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Research paper

The evolutionary history of two lizards (Squamata: Lacertidae) is linked to the geological development of Iran

Faraham Ahmadzadeh[a], Petros Lymberakis[b], Reyhaneh Saberi Pirouz[a], Paschalia Kapli[c,*]

[a] *Department of Biodiversity and Ecosystem Management, Environmental Sciences Research Institute, Shahid Beheshti University, G.C, Tehran, Iran*
[b] *Natural History Museum of Crete, University of Crete, Gr-71409 Heraklio, Crete, Greece*
[c] *The Exelixis Lab, Scientific Computing Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, D-68159 Heidelberg, Germany*

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ABSTRACT

The Iranian Plateau is an area of highly complex landscape and incredibly diverse habitats, from salt deserts to temperate forests. Such a heterogeneous environment, at the crossroads of Asia, Europe, and Africa, fuels the high diversity and uniqueness of the Iranian fauna and flora. Nevertheless, our understanding of the historical processes that shaped the Iranian biodiversity remains limited. In this context, we revisit the evolutionary history of the two most widespread lizard species on the Iranian Plateau (*Eremias persica* and *Mesalina watsonana*) to explore whether these two taxa of similar ecology have been subjected to similar historical processes. To achieve that, we performed a series of phylogenetic analyses and thoroughly tested all possible alternative topologies based on available mitochondrial sequences (cytochrome *b* and 12S ribosomal RNA). Additionally, we estimated the number of mitochondrial clusters based on a novel single-locus delimitation method and the time of their divergence, using recently inferred evolutionary rates for lacertid species. The results indicate that the two taxa have been shaped by similar physiographic and climatic barriers since both phylogenies split into similar geographical clusters. However, *E. persica* has twice as long evolutionary time on the Iranian Plateau than *M. watsonana*. The first divergence of *E. persica* was estimated in the Middle Miocene (~13 Mya), at the time of the major uplift of the Zagros mountain range. The Upper Miocene tectonic rearrangement that resulted in the uplift of the Alborz mountains coincides with the original split in *M. watsonana* (~6.6 Mya) and several contemporaneous splits within *E. persica.*

1. Introduction

The Iranian Plateau is a unique geological formation of Western Asia, located among the continental plates of Eurasia, Arabia, and India. A series of tectonic movements among these plates resulted in the gradual uplift of the Plateau and the extensive mountain system surrounding it (Crawford, 1972; Sborshchikov et al., 1981). Most of the estimates for the initial collision among Arabia and Eurasia range between 35 and 20 Mya (Khadivi, 2010; McQuarrie and Van Hinsbergen, 2013; Van Hinsbergen et al., 2012). At about the same time [25 to 20 Million years ago (Mya)], a “hard” collision episode is presumed to have occurred between the Indian and the Eurasian plates (Van Hinsbergen et al., 2012). Eventually, the combined tectonic movements led to the uplift of the four mountain ranges around Iran, i.e., the Zagros on the west border of the Plateau neighboring Iraq and Turkey, the Alborz range along the coast of the Caspian Sea, the Kopet Dagh on the border of Iran and Turkmenistan, and finally, the mountain ranges of Makran in the southeast (Fig. 2). There has been a broad spectrum of estimates for the initial uplift of the Zagros (Alavi, 1994; Berberian and King, 1981; Jassim and Goff, 2006), but the most recent findings suggest a Middle Miocene (~14Mya) onset of the deformation of the High Zagros (Mouthereau et al., 2012). Nevertheless, the major orogeny did not occur before 12.4 Mya, after which both the Zagros Mountains and the Iranian Plateau were rapidly uplifted in less than 5 My (Khadivi, 2010). During the same period, deformation is also reported in the Alborz (Guest et al., 2007; McQuarrie and Van Hinsbergen, 2013) and the Kopet Dagh mountains (Hollingsworth

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* Corresponding author.
E-mail address: k.pashalia@gmail.com (P. Kapli).

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et al., 2010; McQuarrie and Van Hinsbergen, 2013). Later, at the Miocene-Pliocene border, the latest major tectonic activity shaped the area into its current configuration. During this period, the Alborz Mountains and the entire Plateau were uplifted to their current elevations (Allen et al., 2004; Axen et al., 2001).

The geographical position of Iran at the crossroads of Asia, Africa, and Europe, as well as the surrounding mountains that filter the dispersal in and out of the Plateau, has fueled the high biodiversity and endemicity in the area (Anderson, 1999; Bernor, 1986; Firouz, 2005; Frey and Probst, 1986; Madjnoonian et al., 2005). In addition to the vast mountain systems, the diverse habitat synthesis, from (semi-) arid to temperate (Madjnoonian et al., 2005 and references therein) interrupted by the non-habitable deserts of the Dasht-e Kavir and Dasht-e Lut (Blanford, 1876; Hedge and Wendelbo, 1978) create a dynamic mosaic environment that further reinforces the richness and uniqueness of the Iranian fauna and flora.

In spite of the extensive bibliography regarding the tectonics and paleogeography of the area, the systematic studies on the Iranian fauna lag behind, and our understanding of its phylogeography and biodiversity remains limited (Ahmadzadeh et al., 2013). In this study, we attempt to gain insight into the main biodiversity patterns of the Iranian Plateau by studying the historical biogeography of two of its most widely distributed lizards, *Eremias persica* Blanford, 1876, and *Mesalina watsonana* (Stoliczka, 1872). The two lacertids overlap extensively in the Central Plateau and partially in Pakistan and Afghanistan, however, their congeneric species are distributed allopatrically. West of Iran, *Mesalina* Gray, 1838 is found up to Morocco (Schleich et al., 1996), with a typical Saharo-Arabian distribution while *Eremias* Fitzinger, 1834 occurs east of Iran into central Asia (Sindaco and Jeremčenko, 2008). Several phylogenetic studies on the lacertid family acknowledge the two genera as sister taxa (Fu, 2000; Harris et al., 1998; Mayer and Pavlicev, 2007). Nonetheless, the relationships of the family remain significantly unresolved (Kapli et al., 2011; Mayer and Pavlicev, 2007; Mendes et al., 2016), but see (Pyron et al., 2013). Both *M. watsonana* and *E. persica* are assumed to form species complexes, revealing much higher genetic divergence than expected among congeneric lacertid species (Kapli et al., 2015; Pouyani et al., 2010; Šmíd and Frynta, 2012). The two complexes exhibit similar ecological requirements, occurring in the lowlands and mountain hills of the Iranian Plateau (except very high elevations) in arid and semi-arid habitats (Anderson, 1999; Ebrahimi et al., 2013). Recent estimates (Kapli et al., 2015) suggest that the initial divergence within *Mesalina* occurred in early Miocene and resulted in two lineages east (*M. watsonana*) and west (the rest of the *Mesalina* species) of Zagros. However, the first split within *M. watsonana* was estimated in late Miocene (Kapli et al., 2015) [~7 Mya (Kapli et al., 2015; Šmíd and Frynta, 2012)]. The initial split in the *E. persica* complex was estimated earlier, ca. 11 Mya (Pouyani et al., 2010). However, a later study on the phylogeny of the entire *Eremias* genus placed the event much later, ca. 6 Mya (Guo et al., 2011), an estimate much closer to the equivalent for *M. watsonana*.

