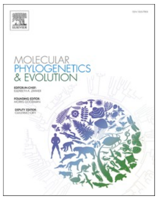
Molecular Phylogenetics and Evolution 180 (2023) 107675

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



Diversification in the mountains: Evolutionary history and molecular phylogeny of Anatolian rock lizards

Sara Kafimola [a,b,1], Maryam Azimi [a,1], Reihaneh Saberi-Pirooz [a], Çetin Ilgaz [c,d], Ghasem Mohammadi Kashani [b], Paschalia Kapli [e], Faraham Ahmadzadeh [a,*]

[a] Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C., Evin, Tehran 1983963113, Iran
[b] Department of Biology, Faculty of Science, University of Zanjan, Zanjan, Iran
[c] Department of Biology, Faculty of Science, Dokuz Eylül University, Buca- İzmir, Turkey
[d] Research and Application Center for Fauna Flora, Dokuz Eylul University, Buca-İzmir, Turkey
[e] Centre for Life's Origins and Evolution, Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK

ARTICLE INFO

Keywords:
Apathya
Zagros Mountains
Anatolia
Molecular systematics
Orogeny
Middle Miocene Climate Transition

ABSTRACT

Mountains play a key role in forming biodiversity by acting both as barriers to gene flow among populations and as corridors for the migration of populations adapted to the conditions prevailing at high elevations. The Anatolian and the Zagros Mountains are located in the Alpine-Himalayan belt. The formation of these mountains has influenced the distribution and isolation of the animal population since the late Cenozoic. *Apathya* is a genus of lacertid lizards distributed along these mountains with two species, i.e., *Apathya cappadocica* and *Apathya yassujica*. The taxonomy status of lineages within the genus is complicated. In this study, we tried to collect extensive samples from throughout the distribution range, especially within the Zagros Mountains. Also, we used five genetic markers, two mitochondrial (COI and Cyt *b)* and three nuclear (C-mos, NKTR, and MCIR), to resolve the phylogenetic relationships within the genus and explain several possible scenarios that shaped multiple genetic structures. The combination of results in the current study indicated eight well-support monophyletic lineages that separated to two main groups; group 1 including *A. c. cappadocica, A. c. muhtari and A. c. wolteri,* group 2 contains four regional clades Turkey, Urmia, Baneh and Ilam, and finally a single clade belonging to the species *A. yassujica*. In contrast to previous studies, *Apathya cappadocica urmiana* was divided into four clades and three clades were recognized within Iranian boundaries. The clades have dispersed from Anatolia to adjacent regions in the south of Anatolia and the western Zagros Mountains. According to the evidence generated in this study this clade is paraphyletic. Based on our assumption, orogeny activities and also climate fluctuations in Middle Miocene and Pleistocene have influenced to formation of lineages. In this study we revisit the taxonomy of the genus and demonstrate that the species diversity was substantially underestimated. Our findings suggest that each of the eight clades corresponding to subspecies and distinct geographic regions deserve to be promoted to species level.

1. Introduction

The dynamics of isolation caused by geological and climatological processes play a fundamental role in shaping the diversity and geographical distribution of all terrestrial life forms (Ali and Aitchison, 2014). Orogenesis, the result of two tectonic plates colliding, can act either as a barrier or as a corridor to organisms' migration (Fouad and Sissakian, 2011; Cox et al., 2016; Trewick, 2017). Climatic fluctuations can play a similarly important role in shaping the genetic diversity and the geographical distribution of species (Taberlet et al., 1998; Avise, 2000; Hewitt, 2004a,b; Himes et al., 2008). Thus, the interaction of geological and climatic changes can lead up to speciation and dramatic redistribution of species across the complex landscape (Gillespie and Roderick, 2014). It is generally assumed that geological events form early interspecies geographic boundaries and climate fluctuations subsequently shape intra-species structures (Ahmadzadeh et al., 2013a; Barata et al., 2012; Joger et al., 2007).

The Anatolian and Zagros Mountains are located in the Alpine-

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* Corresponding author.
E-mail address: f_ahmadzade@sbu.ac.ir (F. Ahmadzadeh).
[1] Both authors have the same contribution.

https://doi.org/10.1016/j.ympev.2022.107675
Received 19 June 2022; Received in revised form 25 August 2022; Accepted 7 December 2022
Available online 14 December 2022

Himalayan belt and were created from the collision of the Arabian plate with the European and Asian plates (Agard et al., 2005; Khadivi, 2010; Mouthereau, 2011). The Zagros Mountains are placed in the west of the Iranian Plateau and are extended about 1200 km along the north-west to southeast between eastern Turkey, where the mountains join the Anatolian Mountain range, and the Makran subduction zone in the Strait of Hormuz (Hatzfeld et al., 2010). The mountains reflect a complex series of tectonic activities. The Zagros orogeny stretches from the Turkish–Iranian border to the Makran area to the southeast (Ellouz-Zimmermann et al., 2007; Smit et al., 2010). It is also important to note that the Zagros probably developed during the Oligocene-Miocene, a period characterized by major tectonic and climatic changes. The tectonic deformation in the northern Zagros was already underway in the Middle Miocene (Khadivi, 2010). The Zagros mountain ranges with deep valleys have been referred to as the major centers of speciation in the Irano-Anatolia area (Mohammadi et al., 2018; Asadi et al., 2019; Goudarzi et al., 2019). The area has a high level of species richness and endemism, thus it is considered as a biodiversity hot-spot (Mittermeier et al., 2004; Ahmadzadeh et al., 2017; Noroozi et al., 2018). The area was affected by both climatic and tectonic factors that influenced the speciation processes. The Middle Miocene Climate Transition (~15–13.7 Ma) was a major event in Cenozoic climate evolution, causing global cooling, (Flower and Kennett, 1994; Foster and Rohling, 2013; Holbourn et al., 2014) and Pleistocene climatic oscillations have played a major role in structuring current biodiversity patterns and shaping the distribution of species and their genetic diversity both at the regional and global scale (Hewitt, 2000; Hewitt, 2004a,b). Many studies have documented that mountains surrounding the Iranian plateau have acted as natural barriers against the dispersal of different lizard species and as the driving force of speciation in the region (Joger et al., 2007; Kapli et al., 2013; Ghaedi et al. 2021). In western Iran and neighboring regions, the species of the small Lacertini often display fragmented ranges (Nilson et al., 2003; Arnold et al., 2007; Pavlicev and Mayer, 2009). Rapid diversification for tribe Lacertini led to their allopatric and often disjunctive ranges (Carranza et al., 2004; Arnold et al., 2007).

