geographic distribution and genetic differentiation of the Iranian *Paradactylodon* (Amphibia: Hynobiidae). Amphib. -Reptil. 41 (4), 519–534.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19 (6), 716–723.

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

Amiri, N., Vaissi, S., Aghamir, F., Saberi-Pirooz, R., Rödder, D., Ebrahimi, E., Ahmadzadeh, F., 2021. Tracking climate change in the spatial distribution pattern and the phylogeographic structure of Hyrcanian wood frog, *Rana pseudodalmatina* (Anura: Ranidae). J. Zool. Syst. Evol. Res. 00, 1–16.

Araújo, M.B., New, M., 2007. Ensemble forecasting of species distributions. Trends Ecol. Evol. 22 (1), 42–47.

Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: a comprehensive review. Infect. Genet. Evol. 29, 164–179.

Arnold, E.N., 1973. Relationships of the Palaeartic lizards assigned to the genera *Lacerta, Algyroides* and *Psammodromus* (Reptilia: Lacertidae). Bull. Brit. Mus. Nat. Hist. Zool. 25, 289–366.

Arnold, E.N., 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 (1), 1–86.

Bartlein, P.J., Harrison, S., Brewer, S., Connor, S., Davis, B., Gajewski, K., Peyron, O., 2011. Pollen-based continental climate reconstructions at 6 and 21 ka: a global synthesis. Clim. Dyn. 37, 775–802.

Bennett, K.D., Provan, J., 2008. What do we mean by 'refugia'? Quat. Sci. Rev. 27, 2449–2455.

Bilgin, R., 2011. Back to the suture: the distribution of intraspecific genetic diversity in and around Anatolia. Int. J. Mol. Sci. 12 (6), 4080–4103.

Braconnot, P., Harrison, S.P., Kageyama, M., Bartlein, P.J., Masson-Delmotte, V., Abe-Ouchi, A., Otto-Bliesner, B., Zhao, Y., 2012. Evaluation of climate models using palaeoclimatic data. Nat. Clim. Change 2 (6), 417–424.

Breiman, L., 2001. Random forests. Mach. Learn 45 (1), 5–32.

Brieman, L., Friedman, J.H., Olshen, R.A., Stone, C.J., 1984. Cart. *Classification and Regression Trees*. Wadsworth and Brooks/Cole: Monterey, CA, USA.

Buerki, S., Callmander, M.W., Bachman, S., Moat, J., Labat, J.N., Forest, F., 2015. Incorporating evolutionary history into conservation planning in biodiversity hotspots. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370, 20140014.

Burke, K.D., Williams, J.W., Chandler, M.A., Haywood, A.M., Lunt, D.J., Otto-Bliesner, B.L., 2018. Pliocene and Eocene provide best analogs for near-future climates. Proc. Natl. Acad. Sci. U. S. A. 115 (52), 13288–13293.

Cadotte, M.W., Jonathan Davies, T., 2010. Rarest of the rare: advances in combining evolutionary distinctiveness and scarcity to inform conservation at biogeographical scales. Divers. Distrib. 16 (3), 376–385.

Canestrelli, D., Nascetti, G., 2008. Phylogeography of the pool frog *Rana* (*Pelophylax*) lessonae in the Italian peninsula and Sicily: multiple refugia, glacial expansions and nuclear–mitochondrial discordance. J. Biogeogr. 35 (10), 1923–1936.

Channell, R., 2004. The conservation value of peripheral populations: the supporting science. In Proceedings of the species at risk 2004 pathways to recovery conference (pp. 1–17). Victoria, British Columbia, Canada: Species at Risk 2004 Pathways to Recovery Conference Organizing Committee.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F., Rodrigues, M.T., Moritz, C., 2009. Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot. Science 323 (5915), 785–789.