In this study, we aim to identify whether the evolutionary history of the two lizard species has been subjected to similar historical process on the Iranian Plateau. In this framework, we perform a series of phylogenetic analyses to reconstruct the evolutionary relationships of the two taxa based on Maximum Likelihood and Bayesian Inference. Additionally, we estimate the number of mitochondrial clusters based on single-locus delimitation methods, and the time of their divergence using recently inferred evolutionary rates for lacertid species (Carranza and Arnold, 2012). The comparative analysis of the two taxa under the same assumptions and methods allows us to evaluate previous findings, identify similarities in their evolutionary history and associate them with the most plausible paleogeographic events in the area.

2. Material and methods

2.1. Sequences

We assembled two sequence datasets for the Iranian *E. persica* and for *M. watsonana* by retrieving sequences from NCBI (www.ncbi.nlm.nih.gov). For *E. persica* we relied on two mitochondrial markers [12S ribosomal RNA (12S) and cytochrome *b* (cyt *b*) sequences] which were originally published in Pouyani et al. (2010). Out of the 129 sequences, 125 belonged to the *E. persica* complex (*E. persica*, *E.* sp., *E. nigrolateralis*, *E. montanus*) and four of them corresponded to three outgroup taxa (*E. velox*, *Ophisops elegans*, and *Mesalina brevirostris*). For the dataset of *M. watsonana*, we combined the cyt *b* sequences produced in two previous phylogenetic studies, one aiming at resolving the relationships of the genus *Mesalina* (Kapli et al., 2015) and the other one focusing on *M. watsonana* (Šmíd and Frynta, 2012). The combined dataset consisted of 29 *M. watsonana* samples that covered the distribution of the species and eight sequences belonging to outgroup taxa (*Eremias brenchleyi*, *Gallotia caesaris*, *G. galloti*, *G. stehlini*, *G. atlantica*, *M. guttulata*). The choice of the outgroup was based on the original articles (Kapli et al., 2011; Pouyani et al., 2010; Šmíd and Frynta, 2012).

2.2. Phylogenetic inference

All alignments were performed with MAFFT (Katoh and Standley, 2013) under the default settings. The best-fit model of DNA substitution for each gene was chosen with jModelTest 2.1.1 (Darriba et al., 2012), under three substitution schemes and accounting for base frequencies, gamma shape and invariable sites (excluding the combination of invariable sites and Γ distribution). For each dataset, we performed all the analyses, including and excluding the outgroup taxa.

Phylogenetic inference for each of the assembled datasets was carried out with Bayesian Inference (BI) and under the Maximum Likelihood (ML) criterion. For both analyses, the alignment sites were used as discrete, unordered characters. We performed three independent BI analyses with the software MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), for 5^*10^6 generations and four chains, and a sampling frequency of 0.01. Subsequently, we assessed the performance of each run with Tracer v1.5 (Drummond and Rambaut, 2007) and discarded 10% of the posterior samples as 'burn-in.' From the remaining trees, we calculated the majority rule consensus topology and the posterior probabilities [($\geq$ 95% indicated significant support according to Huelsenbeck & Ronquist (2001)]. The ML analysis was carried out with RAxML v. 8.0.20 (Stamatakis, 2014) for each dataset under the GTR + Γ evolutionary model. For *M. watsonana* there was only one partition, while for *E. persica* the alignment was partitioned into the two loci (12S and cyt *b*) and a distinct substitution model was assigned to each. To assess the confidence of the ML topology, we performed 1000 bootstrap searches under the GTR + Γ model.

2.3. Single-locus species delimitation

Several previous systematic studies of the two taxa assume a "species complex" status for both *M. watsonana* and *E. persica* (Kapli et al., 2015; Pouyani et al., 2010; Šmíd and Frynta, 2012). So far, however there has been no attempt to draw the limits among the independently evolving lineages involved in each phylogeny. Here, we make a first attempt by quantify the number of mitochondrial clusters for *E. persica* and *M. watsonana* using the recently introduced, single-locus species delimitation method, "multi-rate Poisson Tree Processes model" (mPTP, Kapli et al., 2017). The (m)PTP model attempts to identify the shifting point from the speciation to the coalescent processes, assuming that the two processes produce phylogenies of distinct branching patterns which can be described by a two (PTP) or more (mPTP) parameter model. The mPTP model is a generalization of its predecessor PTP (Zhang et al., 2013) since it additionally assumes that the genetic variation of putative species may differ. The method requires as input a rooted phylogenetic tree, hence, we used the phylogenies recovered by RAxML and rooted it based on the outgroup taxa. A complication that comes up in phylogenetic inference is that the relationships among identical or almost identical sequences (i.e., of different length or involving different or different number of degenerate characters) are represented by very short branch lengths, since zero branch lengths implies multifurcation which is not considered in phylogenetic inference. Such branch lengths are significantly smaller than the remaining ones, thus, mPTP may falsely classify these two sets of branch lengths into within and between species processes, respectively. To avoid such delimitation errors, before the analysis we calculated a threshold for the minimum branch length that should be considered in the delimitation process ("–minbr" option) by providing the phylogeny and the alignment for each dataset.