Apathya is a lacertid genus distributed in southeastern Turkey, western Iran, and northern Iraq and Syria (Eiselt, 1979; Nilson et al., 2003; Arnold et al., 2007) and currently consists of two species: *Apathya cappadocica* (Werner, 1902) and *Apathya yassujica* (Nilson et al. 2003). *Apathya yassujica* is a monotypic species restricted to the southern Zagros Mountains in Iran (Nilson et al., 2003); *A. cappadocica* is a polytypic species and broadly distributed in southeastern Turkey, northern Iraq, Syria, and western Iran (Arnold et al., 2007; Kapli et al., 2013). Up until now, five subspecies including *A. c. cappadocica* (Werner, 1902), *A. c. urmiana* (Lantz and Suchow, 1934), *A. c. wolteri* (Bird, 1936), *A. c. muhtari* (Eiselt, 1979), and *A. c. schmidtlerorum* (Eiselt, 1979) were recognized within the species. In spite of previous attempts to identify morphological traits and genetic structures (Schmidtler and Bischoff, 1995; Nilson et al., 2003; Arnold et al., 2007; Ilgaz et al., 2010), the phylogenetic relationships within the genus remain unclear. Kapli et al. (2013) showed that *Apathya* is a genetically diverse genus, and in fact, the diversity of mitochondrial DNA even between intraspecific lineages was higher than that between recognized lacertid species. Despite the general concordance between morphological and phylogenetic conclusions, they observed some important discrepancies; the most impressive one was the phylogenetic position of *A. c. urmiana* and its relationship with *A. yassujica*. Therefore, they suggested a comprehensive investigation with more samples, in particular from Iran, to resolve ambiguous relationships within the genus.

In the present study, we conduct a much more detailed sampling of localities and loci, combining two mitochondrial (Cyt *b* and COI) and three nuclear (C-mos, NKTR and MC1R) genes, to investigate the relationship within the genus more thoroughly. We, additionally, investigate the geological and climatological processes associated with shaping *Apathyás* diversity and distribution.

2. Material and methods

2.1. Sampling, laboratory procedures and preparing dataset

We did extensive sampling and sequencing of *Apathya* from the west of Iran and the south of Turkey (for more details see Table S1). Also, 44 additional specimens were retrieved from Kapli et al. (2013) and added (see Table S1) to the generated sequence dataset. Overall 66 specimens were analyzed and 92 new sequences were obtained in this study. Therefore, we made a comprehensive dataset throughout of the genus distribution range (Fig. 1). During fieldwork, a small part of the tail tip was removed from each animal and specimens were released at the site of capture. Tissue samples were preserved in 96 % ethanol and stored in a freezer at − 20 ℃ for long-term maintenance.

Total genomic DNA was extracted with standard protocols of high-salt and phenol–chloroform methods (Sambrook et al., 1989). Five partial genes including two mtDNA (Cytochrome *b* (Cyt *b*) and cytochrome *c* oxidase subunit I (COI)) and three nuclear (Oocyte maturation factor Mos (C-mos), MelanoCortin-1 (MC1R) and Natural Killer Cell Triggering Receptor (NKTR)) markers were used. Polymerase chain reactions (PCRs) were performed 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 dd H_2O, and 1 μl of template DNA (50–100 ng). Primers and conditions used in PCR amplification for each gene are demonstrated in Table S2. PCR products were visualized on 1 % agarose gel. The successfully amplified PCR samples were then sent to Macrogen (Macrogen, Seoul, South Korea) for sequencing. Sequences were edited using Geneious Prime® V. 2021.0.0 (Biomatters, www.geneious.com). The generated sequences were submitted to the GenBank database (Table S1).

2.2. Alignments and phylogenetic analyses

To infer the phylogenetic relationship of *Apathya* lineages throughout its distribution, we combined our newly produced sequence data with previous published ones (see Table S1). Two species of *Podarcis* (*Podarcis cretensis* and *Podarcis pityusensis*) were considered as outgroups. The datasets of all genes were aligned with MAFFT v.6 (Katoh et al., 2019) (https://mafft.cbrc.jp/; algorithm: Auto; scoring matrix: 200Pam/k = 2; Gap open penalty: 1.53) and were then combined, resulting in a final 2156 bp alignment (Cyt *b*: 319 bp, COI: 368 bp, C-mos: 351 bp, MCIR: 637 bp, and NKTR: 481 bp).

The best-fit partitioning scheme and the associated nucleotide substitution models for each partition under the Akaike's Information Criterion (Akaike, 1974) were obtained using PartitionFinder v.2 (Lanfear et al., 2016). The best scheme was six partitions. A summary of models for each partition is provided in Table S3.

The Maximum Likelihood (ML) inference was performed using IQ-Tree v.1.6.12 (Nguyen et al., 2015) under the six partitions (Table S3), and the confidence of branch supports were assessed from 1000 pseudoreplicates using the ultrafast Bootstrap (UFB) approach (Hoang et al., 2018).

The Bayesian Inference (BI) analysis was conducted for the combined dataset under the same partitioning scheme as in ML using MrBayes v.3.2 (Huelsenbeck & Ronquist, 2001). The analysis was performed in two independent runs with four chains for five million generations. Subsampling trees and parameters were saved every 100 iterations, which produced 50,001 trees during the analysis. Finally, 10 % of trees were discarded as burn-in, and the remaining trees were used to reconstruct the 50 % majority-rule consensus tree. The final standard deviation (SD) of split frequencies was 0.0025. The parameters were separately calculated for each partition. The performance of each run and evaluate convergence was explored using Tracer v.1.6 (Rambaut & Drummond, 2009).

The statistical significance of alternative tree topologies using the Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa, 1999) was