Clark, P.U., Archer, D., Pollard, D., Blum, J.D., Rial, J.A., Brovkin, V., Mix, A.C., Pisias, N.G., Roy, M., 2006. The middle Pleistocene transition: characteristics, mechanisms, and implications for long-term changes in atmospheric pCO2. Quat. Sci. Rev. 25 (23–24), 3150–3184.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9 (10), 1657–1659.

Davis, P.H., 1971. Distribution patterns in Anatolia with particular reference to endemism. In: Davis, P.H., Harper, P.C., Hedge, I.C. (Eds.), Plant life of South-West Asia. The Botanical Society of Edinburgh, Edinburgh, pp. 15–27.

Djamali, M., de Beaulieu, J.L., Shah-hosseini, M., Andrieu-Ponel, V., Ponel, P., Amini, A., Akhani, H., Leroy, S.A., Stevens, L., Lahijani, H., Brewer, S., 2008. A late Pleistocene long pollen record from Lake Urmia, NW Iran. Quat. Res. 69 (3), 413–420.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7 (1), 214.

Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol. Biol. Evol. 22, 1185–1192.

Ehlers, J., Gibbard, P.L., 2004. *Quaternary glaciations-extent and chronology: part I: Europe.*. Elsevier.

Engström, K.S., Broberg, K., Concha, G., Nermell, B., Warholm, M., Vahter, M., 2007. Genetic polymorphisms influencing arsenic metabolism: evidence from Argentina. Environ. Health Perspect. 115 (4), 599–605.

Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10 (3), 564–567.

Eyring, V., Bony, S., Meehl, G.A., Senior, C.A., Stevens, B., Stouffer, R.J., Taylor, K.E., 2016. Overview of the Coupled Model Intercomparison Project Phase 6 (CMIP6) experimental design and organization. Geosci. Model Dev. 9 (5), 1937–1958.

Farasat, H., Akmali, V., Sharifi, M., 2016. Population genetic structure of the endangered Kaiser's Mountain Newt, Neurergus kaiseri (Amphibia: Salamandridae). PLoS One 11 (2), e0149596.

Fechter, D., Storch, I., 2014. How many wolves (*Canis lupus*) fit into Germany? The role of assumptions in predictive rule-based habitat models for habitat generalists. PloS One 9 (7), e101798.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1–km spatial resolution climate surfaces for global land areas. Int J. Clim. 37 (12), 4302–4315.

Friedman, J.H., 1991. Multivariate adaptive regression splines (with discussion). Ann. Stat. 19 (1), 79–141.

Friedman, J.H., 2001. Greedy function approximation: a gradient boosting machine. Ann. Stat. 29 (5), 1189–1232.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147 (2), 915–925.

Giokas, S., Kumlutaş, Y., Ilgaz, Ç., Kornilios, P., Chondropoulos, B., Fraguedakis-Tsolis, S., 2011. The role of Anatolian refugia in herpetofaunal diversity: an mtDNA analysis of *Typhlops vermicularis* Merrem, 1820 (Squamata, Typhlopidae). Amphib. -Reptil 32 (3), 351–363.

Giovanelli, J., Siqueira, M., de, Haddad, C., Alexandrino, J.-E., 2010. Modeling a spatially restricted distribution in the Neotropics: How the size of calibration area affects the performance of five presence-only methods. Ecol. Model. 221 (2), 215–224.

Godinho, R., Crespo, E., Ferrand, N., Harris, D.J., 2005. Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. Amphib. -Reptil 26, 271–285.

Gómez, A., Lunt, D.H., 2007. Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. Phylogeography of Southern European Refugia. Springer, Dordrecht, pp. 155–188.

Guisan, A., Thuiller, W., 2005. Predicting species distribution: offering more than simple habitat models. Ecol. Lett. 8 (9), 993–1009.

Hansen, J., Sato, M., Russell, G., Kharecha, P., 2013. Climate sensitivity, sea level and atmospheric carbon dioxide. Philos. Trans. A Math. Phys. Eng. Sci. 371 (2001), 20120294, 20120294.