Multiple studies show that single-locus delimitation methods provide reliable species boundaries estimates (Esselstyn et al., 2012; Fujisawa and Barraclough, 2013; Kapli et al., 2017; Tang et al., 2014). However, it is also broadly known that they fail to detect several evolutionary processes, such as recent speciation, mitochondrial introgression events or sex biased dispersal, while they can be vulnerable to sampling biases or deep population substructure. Hence, we will consider the clusters delimited by mPTP as an initial re-assessment of the underestimated diversity of the two complexes that should be explored further based on additional data. Multiple unlinked molecular loci combined with morphological and distributional data, in the framework of integrative taxonomy (Dayrat, 2005), could refine the species delimitation estimates for each complex.

2.4. Divergence time estimation – alternative topology tests

We used BEAST (Drummond et al., 2012) to estimate the divergence times among the major splits within each species complex and assess how the paleogeography of the Iranian Plateau may have driven their evolution. For this analysis we used a subset of the original sequence-data representing the interspecific diversity in each case, to avoid estimation errors due to different (speciation and population) time-scales (Ho et al., 2011). In this framework, based on the clusters identified by mPTP we selected a single representative per cluster to form a subset of sequences that reflects the speciation process of each dataset. In the absence of calibration points in both datasets, we estimated the divergence times, using the most recently updated substitution rates for cyt *b* and 12S inferred for the *Gallotia* species in Carranza and Arnold (2012) under a relaxed lognormal clock prior. Each analysis was run twice for 10^7 generations with a sampling frequency of 10^{-3}. To assess convergence between the independent runs we used likelihood trace plots and ESS values, provided in Tracer v.1.6.0 (Drummond and Rambaut, 2007).

The BEAST analysis was ran for all alternative topologies resulting from the phylogenetic inference. To assess which of the topologies fit the data better, we calculated the marginal likelihood under the topological constraints of each scenario, using only representatives of the ingroup sequences. For the marginal likelihood estimation (MLE) we used the two sampling techniques available in BEAST, i.e., the Stepping Stone and the Path Sampling (Baele et al., 2012). For each analysis we performed 20 path sampling steps each of which was ran for $5*10^6$ MCMC steps. To compare the estimated marginal likelihoods we used the Bayes Factor (BF, Kass and Raftery, 1995).

3. Results

3.1. Alignment – phylogenetic trees – alternative topologies

For *E. persica* we obtained 1535 alignment sites (cyt *b*: 1144; 12S: 391) out of which 673 were informative (cyt *b*: 546; 12S: 127) including the outgroup sequences, while for the ingroup sequences only, it was 512 (cyt *b*: 434; 12S: 77). For *M. watsonana* the alignment length of cyt *b* was 821 bases out of which 306 and 200 were variable with and without the outgroup taxa, respectively. All phylogenetic inference analyses for both datasets identified four major clades: the (A) Western, (B) the Quom ("Tehran" in Pouyani et al., 2010), (C) the North-Eastern (D) and the South-Eastern Iran (Figs. 1 and 2). The topology recovered for *E. persica* (Fig. 1A), when the outgroup sequences were included in the analyses, was highly supported by both posterior probabilities and bootstrap values for the majority of clades. Fig. 1A shows the retrieved phylogeny and the correspondence of the lineages to four major geographic areas. The Western clade (A) appears as sister to all the remaining clades of *E. persica* while the second split of the genus leads to the North-Eastern (C) and the two sister clades, the Quom (B) and the South-Eastern (D). In a newick representation, the phylogenetic relationships of the four phylogeographic areas for *E. persica* would be (((D,B),C),A). When the outgroup taxa were not included in the analysis the resulting topology was different. In particular, none of the five rooting possibilities of the recovered unrooted tree (Table 1), would resemble the rooted topology based on the outgroup taxa.

Regarding *M. watsonana*, the different analyses resulted in similar topologies, including the four primary clades (A, B, C and D), regardless if the outgroups were included or not. The four clades were highly supported as well as the relationships among them with the exception of the position of clade B (Quom) which was unresolved based on both BI and ML (posterior probability <0.95 and bootstrap support < 50). On the contrary, the Eastern (D) and the North-Eastern (C) always appeared as sister clades. Taking into account the ambiguity of placing the Quom clade, there are three possible rooted tree topologies, i.e., (A,(B,(C,D))), ((A,B),(C,D)), and (B, (A,(C,D)).

Using BEAST under the settings described earlier, we calculated the MLE for all the alternative scenarios stemming from the phylogenetic analyses for each taxon. For *E. persica* we compared six alternative scenarios, one of which corresponds to the rooted topology based on the outgroup taxa and five to the potential rooted phylogenies compatible with the topology inferred based on the ingroup taxa alone (Table 1). For *M. watsonana* we calculated the MLE for three topologies which correspond to all possible scenarios regarding the position of clade B (Table 1). The MLE comparison based on the BF favored the topological configurations presented in Fig. 3, which are congruent with the topologies in Fig. 1. Based on these results, the two phylogenies agree on identifying the Western clade as the sister to all the rest but differ in the relationship among the remaining three lineages.

3.2. mtDNA clusters – divergence times

The mPTP model identified six and 13 clusters for the *M. watsonana* and the *E. persica* mitochondrial phylogenies, respectively. The most divergent lineage of *E. persica* was the South-Eastern (D) which comprises five clusters, followed by the Western (A) and the Quom (B) with three clusters each, while the least variable was the North-Eastern one (C) with two clusters. For *M. watsonana*, two