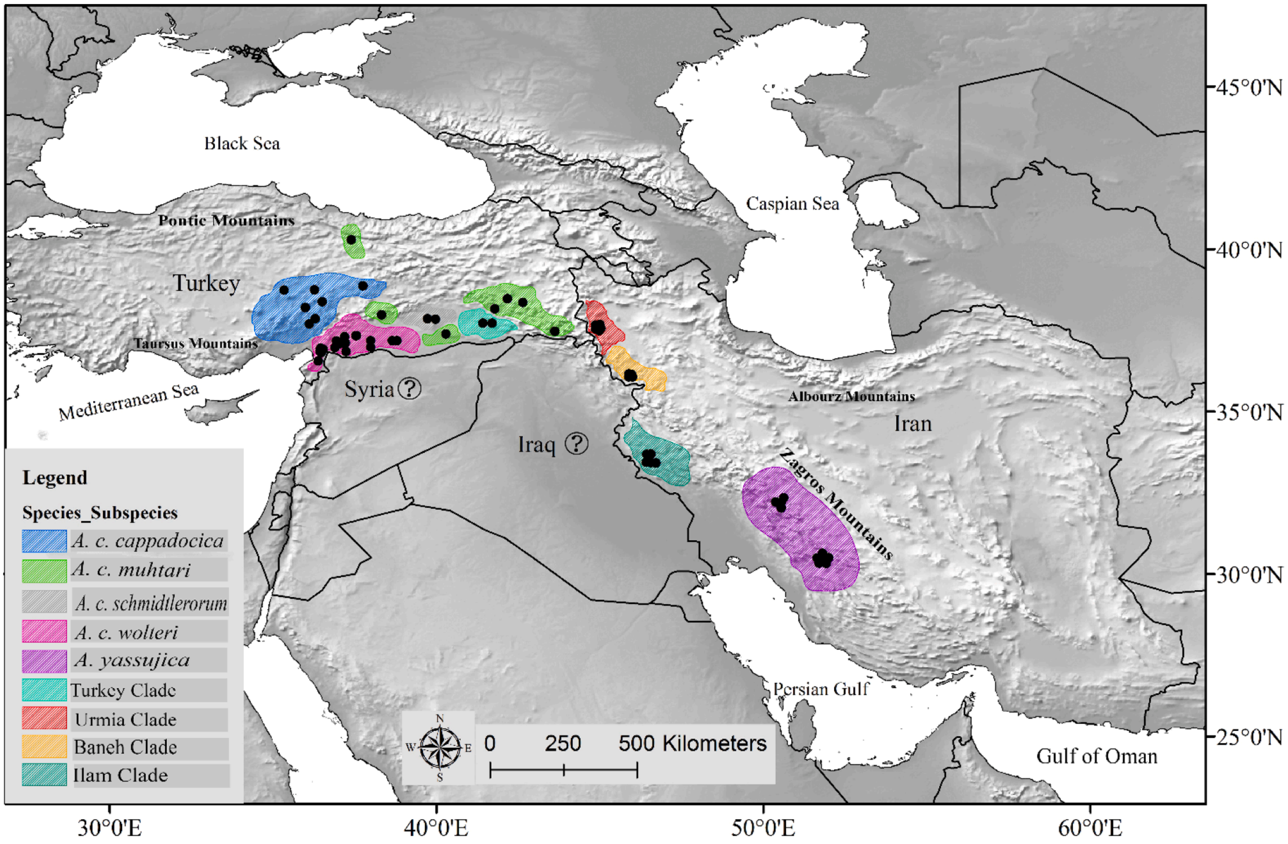

Fig. 1. The distribution map of *Apathya*. The black circles represent locations of samples that were used in the genetic analyses. The ranges of *A. c. muhtari, A. c. schmidtlerorum, A. c. cappadocica, A. c. wolteri*, Turkey Clade, Urmia Clade, Baneh Clade, Ilam Clade, and *A. yassujica* are illustrated with light green, gray, dark blue, pink, light blue, red, orange, dark green, and purple crosshatches, respectively. The sign of (?) shows missing data for these countries (Iraq and Syria). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

evaluated with 1000 bootstrap pseudoreplicates likelihood ratio test (SH-aLRT; Anisimova et al., 2011) as implemented in IQ-Tree v.1.6.12.

Uncorrected genetic distances were calculated among clades with Mega X (Kumar et al., 2018) for mtDNA (Cyt *b* and COI) datasets, separately.

2.3. Species delimitation

The *Apathya* genus was delimited by the General Mixed Yule Coalescent (GMYC) model (Pons et al., 2006), and Bayesian implementation of the Poisson tree processes model (bPTP: Zhang et al., 2013) using the mtDNA sequence dataset (Cyt *b* and COI). The GMYC model was run in R package SPLITS (SPecies'Limits by Threshold Statistics). The method is available as part of the R package 'splits' (https://r-forge.r-project.org/projects/splits/). An ultrametric tree obtained by BEAST was employed for the analysis. The bPTP infers species boundaries based on phylogenetic trees with branch lengths representing substitutions per site, therefore, we use the ML tree inferred with IQTree as the input file. The analysis was run on the bPTP online web server (https://species.h-its.org/ptp/) with 5×10^{5} MCMC generations, with a thinning of 100 and burn-in of 10 %.

2.4. Estimation of divergence times

Divergence times were estimated with BEAST v.1.7.2 (Drummond and Rambaut, 2007) using the combined dataset (five genes, 2156 bp). To calibrate the molecular clock, a secondary calibration approach was taken based on the age estimated for the *Apathya* genus (13.6 million years ago (henceforth Mya); Kapli et al. 2013). This calibration point was applied at the *Apathya* node (normal distribution, M: 13.7, SD: 0.2). A lognormal relaxed clock (uncorrelated) was used for all markers (because primary runs using an uncorrelated lognormal clock revealed ucld.stdev ($\delta > 0.1$) for all genes) with the Yule model for the speciation prior. The analysis was run for two million generations and sampled every 1,000 generations. Convergence diagnostics for the MCMC analyses were assessed using Tracer v. 1.6.1.

Lineage Through Time plotting (LTT) was constructed using Tracer v.1.6 within *Apathya* based on the combined dataset (five genes) to show the diversification of extant lineages through time.

2.5. Biogeographic analysis

Biogeographic reconstructions were executed using RASP 2.1 beta (Yu et al., 2015) for mtDNA markers (Cyt *b* and COI) because of the adequate available samples and comparatively higher mutation rate. For reconstructing the possible ancestral range of *Apathya* species, we used two different methods including the statistical dispersal-vicariance (S-DIVA, Yu et al., 2010) and Bayesian binary MCMC (BBM, Yu et al., 2010) analyses. As a result of phylogenetic analysis, nine different areas were considered within their distribution range (see results). Four areas settled in Iran through the north–south Zagros Mountains included Urmia, Baneh, Ilam and Yassuj and five regions were placed mostly in the south of Turkey (see Fig. 1). To take into account phylogenetic uncertainty, 20,000 trees generated from the Mr Bayes 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 Jukes-Cantor model with equal among-site rate variation was used for the BBM analysis.

TCS network constructed in PopART v1.7 (Leigh and Bryant, 2015) for combined of three nuDNA genes.

3. Results

3.1. Phylogenetic analyses

The constructed phylogenetic trees from the combined genes showed the same topologies for both ML and BI trees. Eight strongly supported major clades were recovered within *Apathya* which were divided into two monophyletic clades referred to as groups. Group 1 included *A. c. cappadocica*, *A. c. muhtari and A. c. wolteri* with high support values (BS = 100 %, PP = 1.00), which occur in Anatolia. Group 2 containing four clades (Urmia Clade, Baneh Clade, Ilam Clade, and *A. yassujica*) from Iran and one clade (Turkey Clade) from Anatolia were well separated from each other with high support values (Fig S1). Based on the phylogenetic tree, *A. cappadocica* (including *A. c. cappadocica, A. c. muhtari, A. c. wolteri,* Urmia Clade, Baneh Clade and Ilam Clade) and *A. c. urmiana* (i.e. Urmia Clade, Baneh Clade, Ilam Clade) are paraphyletic groups.