Hastie, T., Tibshirani, R., Buja, A., 1994. Flexible discriminant analysis by optimal scoring. J. Am. Stat. Assoc. 89 (428), 1255–1270.

Hastie, T.J., Tibshirani, R.J., 1986. Generalized additive models. Stat. Sci. 1 (3), 297–318.

Heidari, N., 2020. Ecological niche differentiation between *Lacerta media* and *Lacerta strigata* (Sauria: Lacertidae) in Iran. Biologia 1–5.

Hendrix, R., Fleck, J., Schneider, W., Schneider, C., Geller, D., Avci, A., Olgun, K., Steinfartz, S., 2014. First comprehensive insights into nuclear and mitochondrial DNA based population structure of Near East Mountain brook newts (Salamandridae: genus *Neurergus*) suggest the resurrection of *Neurergus derjugini*. Amphib. -Reptil 35 (2), 173–187.

Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. R. Soc. Lond. B Biol. Sci. 359 (1442), 183–195.

Hijmans, R.J., Cameron, S., Parra, J., 2005. BIOCLIM. Availabe online: ⟨http://www.worldclim.Org/bioclim⟩ (accessed on 15 June 2018).

Hofreiter, M., Stewart, J., 2009. Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr. Biol. 19 (14), R584–R594.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Jeschke, J.M., Strayer, D.L., 2008. Usefulness of bioclimatic models for studying climate change and invasive species. Ann. N. Y. Acad. Sci. 1134 (1), 1–24.

Kafash, A., Yousefi, M., 2017. Negative impacts of the future climate change on mountain dweller lacertid lizards in Iran. J. Ecol. Nat. Environ. 70 (1), 149–160.

Kafash, A., Ashrafi, S., Yousefi, M., Rastegar-Pouyani, E., Rajabizadeh, M., Ahmadzadeh, F., Grünig, M., Pellissier, L., 2020. Reptile species richness associated to ecological and historical variables in Iran. Sci. Rep. 10 (1), 1–11.

Katoh, K., Rozewicki, J., Yamada, K.D., 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. BRIEF BIOINFORM.

Kaufman, D.S., Ager, T.A., Anderson, N.J., Anderson, P.M., Andrews, J.T., Bartlein, P.J., Brubaker, L.B., Coats, L.L., Cwynar, L.C., Duvall, M.L., Dyke, A.S., 2004. Holocene thermal maximum in the western Arctic (0–180 W). Quat. Sci. Rev. 23 (5–6), 529–560.

Kehl, M., 2009. Quaternary climate change in Iran—the state of knowledge. Erdkunde 63, 1–17.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA. 86 (16), 6196–6200.

Kosswig, C., 1955. Zoogeography of the Near East. Syst. Zool. 4 (2), 49–73.

Kuhle, M., 2008. The Pleistocene Glaciation (LGP and pre-LGP, pre-LGM) of SE Iranian mountains Exemplified by the Kuh-i-Jupar, Kuh-i-Lalezar and Kuh-i-Hezar Massifs in the Zagros. Polarforschung 77 (2/3), 71–88.

Lesica, P., Allendorf, F.W., 1995. When are peripheral populations valuable for conservation? Conserv. Biol. 9 (4), 753–760.

Lisiecki, L.E., Raymo, M.E., 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18O records. Paleoceanography 20 (1), PA1003.

Loos, J., Dayan, T., Drescher, N., Levanony, T., Maza, E., Shacham, B., Talbi, R., Assmann, T., 2011. Habitat preferences of the Levant Green Lizard, *Lacerta media israelica* (Peters, 1964) (Reptilia: Lacertidae). Zool. Middle East 52 (1), 17–28.

Malekoutian, M., Sharifi, M., Vaissi, S., 2020. Mitochondrial DNA sequence analysis reveals multiple Pleistocene glacial refugia for the Yellow-spotted Mountain newt, *Neurergus derjugin*i (Caudata: Salamandridae) in the mid-Zagros range in Iran and Iraq. Ecol. Evol. 10 (5), 2661–2676.