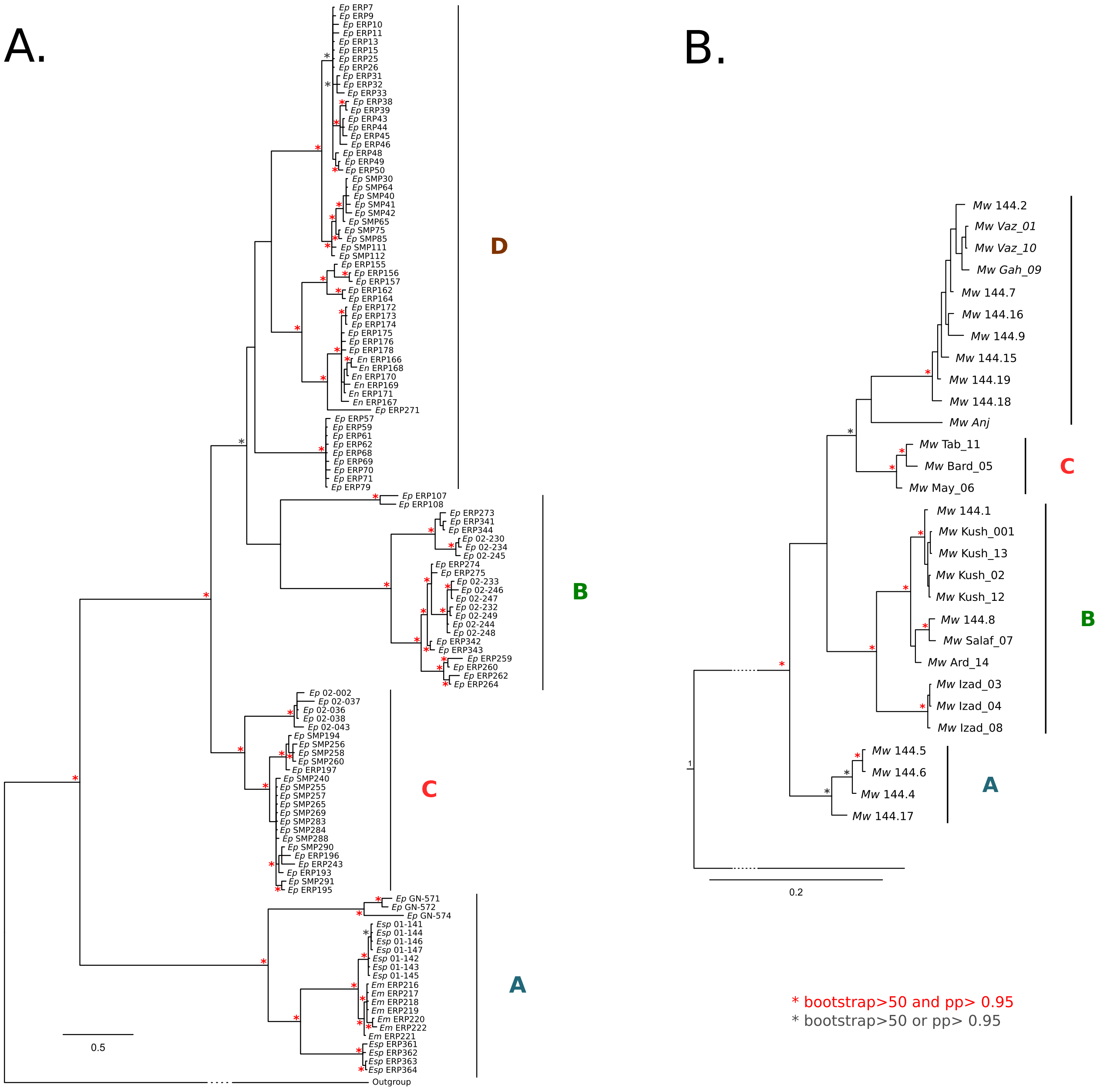

Fig. 1. Phylogenetic relationship of the two species complexes A) *E. persica* and of B) *M. watsonana* based on the Bayesian inference method. The asterisk symbols (*) on the nodes indicate the bootstrap support values and the posterior probabilities, red asterisks stand for bootstrap values higher than 50% and posterior probabilities higher than 0.95, while gray asterisks stand for either bootstrap values higher than 50% or posterior probabilities higher than 0.95. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Marginal likelihood Estimates (MLE) for the three alternative topologies of *M. watsonana* and the six of *E. persica* (the first one corresponds to the rooted topology based on the outgroup and the remaining five to all possible rooting scenarios based on the phylogeny of the ingroup taxa alone). The MLE was estimated using the two sampling strategies “Path Sampling” and “Stepping Stone”. “Rank” shows the ranking of the alternative topologies sorted by highest to lowest MLE, while “BF” stands for “Bayes Factors”, the measure for comparing the best with the alternative hypotheses.

Taxon	Phylogeny	Rank	Path Sampling	BF	Rank	Stepping Stone	BF
M. watsonana	(A,(B,(C,D)))	1	−1991.01861		1	−1993.196089	
	((A,B),(C,D))	2	−1993.171747	4.306274161	2	−1993.995615	1.599051912
	(B, (A,(C,D)))	3	−1995.378404	8.71958942	3	−1995.559165	4.726151176
E. persica	(A,(C, (D,B)))	1	−5964.742493		1	−5954.263503	
	((D,C), (A,B))	2	−5971.589461	13.69393547	5	−5972.443973	36.36094012
	(D,(C, (A,B)))	3	−5972.916007	16.34702858	3	−5968.324814	28.12262219
	(A,(B,(C,D)))	4	−5974.142525	18.80006389	4	−5969.168507	29.81000722
	(C, (D, (A,B)))	5	−5974.5694	19.65381438	6	−5981.674807	54.82260735
	(B, (A,(C,D)))	6	−5974.629317	19.77364775	2	−5968.200791	27.87457677