The topological analyses indicated that SH-aLRT in the main clade was higher than 90 %. Also, eight clades (*A. c. cappadocica, A. c. muhtari and A. c. wolteri* (group 1); Turkey Clade, Urmia Clade, Baneh Clade, Ilam Clade and *A. yassujica* (group 2) well supported.

Uncorrected genetic distances between clades were approximately 5–17 % (Cyt *b*) and 4–19 % (COI). *Apathya yassujica* had the maximum genetic distance among other clades, and the genetic distance of *A. c. schmidtlerorum* with *A. c. muhtari* was the lowest for COI gene (Table 1).

3.2. Species delimitation

The GMYC model identified 19 genetic clusters, while PTP identified nine putative species within the genus.

3.3. Estimation of divergence times

Based on the calibrated tree, *Apathya* species diverged two main groups at 13.79 Mya (95 % highest posterior density (henceforth HPD): 13.31 – 14.07 Mya). The diversification within group 1 happened at 6.28 Mya (95 % HPD: 4.67 – 7.89 Mya). *A. c. cappadocica*, and *A. c. wolteri* were separated from each other at 2.76 Mya (95 % HPD: 1.93 – 3.94 Mya). Diversification within group 2 occurred 9.85 Mya (95 % HPD: 7.78 – 11.72 Mya), Baneh Clade derived from Turkey Clade-Urmia Clade at 4.05 (95 % HPD: 3.19 – 5.82 Mya), and the split of Turkey Clade/Urmia Clade happened almost 2.86 Mya (95 % HPD: 1.84 – 3.98 Mya). Ilam Clade, and *A. yassujica* split 5.95 Mya (95 % HPD: 4.31 – 7.69 Mya, Fig. 2).

The LTT plot indicated that diversification over time within the *Apathya* was happened around 13 Mya with an increasing slope to present (Fig. 3).

3.4. Biogeographic analysis

S-DIVA analysis indicated four nodes of dispersal (69, 92, 117 and 118) and eight nodes of vicariance (68, 91, 92, 99, 102, 116, 117 and 118) events (Fig. 4a). The BBM analysis showed both dispersal and vicariance events in eight nodes (i.e. 68, 91, 92, 99, 102, 116, 117 and 118 (Fig. 4b).

The TCS network showed that major clades were placed far from others without any shared haplotypes. Two main groups separate completely with several step-mutations (Fig. S2).

4. Discussion

We carried out an extensive study on the genus *Apathya* to reconstruct its evolutionary history and assess the genus' species diversity. Our results suggest that several clades within the two currently species of the genus may correspond to distinct species and do not match the subspecific arrangement based on morphology.

4.1. Phylogenetic relationships

The phylogenetic trees using multilocus DNA sequences revealed eight monphyletic clades within the genus (Fig. S1) without any shared haplotypes for nuclear genes (Fig. S2). All clades were divided into two main groups. The first one comprised of Anatolian lineages (*A. c. cappadocica*, *A. c. muhtari*, *A. c. wolteri*), and the second included Iranian clades (Urmia Clade, Baneh Clade, Ilam Clade, and *A. yassujica*) with one clade from Anatolia (Turkey Clade). Based on the study, *A. c. schmidtlerorum* and *A. c. muhtari* have low genetic distances and phylogenetic differentiation (Fig. S1 and Table 1). Despite this, according to the pervious researches, they form distinct clades with proteins methods and morphologies features (Nilson et al., 2003; Ilgaz et al., 2010). Besides, Hosseinian Yousefkhani et al. (2019) studied niche overlap and niche differentiation between clades of *Apathya*. They indicated that *A. c. schmidtlerorum* has niche similarity with other subspecies. Also, Kapli et al. (2013) mentioned that there is not any genetic distance between these two subspecies (*A. c. muhtari* and *A. c. schmidtlerorum*).

In contrast to previous studies (Nilson et al., 2003; Kapli et al., 2013; Hosseinian Yousefkhani et al., 2019), the results of the current study showed four clades within *A. c. urmiana* (i.e. throughout the distribution range of the subspecies). Although, the lineages are not grouped according to the current taxonomy in the case of *A. c. urmiana*. Based on previous reports, two subspecies of *A. cappadocica* were recorded from Iran (i.e. *A. c. urmiana* and *A. c. muhtari*) (Bahmani et al., 2012). However, our investigation indicated that some samples from Baneh in Kurdistan Province of Iran which were assigned to *A. c. muhtari*, were placed in a separate clade near the clade of *A. c. urmiana* (Fig. 2). Also, the Baneh samples showed a high genetic distance from *A. c. muhtari* (about 16 %).

Contrary to the previous expectation, more genetic structuring was recognized within the genus. The previous assumption consisted of five subspecies within the polytypic species (*A. cappadocica*) including *A. c. cappadocica*, *A. c. muhtari*, *A. c. schmidtlerorum*, *A. c. urmiana* and *A. c. wolteri*. In the current research, the results of GMYC and bPTP indicated 19 and nine lineages within the genus, respectively. Lohse (2009) mentioned that the results of GMYC model may overestimate the species number and it could also artificially yield clusters recognized as separate species when approximately less than 20 % of all demes were sampled.

Table 1
Uncorrected genetic *p*-distances within *Apathya* clades using Cyt *b* (above matrix) and COI (below matrix).

	A. c. cappadocica	*A. c.wolteri*	*A.c. muhtari*	*A. c. schmidtlerorum*	Turkey Clade	Urmia Clade	Baneh Clade	*A. yassujica*
A.c. cappadocica		0.050	0.115	0.122	0.159	0.134	0.135	0.144
A. c.wolteri	0.063		0.109	0.118	0.161	0.127	0.151	0.146
A.c. muhtari	0.114	0.115		0.051	0.157	0.144	0.139	0.175
A. c. schmidtlerorum	0.110	0.112	0.042		0.167	0.141	0.138	0.168
Turkey Clade	0.187	0.188	0.167	0.178		0.093	0.081	0.150
Urmia Clade	0.161	0.169	0.163	0.169	0.061		0.076	0.136
Baneh Clade	0.172	0.175	0.163	0.167	0.089	0.072		0.150
A. yassujica	0.154	0.158	0.147	0.148	0.128	0.119	0.105	
Ilam Clade	0.155	0.161	0.143	0.153	0.116	0.116	0.117	0.089