Maslin, M.A., Brierley, C.M., 2015. The role of orbital forcing in the Early Middle Pleistocene Transition. Quat. Int. 389, 47–55.

Maslin, M.A., Ridgwell, A.J., 2005. Mid-Pleistocene revolution and the 'eccentricity myth'. Geol. Soc. Spec. Publ. 247 (1), 19–34.

Mazanaeva, L.F., Askenderov, A.D., 2016. The distribution and ecology of the middle lizards *Lacerta media* Lantz et Cyren, 1920 (Reptilia: Sauria: Lacertidae) in Dagestan. Biol. Commun. 3 (3), 93–99 ([In: Russian with English abstract]).

McCullagh, P., Nelder, J.A., 1989. Generalized Linear Models. Chapman and Hall, New York.

Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T.G.M.C.J., Mittermeier, C.G., Lamoreux, J., 2005. FONSECA & GAB 2005. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. Conservation International,, Washington, DC.

Morán-Ordóñez, A., Lahoz-Monfort, J.J., Elith, J., Wintle, B.A., 2017. Evaluating 318 continental-scale species distribution models over a 60–year prediction horizon: what factors influence the reliability of predictions? Glob. Ecol. Biogeogr. 26 (3), 371–384.

Morellón, M., Aranbarri, J., Moreno, A., González-Sampériz, P., Valero-Garcés, B.L., 2018. Early Holocene humidity patterns in the Iberian Peninsula reconstructed from lake, pollen and speleothem records. Quat. Sci. Rev. 181, 1–18.

Naimi, B., Araújo, M.B., 2016. sdm: a reproducible and extensible R platform for species distribution modelling. Ecography 39 (4), 368–375.

Naimi, B., Hamm, N.A., Groen, T.A., Skidmore, A.K., Toxopeus, A.G., 2014. Where is positional uncertainty a problem for species distribution modelling? Ecography 37 (2), 191–203.

Noroozi, J., Talebi, A., Doostmohammadi, M., Rumpf, S.B., Linder, H.P., Schneeweiss, G.M., 2018. Hotspots within a global biodiversity hotspot-areas of endemism are associated with high mountain ranges. Sci. Rep. 8 (1), 1–10.

Noroozi, J., Naqinezhad, A., Talebi, A., Doostmohammadi, M., Plutzar, C., Rumpf, S.B., Asgarpour, Z., Schneeweiss, G.M., 2019. Hotspots of vascular plant endemism in a global biodiversity hotspot in Southwest Asia suffer from significant conservation gaps. Biol. Conserv. 237, 299–307.

Nylander, J., 2004. 'MrModeltest 2.2′, Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

de Oliveira, G., Lima-Ribeiro, M.S., Terribile, L.C., Dobrovolski, R., Telles, M.P.D.C., Diniz-Filho, J.A.F., 2015. Conservation biogeography of the Cerrado's wild edible plants under climate change: linking biotic stability with agricultural expansion. Am. J. Bot. 102 (6), 870–877.

Palumbi, S., 1991. 'Simple fool's guide to PCR'. Dept. of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pavlicev, M., Mayer, W., 2009. Fast radiation of the subfamily Lacertinae (Reptilia: Lacertidae): History or methodical artefact? Mol. Phylogenet. Evol. 52 (3), 727–734.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190 (3–4), 231–259.

Pollock, L.J., Rosauer, D.F., Thornhill, A.H., Kujala, H., Crisp, M.D., Miller, J.T., McCarthy, M.A., 2015. Phylogenetic diversity meets conservation policy: small areas are key to preserving eucalypt lineages. Philos. Trans. R. Soc. Lond. b Biol. Sci. 370 (1662), 20140007, 20140007.

Provan, J., Bennett, K.D., 2008. Phylogeographic insights into cryptic glacial refugia. Trends Ecol. Evol. 23 (10), 564–571.