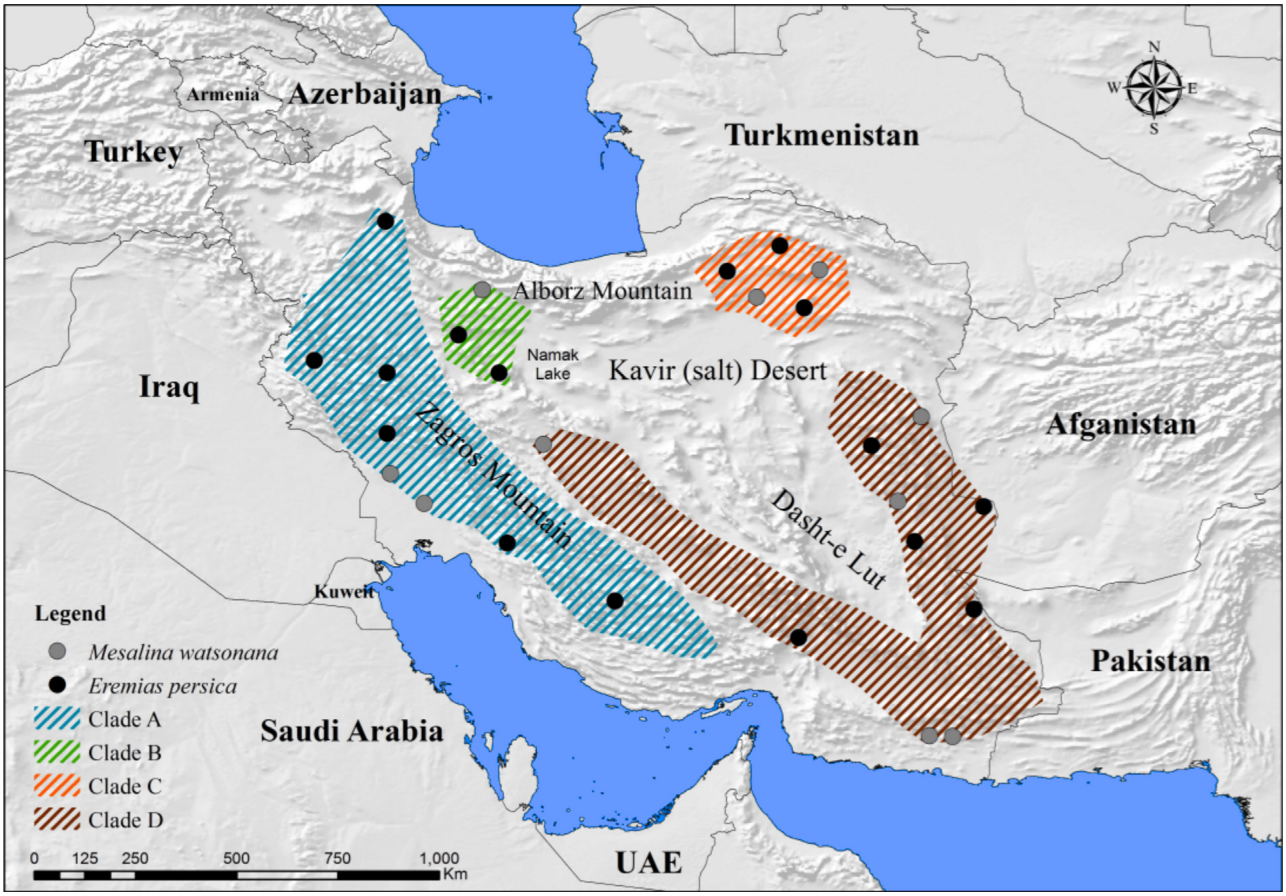

Fig. 2. The map shows the geographic region of the four main clades (oblique lines) recovered in the phylogeny of *M. watsonana* and *E. persica* as well as the main mountain ranges and the extensive deserts Kavir and Dasht-e-Lut. The localities of the samples are shown in gray circles for *M. watsonana* and in black for *E. persica.*

clusters were identified in Quom (B) and South-Eastern (D) Iran while th Western (A) and the North-Eastern (C) lineages comprise a single cluster each.

The independent BEAST runs for each dataset under the best topological hypothesis (based on the BF) produced congruent results. The trace plots indicated that all analyses had reached convergence for all parameters with good mixing (effective sample size >200). The inferred divergence times suggest that the origin of *E. persica* dates back to the mid-Miocene (~13Mya) and *M. watsonana* later on, to the late Miocene (~6-7 Mya).

4. Discussion

The study of molecular phylogenies in the framework of comparative biogeography is a powerful tool for identifying broad biodiversity patterns and the processes that shaped them. Such comparative studies are particularly important in poorly studied areas, such as the Iranian Plateau, and enhance both the systematic and evolutionary biogeographic knowledge of the area. In the present study, we identify four major regions in Iran based on the mitochondrial phylogenies of *M. watsonana* and *E. persica*, i.e., Quom, Eastern, North-Eastern, and Western Iran. The fact that the relationships among these regions and the times of their origins differ indicates that the similarity in the geographical clusters is due to ecological and geophysical barriers rather than shared evolutionary history among the two taxa. In the following text, we outline the major paleogographic events that correspond to the divergence times among the four areas for each taxon and comment on their taxonomy based on the mitochondrial clusters delimited by mPTP.

4.1. *Historical biogeography*

The *E. persica* complex dates back to the mid-Miocene and has been in the Iranian Plateau for twice as long as *M. watsonana,* which originated much later, in the upper Miocene. The two complexes speciate at a similar pace based on the rates recovered by BEAST, even though it is slightly higher for *M. watsonana* (0.2 speciation events/My) than for *E. persica* (0.14 speciation events/My). The similarity in speciation rates further support the longer evolutionary time of *E. persica* compared to *M. watsonana* given the number of independent mtDNA clusters found for *E. persica* which is twice as high, i.e., six versus 13 clusters, respectively.

The current assumption for a Middle Miocene origin of *E. persica* is far older than a previous estimation that placed the event earlier, in the Early Pliocene (~5.3 Mya Guo et al., 2011). Both attempts are indirectly based on the divergence of the *Gallotia* species which were estimated using the time of emergence of the Canary islands. With reference to Arnold et al. (2007), Guo et al. (2011) used the split among Lacertinae and Eremiadinae as a calibration point, while we used the evolutionary rates reported in Carranza et al. (2012). Both reference papers, Arnold et al. (2007) and Carranza et al. (2012), estimated the divergence times of the lacertid species using the emergence of the Canary islands as calibration points. However, Carranza et al. (2012) included multiple calibration points with more appropriate time constraints (Cox et al., 2010) which yielded older divergence times among the *Gallotia* species. The phylogeographic scenario for *E. persica* in Pouyani et al. (2010) assumes more similar divergence times to the ones inferred here, though still younger. However, these estimates were calculated assuming a strict clock and using directly the uplift of