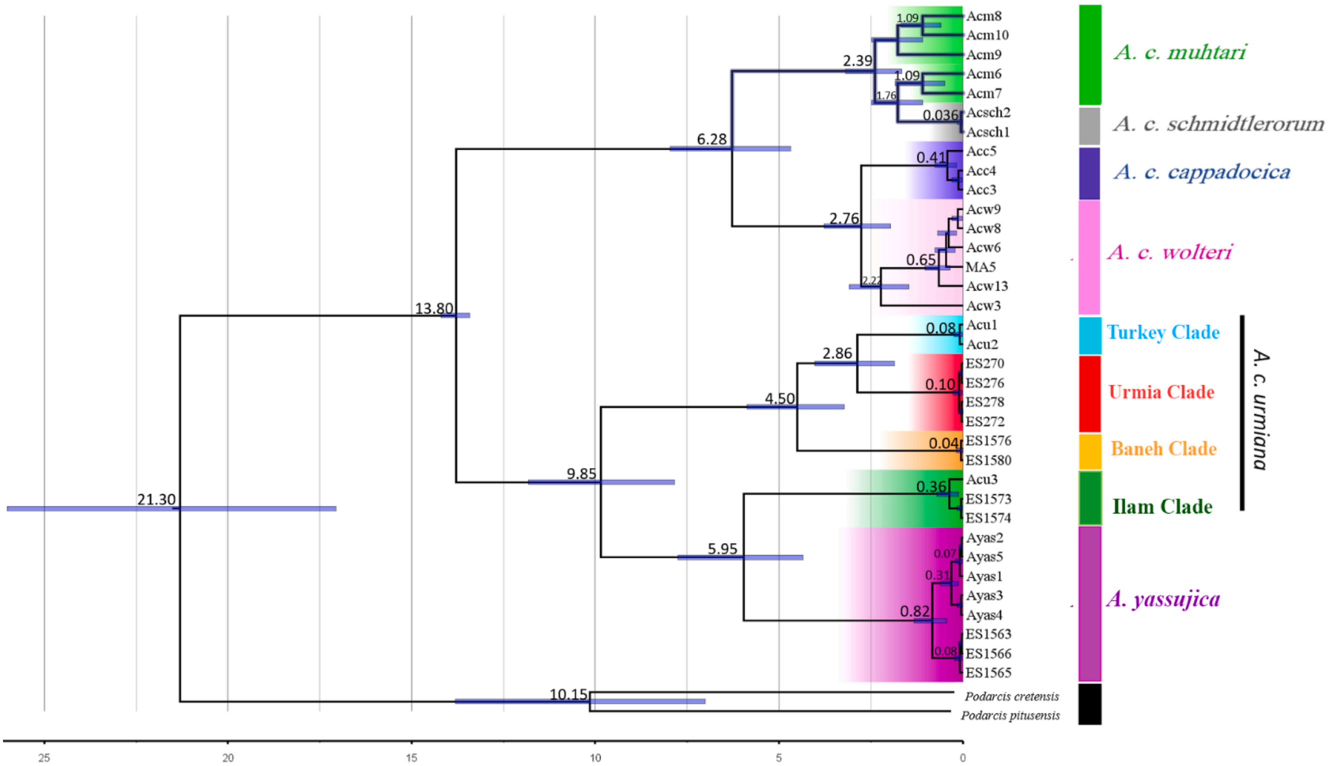

Fig. 2. The dated phylogenetic trees using the combined dataset (mtDNA and nuDNA), within *Apathya*. Blue bars show 95% highest posterior density intervals of the estimated node ages; numbers under the branches are mean node ages (Mya). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

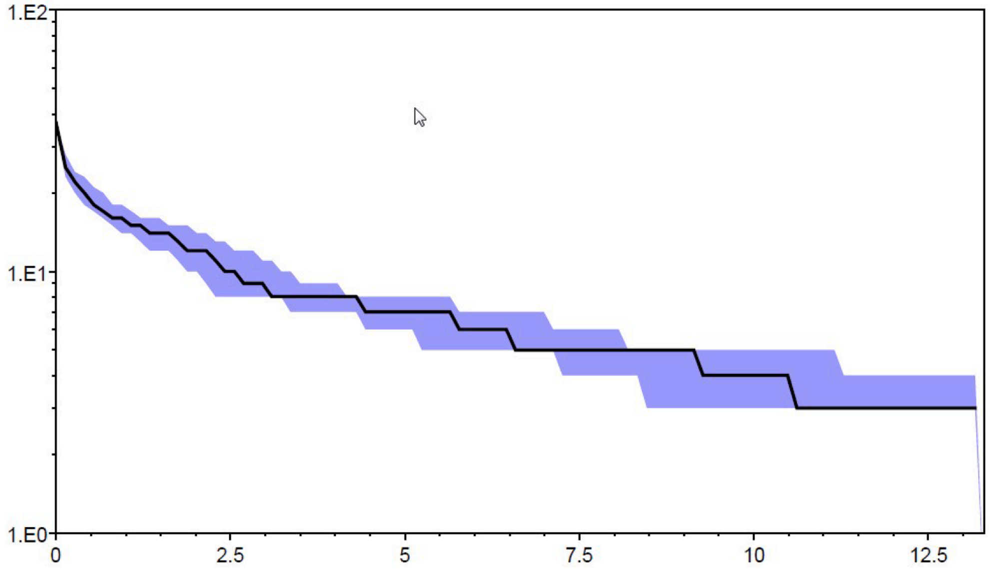

Fig. 3. Lineage through time plot within *Apathya*. The time axis indicates millions of years.

However, the bPTP model is more conservative. Taking together, our findings of species delimitations and genetic distances, along with the phylogeny of multiple genes and the haplotype network, especially unresolved the branch of *A. c. schmidtlerorum* (Fig S1) indicated eight resolved evolutionary entities within *Apathya*. Kapli et al. (2013) identified five supported lineages for the genus and reported a new population from Syria (*A. c. cf. wolteri*). Since there is no access to the comprehensive dataset from this region, we excluded the population. Extensive sampling in the current study (especially within Iranian boundaries) provides a more robust phylogenetic inference and clarified the mystery phylogenetic status of lineages within the genus.

4.2. *Evolutionary history of Apathya*

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). The diversification within *Apathya*