Rambaut, A., Drummond, A.J., 2009. 'Tracer: MCMC trace analysis tool, version 1.5′, Available at website ⟨http://tree.bio.ed.ac.uk/software/tracer⟩.

Richardson, D.M., Whittaker, R.J., 2010. Conservation biogeography–foundations, concepts and challenges.

Rubinoff, D., Holland, B.S., 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Syst. Biol. 54 (6), 952–961.

Saberi-Pirooz, R., Rajabi-Maham, H., Ahmadzadeh, F., Kiabi, B.H., Javidkar, M., Carretero, M.A., 2021. Pleistocene climate fluctuations as the major driver of genetic diversity and distribution patterns of the Caspian green lizard, *Lacerta strigata* Eichwald, 1831. Ecol. Evol. 11 (11), 6927–6940.

Sagonas, K., Poulakakis, N., Lymberakis, P., Parmakelis, A., Pafilis, P., Valakos, E.D., 2014. Molecular systematics and historical biogeography of the green lizards (Lacerta) in Greece: Insights from mitochondrial and nuclear DNA. Mol. Phylogenet. Evol. 76, 144–154.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular cloning: a laboratory manual* (No. Ed. 2). Cold spring harbor laboratory press.

Schuster, M., Duringer, P., Ghienne, J.F., Vignaud, P., Mackaye, H.T., Likius, A., Brunet, M., 2006. The age of the Sahara Desert. Science 311 (5762), 821, 821-821.

Shahabi, S., Akmali, V., Sharifi, M., 2017. Taxonomic evaluation of the greater horseshoe bat *Rhinolophus ferrumequinum* (Chiroptera: Rhinolophidae) in Iran inferred from the mitochondrial D-loop gene. Zool. Sci. 34 (4), 361–367.

ŠMÍD, J., Moravec, J., Kodym, P., Kratochvíl, L., Yousefkhani, S.S.H., Frynta, D., 2014. Annotated checklist and distribution of the lizards of Iran. Zootaxa 3855 (1), 1–97.

Soberón, J., 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecol. Lett. 10 (12), 1115–1123.

Srinivas, Y., Pande, A., Gole, S., Jothi, P.P., Magesh, K.M., Pathan, S., Dudhat, S., Shekar, H., Ghanekar, C., Kukadia, D., Johnson, J.A., 2021. Mitochondrial phylogeography reveals high haplotype diversity and unique genetic lineage in Indian dugongs (*Dugong dugon*). Aquat. Conserv. 31 (4), 818–829.

Stamatakis, A., 2006. RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models, Bioinformatics, 22. Oxford University Press, pp. 2688–2690.

Swofford, D.L., 2003. 'PAUP*: phylogenetic analysis using parsimony, version 4.0 b10′.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595, 585_595.

Torki, F., Ahmadzadeh, F., Ilgaz, Ç., Avci, A., Kumlutacs, Y., 2011. Description of four new *Asaccus* Dixon and Anderson, 1973 (Reptilia: Phyllodactylidae) from Iran and Turkey. Amphib. -Reptil. 32, 185–202. https://doi.org/10.1163/017353711×556998.

Vapnik, V., 1995. The Nature of Statistical Learning Theory. Springer-Verlag.

Wanner, H., Beer, J., Bütikofer, J., Crowley, T.J., Cubasch, U., Flückiger, J., Goosse, H., Grosjean, M., Joos, F., Kaplan, J.O., Küttel, M., 2008. Mid-to Late Holocene climate change: an overview. Quat. Sci. Rev. 27 (19–20), 1791–1828.

Warren, D.L., Glor, R.E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography 33 (3), 607–611.

Willeit, M., Ganopolski, A., Calov, R., Brovkin, V., 2019. Mid-Pleistocene transition in glacial cycles explained by declining CO_2 and regolith removal. Sci. Adv. 5 (4), eaav7337.

Yu, Y., Harris, A.J., Blair, C., He, X., 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49.