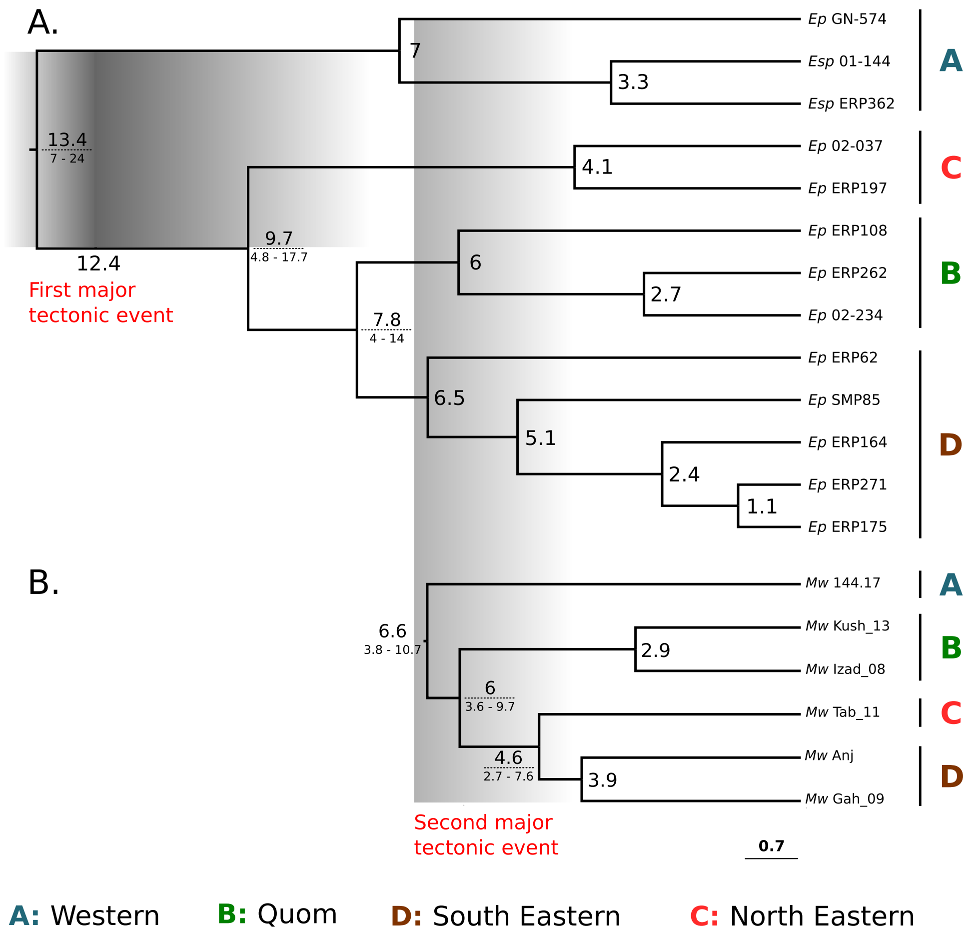

Fig. 3. Calibrated trees of A) *E. persica* and B) *M. watsonana*. The values of the nodes correspond to the median posterior estimation of the divergence time, while for the splits of the major clades the 95% Highest Posterior Density is also provided under the dashed line. The gray shades indicate the two major orogenetic events occurring on the Iranian Plateau since the Miocene, the first one is estimated to have started in the Middle Miocene (∼14 Mya) but the activity did not pick before the 12.7 Mya, the second major tectonic activity occurred later, at the Miocene-Pliocene boundary.

the Zagros Mountains as the calibration point. Based on the mid-Miocene origin of *E. persica*, the ancestral populations have been present in the broader area at the onset of the major paleotectonic and paleoclimatic changes that shaped the Plateau into its current form. The original split of the complex was estimated at ∼13.4 Mya, which coincides with the initial folding along the Zagros mountain range (Khadivi, 2010). The major uplifting of the range was initiated shortly after that (∼12.4 Mya, Khadivi, 2010) and lasted ∼5 My. Taking into account that this split separates the *E. persica* lineages east and west of the Zagros mountain range, the data corroborate the assumption (Pouyani et al., 2010) that the mid-Miocene exhumation of Zagros caused a vicariant event among the ancestral populations of the complex. The second split of *E. persica* was estimated much later, in the upper Miocene (∼10 Mya), and led to the isolation of the North-Eastern from the Eastern and the Quom populations. Previous studies assume that this split was associated with the latest major tectonic events of the Iranian Plateau (Pouyani et al., 2010). Nevertheless, the estimation here predates those events significantly. The effect of the orogenetic events in the diversification of *E. persica* was possibly amplified by the coeval climatic changes occurring in the Plateau, since the Mid-Miocene climatic optimum. In particular, evidence from stable oxygen and carbon isotopes in Northern Iran (Ballato et al., 2010), suggest that there have been three major climatic phases during the Miocene, a) of increased aridity, particularly between 17.5 and 13.2 Mya, followed by 2) a period (13.2 and 10.3 Mya) with increased precipitation [resembling the record from southern Europe (Böhme et al., 2008; Van Dam, 2006)], inducing semi-arid conditions, and a period of 3) further decreased aridity from ca. 9.6–7.6 Mya, which coincides with the similar climatic changes recorded in Asia and India.

Similarly to *E. persica*, the initial split of the Iranian *M. watsonana* was among the Western and the rest of the Iranian lineages, albeit much later. The estimated time of this split is ∼6.6 Mya, which corresponds very well to the second major phase of the tectonic activity in the Iranian Plateau. During this period, the major uplift of the Alborz mountain range occurred (Axen et al., 2001) and it was followed by the uplift of the entire Plateau (Allen et al., 2004). The isolation of the Quom from the remaining populations could be due to these tectonic events coupled with the presence of the salt flats in the Dasht-e Kavir. The salt desert, in the lowland of area southeast of Quom, had already formed since the last seawater retreat (Popov et al., 2004). It is worth noting that the almost simultaneous divergence of the Western and the Quom lineages of *M. watsonana* from the others is the potential reason for the ambiguous resolution of the Quom clade with respect to the remaining phylogeny. The isolation of the equivalent Quom lineage in *E. persica* indicates that the combined effects of the Zagros, the Alborz, and the Dasht-e Kavir desert have led to a particularly isolated lowland area with potentially high endemicity. At approximately the same period, several splitting events in *E. persica* occur, potentially driven by the uplift of the main Plateau.

The Pliocene-Pleistocene divergence in the two complexes may be the result of climatic rather than tectonic changes in the area.