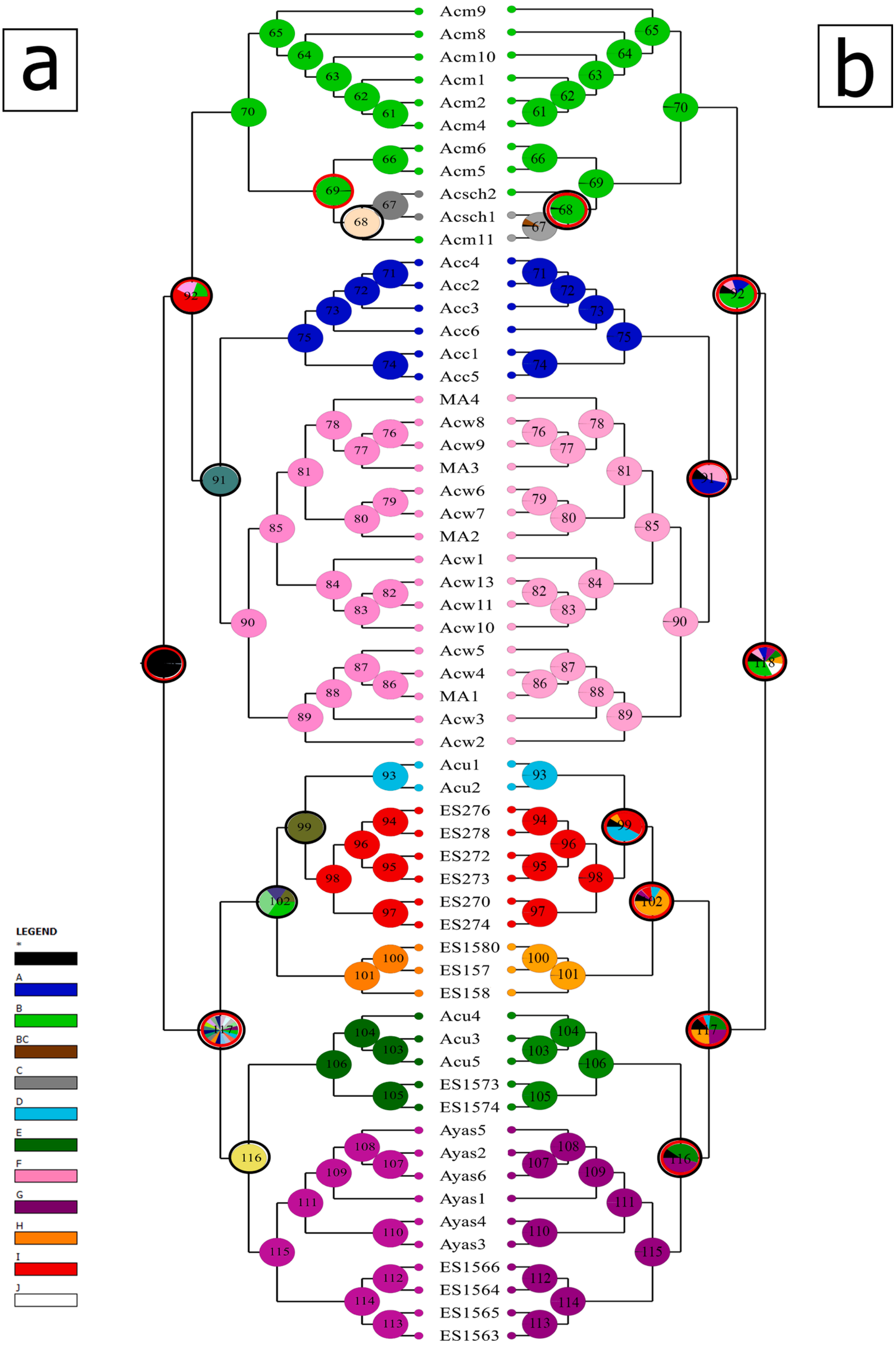

Fig. 4. The biogeographic analysis of *Apathya* using S-DIVA (a) and BBM (b) based on mtDNA sequences. For these analyses, five regions were considered in the south of Turkey: (A: blue nodes) distribution of *A. c. cappadocica*, (B: light green nodes) distribution *A. c. muhtari*, (C: grey nodes) distribution of *A. c. schmidtlerorum*, (F: pink nodes) distribution of *A. c. wolteri*, and (C: light blue nodes) distribution of Turkey Clade and four areas were placed in Iran through the north-south Zagros Mountains included: (I: red nodes) distribution of Urmia Clade, (H: orange nodes) distribution of Baneh Clade, (E: green nodes) Ilam Clade and (G: perpel nodes) distribution of *A. yassujica*. The red and black circles around the nodes show dispersal and vicariance events, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

began mid-Miocene (13.79 Mya; 95 % HPD: 13.31 – 14.07) and at that time two main clades of the genus were separated from each other. LTT plot shows the diversification within the genus happened about 13 Mya rapidly with a significant level of diversification over time (Fig. 3). At this time Middle Miocene Climate Transition (MMCT) occurred and the global temperature was significantly reduced (Zachos et al., 2001; Shevenell et al., 2004) and influenced to the separation of main clades. Besides an early phase of contractional deformation of the Zagros Mountains happened in the Middle Miocene (since 15 Mya; Carminati et al., 2016).

According to several studies Anatolia was the origin of many lacertid species (Ghane-Ameleh et al., 2021; Ahmadzadeh et al., 2013a). Therefore, it is assumed that the ancestral of *Apathya* has colonized Iran and spread to Anatolia was then split into several clades. Considering previous study (Rögl, 1999), the Tethys passage has been opened during the Langhian (15.97–13.65 Mya). It is suggested that the hypothesized ancestral of *Apathya* could disperse to Iran and were then diverged two clades of Iran and Anatolia after closing the way during the Seravalian (13.65–11.62) (Kapli et al., 2013). Therefore, the area was contracted by both climatic and tectonic factors that influenced on the speciation. Anatolian clades diverged from each other because of the orogeny activity of Pontides, Lesser Caucasus, and Taurus mountains. *A. c. muhtari*-separated from *A. c. cappadocica* and *A. c. wolteri* during 6.28 (95 % HPD: 4.67 – 7.89 Mya). Subsequently, some groups fragmented in Iran boundaries by forming the Zagros Mountains.

Also, Pleistocene climatic oscillations have the role of microevolutionary processes and also responsible for shaping intra-species structures by isolation populations in different refugia (Hewitt, 2000; Widmer and Lexer, 2001; Ahmadzadeh et al., 2012; Saberi-Pirooz et al., 2021). Several studies indicated that many species' genetic structures and distribution patterns are related to past climate change in the Irano-Anatolian region (Farasat et al., 2016; Afroosheh et al., 2019; Aghbolaghi et al., 2020). The uplifting of the Zagros mountains has happened by the Arabian plate indentation into Iran during the late Miocene during that time another phase of contractional tectonics was generated (Macey et al., 1998; Carminati et al., 2016), and led to the separation of *A. yasujica* and Ilam Clade from other clades in the second main clade. Following that due to the continuing southern Zagros mountain uplift activity the separation of *A. yasujica* and Ilam Clade has happened. Also, the role of the Zagros Mountains orogeny in the separation of Baneh, Urmia, and Turkey clades is undeniable. It seems the uplifting of the mountains has probably contributed to allopatric speciation and diversification within aridification as well as deep valley as a result of providing different macroclimate conditions for each clade (Hosseinian Yousefkhani et al., 2019). The results of BBM and SDIVA confirmed that multiple vicariance events occurred within the genus (Fig. 4). The mountain acted as a natural barrier to the dispersal of the genus to the Iranian plateau. This fact reinforces by other lizard genera (*Iranolacerta*, *Saara*, *Timon*) which have been distributed in this region (Ahmadzadeh et al., 2012; Ahmadzadeh et al., 2013b; Ghaedi et al. 2021). Taking together, Irano-Anatolian region plays a crucial role in the diversification of small vertebrates (Torki et al., 2011; Ahmadzadeh et al., 2012; Hendrix et al., 2014; Aghbolaghi et al., 2019). Past climate change and orogeny activities have had an impact on the genetic structure and distribution patterns of multiple species in the Irano-Anatolian region (Farasat et al., 2016; Afroosheh et al., 2019; Aghbolaghi et al., 2020).