Climatic geochronological data of Iran are mostly available until the Holocene and the Upper Pleistocene (Djamali et al., 2012, 2011, 2008; Kehl, 2009). For the earlier Quaternary period and the Pliocene the data for the climate conditions and the age estimates of the climate shifts remain sparse. Overall, researchers agree that there have been several Quaternary climatic changes with periodical changes from dry and cold to moist and warm during glacial and interglacial periods, respectively (Kehl, 2009). Such climatic oscillations could result in the retreating to allopatric refugia during glacial periods and consequent further isolation of the populations of the Plateau.

4.2. Comments on taxonomy

The taxonomy of both species has been considered problematic in several previous studies (Kapli et al., 2015; Pouyani et al., 2010; Šmíd and Frynta, 2012). The delimited mtDNA clusters, based on the mPTP model, corroborate the species complex status for both *M. watsonana* and *E. persica* and call for further systematic studies and potential taxonomic revisions. The estimated number of clusters for *E. persica* is twice that of *M. watsonana,* which is further supported by the independent estimation of the divergence times which, as discussed earlier, yielded double the evolutionary time for *E. persica* in comparison to *M. watsonana*. Partial morphological data on the former, support the phenotypic divergence among some clusters (Pouyani et al., 2010), which may additionally imply longer evolutionary isolation among them. A thorough morphological revision would add significant insight to the taxonomic status of the delimited clusters. On the contrary, *M. watsonana* is morphologically much more homogeneous, and slight differences have mostly been attributed to sexual dimorphism and geographical latitude (Yousefkhani et al., 2013). The lack of morphological clustering in the *M. watsonana* populations may be due to the limited time since divergence. However, cryptic morphology should not be ignored for this taxon, especially if we take into consideration the limited pholidotic variation across the entire genus. For instance, *M. watsonana* and *M. guttulata* are identical except for the shape of the collar scales (Anderson, 1999), and until recently they were regarded as the same species (Arnold, 1986a) even though they are two of the most distantly related lineages of the genus (Kapli et al., 2015). Similarly, several *Mesalina* species are not recognizable by pholidotic characters and they can only be distinguished based on their geographic origin or their hemipenial structure [e.g., *M. simoni* vs. *M. olivieri*, *M. bahaeldini* vs. *M. guttulata* (Arnold, 1986a, 1986b)]. The evolutionary process responsible for the limited morphological divergence in *Mesalina* could be convergent evolution as an adaptive response to the homogenous xeric habitats across the distribution of the entire genus (even though morphological stasis could not be ruled out). In either case, the systematic study of the complex based on extensive sampling and multi-locus data would be particularly useful in quantifying its species number (Ahmadzadeh et al., in progress).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcz.2017.09.003.

References

Ahmadzadeh, F., Flecks, M., Carretero, M.A., Mozaffari, O., Böhme, W., Harris, D.J., Freitas, S., Rödder, D., 2013. Cryptic speciation patterns in Iranian rock lizards uncovered by integrative taxonomy. PLoS One, 8, http://dx.doi.org/10.1371/journal.pone.0080563.

Alavi, M., 1994. Tectonics of the zagros orogenic belt of Iran: new data and interpretations. Tectonophysics 229, 211–238, http://dx.doi.org/10.1016/0040-1951(94)90030-2.

Allen, M., Jackson, J., Walker, R., 2004. Late Cenozoic reorganization of the Arabia-Eurasia collision and the comparison of short-term and long-term deformation rates. Tectonics 23.

Anderson, S.C., 1999. The Lizards of Iran. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA.

Arnold, E.N., 1986a. The hemipenis of lacertid lizards (Reptilia: lacertidae): structure, variation and systematic implications. J. Nat. Hist. 20, 1221–1257.

Arnold, E.N., 1986b. Why copulatory organs provide so many useful taxonomic characters: the origin and maintenance of hemipenial differences in lacertid lizards (Reptilia: lacertidae). Biol. J. Linn. Soc. 29, 263–281.

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, 2007.

Axen, G.J., Lam, P.S., Grove, M., Stockli, D.F., Hassanzadeh, J., 2001. Exhumation of the west-central Alborz Mountains, Iran, Caspian subsidence, and collision-related tectonics. Geology 29, 559–562, http://dx.doi.org/10.1130/0091-7613(2001)029<0559.

Böhme, M., Ilg, A., Winklhofer, M., 2008. Late miocene washhouse climate in europe. Earth Planet. Sci. Lett. 275, 393–401, http://dx.doi.org/10.1016/j.epsl.2008.09.011.

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A.V., 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29, 2157–2167.

Ballato, P., Mulch, A., Landgraf, A., Strecker, M.R., Dalconi, M.C., Friedrich, A., Tabatabaei, S.H., 2010. Middle to late Miocene Middle Eastern climate from stable oxygen and carbon isotope data, southern Alborz mountains, N Iran. Earth Planet. Sci. Lett. 300, 125–138, http://dx.doi.org/10.1016/j.epsl.2010.09.043.

Berberian, M., King, G.C.P., 1981. Towards a paleogeography and tectonics evolution of Iran. Can. J. Earth Sci. 18, 210–265, http://dx.doi.org/10.1017/CBO9781107415324.004.

Bernor, R.L., 1986. Mammalian biostratigraphy, geochronology, and zoogeographic relationships of the Late Miocene Maragheh fauna, Iran. J. Vertebr. Paleontol. 6, 76–95.

Blanford, W.T., 1876. Eastern Persia an Account of the Journeys of the Persian Boundary Commission: The Zoology and Geology. Gale and the British Library, USA.

Carranza, S., Arnold, E.N., 2012. A review of the gecko genus *Hemidactylus* (Squamata: gekkonidae) from Oman based on morphology, mitochondrial and nuclear data, with descriptions of eight new species. Zootaxa 3378 (May), 1–95.

Cox, S.C., Carranza, S., Brown, R.P., 2010. Divergence times and colonization of the Canary Islands by *Gallotia* lizards. Mol. Phylogenet. Evol. 56, 747–757, http://dx.doi.org/10.1016/j.ympev.2010.03.020.

Crawford, A., 1972. Iran, continental drift and plate tectonics. 24th International Geological Congress, 106–112.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Dayrat, B., 2005. Towards Integrative Taxonomy. Biol. J. of the Linn. Soc. 85 (3), 407–415, http://dx.doi.org/10.1111/j.1095-8312.2005.00503.x.