In this study, we investigated a comprehensive dataset from the most distribution range of *Apathya* with different genetically aspects. Our data indicated that eight lineages are well-established within the genus that are separated each other completely. Recent studies about niche overlap and niche differentiation (Hosseinian Yousefkhani et al., 2019) and also other genetic studies (Kapli et al., 2013) are in line with our findings. Therefore, we suggest that three subspecies (*A. c. cappadocica*, *A. c. muhtari*, and *A. c. wolteri*) promote at the species level. Also, the distribution boundary of *A. c. urmiana* modify and just restrict to the north east of Iran. Afterwards, Turkey, Baneh and Ilam clades describe as a new species.

5. Conclusion

We investigated the phylogenetic relationship within *Apathya* with extensive sampling along the distribution range of the species especially the Iranian boundaries. We identified eight well supported clades within the genus and based on the genetic data these clades are separated from each other with high genetic distances. Furthermore, our results suggest that the lineages within the genus colonized to the west of the Zagros Mountains from Anatolia. We assumed that orogenic activities and climate fluctuations caused clades within the genus to separate. Our comprehensive molecular dataset reveals eight distinct clades within the genus, and we suggest that they should be classified at the species level.

CRediT authorship contribution statement

Sara Kafimola: Writing – original draft, Writing – review & editing. **Maryam Azimi:** Writing – original draft, Writing – review & editing. **Reihaneh Saberi-Pirooz:** Conceptualization, Writing – original draft, Writing – review & editing. **Çetin Ilgaz:** Writing – review & editing. **Ghasem Mohammadi Kashani:** Conceptualization, Writing – original draft, Writing – review & editing. **Paschalia Kapli:** Conceptualization, Writing – review & editing. **Faraham Ahmadzadeh:** Conceptualization, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank those individuals who have assisted with the fieldwork, the preparation of the specimens, and the provision of lizard tissues for this study: Farshad Nejat, Mostafa Keshtkar, and Saleh Mahmoodi. We would also like to thank Saeedeh Ataei for her help during the laboratory process.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2022.107675.

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. Zoolog. Syst. Evol. 57, 580–590.

Agard, P.J., Omrani, L., Jolivet, F., Mouthereau, 2005. Convergence history across Zagros (Iran): Constraints from collisional and earlier deformation. Int. J. Earth Sci. 94, 401–419.

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.

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

Ahmadzadeh, F., Carretero, M.A., Rödder, D., Harris, D.J., Freitas, S.N., Perera, A., et al., 2013b. 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, 141–148.

Ahmadzadeh, F., Lymberakis, P., Pirouz, R.S., Kapli, P., 2017. The evolutionary history of two lizards (Squamata: Lacertidae) is linked to the geological development of Iran. Zool. Anz. 270, 49–56.

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

Ali, J.R., Aitchison, J.C., 2014. Exploring the combined role of eustasy and oceanic island thermal subsidence in shaping biodiversity on the Galápagos. J. Biogeogr. 41, 1227–1241.

Anisimova, M., Gil, M., Dufayard, J.F., Dessimoz, C., Gascuel, O., 2011. Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Syst. Biol. 60, 685–699.

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, 3–86.

Asadi, A., Montgelard, C., Nazarizadeh, M., Moghaddasi, A., Fatemizadeh, F., Simonov, et al., 2019. Evolutionary history and postglacial colonization of an Asian pit viper (*Gloydius halys caucasicus*) into Transcaucasia revealed by phylogenetic and phylogeographic analyses. Sci. Rep. 9, 1224.

Avise, J.C., 2000. Phylogeography: The history and formation of species. Harvard University Press, USA.

Bahmani, Z., Karamiani, R., Gharzi, A., 2012. The lizard fauna of Kurdistan Province, western Iran. Iran. J. Anim. Biosyst. 8 (1), 1–11.

Barata, M., Carranza, S., Harris, D.J., 2012. Extreme genetic diversity in the lizard *Atlantolacerta* andreanskyi (Werner, 1929): A montane cryptic species complex. BMC Evol. Biol. 12 (1), 167.

Bird, C.G., 1936. The distribution of Reptiles and Amphibians in Asiatic Turkey, with notes on a Collection from the Vilayets of Adana, Gaziantep and Malatya. Ann. Mag. Nat. 18 (10), 257–281.

Carminati, E., Aldega, L., Bigi, S., Minelli, G., Shaban, A., 2016. Not so simple "simply-folded Zagros": the role of pre-collisional extensional faulting, salt tectonics and multi-stage thrusting in the Sarvestan transfer zone (Fars, Iran). Tectonophysics 671, 235–248.

Carranza, S., Arnold, E.N., Amat, F., 2004. DNA phylogeny of *Lacerta* (*Iberolacerta*) and other lacertinae lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? System. Biodivers. 2, 57–77.

Cox, C.B., Moore, P.D., Ladle, R.J., 2016. *Biogeography: an ecological and evolutionary approach.* John Wiley & Sons.

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

Eiselt, J., 1979. Ergebnisse zoologischer Sammelreisen in der Türkei *Lacerta cappadocica* Werner., 1902 (Lacertidae, Reptilia). Annalen des Naturhistorischen Museums in Wien 82, 387–421.

Ellouz-Zimmermann, N., Deville, E., Müller, C., Lallemant, S., Subhani, A.B., Tabreez, A. R., 2007. Impact of sedimentation on convergent margin tectonics: Example of the Makran accretionary prism (Pakistan). In: Thrust belts and foreland basins. Springer, Berlin, Heidelberg, pp. 327–350.

Farasat, H., Akmali, V., Sharifi, M., 2016. Population genetic structure of the endangered Kaiser's Mountain Newt, *Neurergus kaiseri* (Amphibia: Salamandridae). PLoS One 11, e 0149596.

Flower, B.P., Kennett, J.P., 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. Palaeogeogr. Palaeoclimatol. Palaeoecol. 108 (3–4), 537–555.

Foster, G.L., Rohling, E.J., 2013. Relationship between sea level and climate forcing by CO2 on geological timescales. PNAS 110 (4), 1209–1214.

Fouad, S.F., Sissakian, V.K., 2011. Tectonic and structural evolution of the Mesopotamia Plain. Iraqi Bull. Geol. 4, 33–46.

Ghaedi, Z., Badri, S., Saberi-Pirooz, R., Vaissi, S., Javidkar, M., Ahmadzadeh, F., 2021. The Zagros Mountains acting as a natural barrier to gene flow in the Middle East: more evidence from the evolutionary history of spiny-tailed lizards (Uromasticinae: *Saara*). Zool. J. Linn. Soc. 192 (4), 1123–1136.

Ghane-Ameleh, S., Khosravi, M., Saberi-Pirooz, R., Ebrahimi, E., Asadi Aghbolaghi, M., Ahmadzadeh, F., 2021. 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. GECCO. 31, e01839.

Gillespie, R., Roderick, G.K., 2014. Geology and climate drive diversification. Nature 509 (297).

Goudarzi, F., Hemami, M.R., Rancilhac, L., Malekian, M., Fakheran, S., Elmer, K.R., Steinfartz, S., 2019. Geographic separation and genetic differentiation of populations are not coupled with niche differentiation in threatened Kaiser's spotted newt (*Neurergus kaiseri*). Sci. Rep. 9, 6239.

Hatzfeld, D., Authemayou, C., Van der Beek, P., Bellier, O., Lavé, J., Oveisi, B., Tatar, M., Tavakoli, F., Walpersdorf, A., Yamini-Fard, F., 2010. The kinematics of the ZagrosMountains (Iran). Geol. Soc. Lond. Spec. Publ. 330, 19–42.

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., 2000. The genetic legacy of the Quaternary ice ages. Nature 405 (6789), 907–913.

Hewitt, G.M., 2004a. The structure of biodiversity–insights from molecular phylogeography. Front. Zool. 1 (1), 1–16.

Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London. Proc. Royal Soc. B. 359 (1442), 183–195.

Himes, C.M.T., Gallardo, M.H., Kenagy, G.J., 2008. Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial Dromiciops gliroides. J. Biogeogr. 35 (8), 1415–1424.

Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L., 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35, 518–522.

Holbourn, A., Kuhnt, W., Lyle, M., Schneider, L., Romero, O., Andersen, N., 2014. Middle Miocene climate cooling linked to intensification of eastern equatorial Pacific upwelling. Geology 42 (1), 19–22.

Hosseinian Yousefkhani, S.S., Rastegar-Pouyani, E., Ilgaz, Ç., Kumlutaş, Y., Avcı, A., Wink, M., 2019. Evidences for ecological niche differentiation on the Anatolian lizard (*Apathya cappadocica* ssp.) (Reptilia: Lacertidae) in western Asia. Biologia 74 (12), 1661–1667.

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

Ilgaz, C., Arikan, H., Kumlutas, Y., Avci, A., 2010. Electrophoretic comparison of blood-serum proteins of *Apathya cappadocica* (Sauria, Lacertidae) subspecies from Anatolia. Acta Herpetol. 5, 207–215.

Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagy, Z.T., Wink, M., 2007. Phylogeography of western Palaearctic reptiles–Spatial and temporal speciation patterns. Zool. Anz. 246 (4), 293–313.

Kapli, P., Botoni, D., Ilgaz, Ç., Kumlutaş, Y., Avcı, 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). Mol. Phylogenet. Evol. 66 (3), 992–1001.

Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinformatics. 20 (4), 1160–1166.

Khadivi, S., 2010. Tectonic evolution and growth of the Zagros Mountain Belt (Fars, Iran): constraints from magnetostratigraphy, sedimentology and low-temperature thermochronometry (Doctoral dissertation, Université Pierre et Marie Curie-Paris VI).

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34, 772–773.

Lantz, L.A., Suchow, G.F., 1934. *Apathya cappadocica urmiana* susp. nov., eine neue Eidechsenform aus dem persischen Kurdistan. Zool. Anz. 106, 294.

Leigh, J.W., Bryant, D., 2015. POPART: full-feature software for haplotype network construction. Methods Ecol. Evol. 6 (9), 1110–1116.

Lohse, K., 2009. Can mtDNA barcodes be used to delimit species? A response to Pons et al. (2006). Syst. Biol. 58, 439-442.

Macey, J.R., Schulte II, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M., Papenfuss, T.J., 1998. Phylogenetic Relationships among Agamid Lizards of the *Laudakia caucasia* Species Group: Testing Hypotheses of Biogeographic Fragmentation and an Area Cladogram for the Iranian Plateau. Mol. Phylogenet. Evol. 10 (1), 118–131.

Mittermeier, R.A., Robles Gil, P., Hoffmann, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., et al., 2004. Hotspots revisited: Earth's biologically richest and most endangered ecoregions. CEMEX, Mexico City.

Mohammadi, A., Naderi, M., Rezaei Hamid, R., Kaboli, M., 2018. Population genetic structure of Transcaucasian mole vole (*Ellobius lutescens*) along Zagros Mountains. Iran. Contemp. Probl. Ecol. 11, 239–245.

Mouthereau, F., 2011. Timing of uplift in the Zagros belt/Iranian plateau and accommodation of late Cenozoic Arabia–Eurasia convergence. Geol. Mag. 148 (5–6), 726–738.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.

Nilson, G., Rastegar-Pouyani, N., Rastegar-Pouyani, E., Andrén, C., 2003. Lacertas of South and Central Zagros Mountains, Iran, with descriptions of two new taxa. Russ. J. Herpetol. 10, 11–24.

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.

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

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55 (4), 595–609.

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

Rögl, F., 1999. Palaeogeographic considerations for Mediterranean and Paratethys seaways. Ann. Naturhist. Mus. Wien 99A, 279–310.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular cloning: a laboratory manual. Cold spring harbor laboratory press.

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.

Schmidtler, J.F., Bischoff, W., 1995. Beziehungen zwischen Lebensraum und Morphologie bei *Lacerta cappadocica* Wener, 1902 in der Türkei. Die Eidechse 6, 13–21.

Shevenell, A.E., Kennett, J.P., Lea, D.W., 2004. Middle Miocene Southern Ocean cooling and Antarctic cryosphere expansion. Science 305, 1766–1770.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Smit, J., Burg, J.-P., Dolati, A., Sokoutis, D., 2010. Effects of mass waste events on thrust wedges: Analogue experiments and application to the Makran accretionary wedge. Tectonics 29, TC3003.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F., 1998. Comparative phylogeography and postglacial colonization routes in Europe. Mol. Ecol. 7 (4), 453–464.

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.

Trewick, S., 2017. Plate tectonics in biogeography. In: Richardson, D., Castree, N., Goodchild, M., Kobayashi, A., Liu, W., Marston, R., (Eds.), Int. Encycl. Geogr. People, Earth, Environ. Technol. pp. 1–9.

Werner, F., 1902. Die Reptilien und Amphibienfauna von Kleinasien. - Sitzungsberichte der Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe. Wien, Abteilung. 1, 1057-1121.

Widmer, A., Lexer, C., 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends Ecol. Evol. 16 (6), 267–269.

Yu, Y., Harris, A.J., He, X., 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. Mol. Phylogenet. Evol. 56 (2), 848–850.

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.

Zachos, J.C., Pagani, M., Sloan, L.C., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.