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

Djamali, M., Biglari, F., Abdi, K., Andrieu-Ponel, V., de Beaulieu, J.L., Mashkour, M., Ponel, P., 2011. Pollen analysis of coprolites from a late Pleistocene-Holocene cave deposit (Wezmeh Cave, west Iran): insights into the late Pleistocene and late Holocene vegetation and flora of the central Zagros Mountains. J. Archaeol. Sci. 38, 3394–3401, http://dx.doi.org/10.1016/j.jas.2011.08.001.

Djamali, M., Baumel, A., Brewer, S., Jackson, S.T., Kadereit, J.W., López-Vinyallonga, S., Mehregan, I., Shabanian, E., Simakova, A., 2012. Ecological implications of *Cousinia* Cass. (Asteraceae) persistence through the last two glacial-interglacial cycles in the continental Middle East for the Irano-Turanian flora. Rev. Palaeobot. Palynol. 172, 10–20.

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

Drummond, A.J., Suchard a, M., Xie, D., Rambaut, A., 2012. Bayesian P hylogenetics with BEAUti and the BEAST 1. 7. Mol. Biol. Evol. 29, 1969–1973, http://dx.doi.org/10.1093/molbev/mss075.

Ebrahimi, M., Ahmadzadeh, F., Mostafavi, H., Mehrabian, A.R., Abdoli, A., Mahini, A.S., 2013. The ecological associations of surface-dwelling lizards in Qom Province in the northwest of Central Plateau of Iran. PLoS One 8, 1–10, http://dx.doi.org/10.1371/journal.pone.0083890.

Esselstyn, J.A., Evans, B.J., Sedlock, J.L., Anwarali Khan, F.A., Heaney, L.R., 2012. Single-locus species delimitation: a test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. Proc. R. Soc. B Biol. Sci. 279, 3678–3686, http://dx.doi.org/10.1098/rspb.2012.0705.

Firouz, E., 2005. The Complete Fauna of Iran. IB Tauris and Co., London.

Frey, W., Probst, W., 1986. A Synopsis of the Vegetation of Iran Contrib. to Veg. Southwest Asia. Dr. Ludwig Reichert Verlag, pp. 9–43.

Fu, J.Z., 2000. Toward the phylogeny of the family Lacertidae – Why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol. J. Linn. Soc. 71, 203–217, http://dx.doi.org/10.1006/bijl.1999.0431.

Fujisawa, T., Barraclough, T.G., 2013. Delimiting species using single-locus data and the generalized mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. Syst. Biol. Syt033 62 (5), 707–724.

Guest, B., Horton, B.K., Axen, G.J., Hassanzadeh, J., McIntosh, W.C., 2007. Middle to late Cenozoic basin evolution in the western Alborz Mountains: implications for the onset of collisional deformation in northern Iran. Tectonics 26.

Guo, X., Dai, X., Chen, D., Papenfuss, T.J., Ananjeva, N.B., Melnikov, D.A., Wang, Y., 2011. Phylogeny and divergence times of some racerunner lizards (Lacertidae: *Eremias*) inferred from mitochondrial 16S rRNA gene segments. Mol. Phylogenet. Evol. 61, 400–412.

Harris, D.J., Arnold, E.N., Thomas, R.H., 1998. Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proc. R. Soc. London Ser. B-Biological Sci. 265, 1939–1948, http://dx.doi.org/10.1098/rspb.1998.0524.

Hedge, I.C., Wendelbo, P., 1978. Patterns of distribution and endemism in Iran. Notes from the Royal Botanic Garden Edinburgh. 36, 441–464.

Ho, S.Y.W., Lanfear, R., Bromham, L., Phillips, M.J., Soubrier, J., Rodrigo, A.G., Cooper, A., 2011. Time-dependent rates of molecular evolution. Mol. Ecol., http://dx.doi.org/10.1111/j.1365-294X.2011.05178.x.

Hollingsworth, J., Fattahi, M., Walker, R., Talebian, M., Bahroudi, A., Bolourchi, M.J., Jackson, J., Copley, A., 2010. Oroclinal bending, distributed thrust and strike-slip faulting, and the accommodation of Arabia-Eurasia convergence in NE Iran since the Oligocene. Geophys. J. Int. 181, 1214–1246.

Jassim, S.Z., Goff, J.C., 2006. Geology of Iraq. DOLIN, sro, distributed by Geological Society of London.

Kapli, P., Poulakakis, N., Lymberakis, P., Mylonas, M., 2011. A re-analysis of the molecular phylogeny of Lacertidae. Basic Appl. Herpetol. 25, 97–104.

Kapli, P., Lymberakis, P., Crochet, P.A., Geniez, P., Brito, J.C., Almutairi, M., Ahmadzadeh, F., Schmitz, A., Wilms, T., Pouyani, N.R., Poulakakis, N., 2015. Historical biogeography of the lacertid lizard *Mesalina* in North Africa and the Middle East. J. Biogeogr. 42, 267–279.

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., Flouri, T., 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33, 1630–1638, http://dx.doi.org/10.1093/bioinformatics/btx025.

Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kehl, M., 2009. Quaternary climate change in Iran – the state of knowledge. Erdkunde 63, 1–17, http://dx.doi.org/10.3112/erdkunde.2009.01.01.

Khadivi, S., 2010. Tectonic Evolution and Growth of the Zagros Mountain Belt (Fars, Iran): Constraints from Magnetostratigraphy, Sedimentology and Low-Temperature Thermochronometry. Univ. Pierre Marie Curie – Paris VI, pp. 1–242.

Madjnoonian, H.H., Kiabi, B., Danesh, M., 2005. Readings in Zoogeography of Iran (Part II). Department of Environment, Tehran.

Mayer, W., Pavlicev, M., 2007. The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. Mol. Phylogenet. Evol. 44, 1155–1163, http://dx.doi.org/10.1016/j.ympev.2007.05.015.

McQuarrie, N., Van Hinsbergen, D.J.J., 2013. Retrodeforming the Arabia-Eurasia collision zone: age of collision versus magnitude of continental subduction. Geology 41, 315–318, http://dx.doi.org/10.1130/G33591.1.

Mendes, J., Harris, D.J., Carranza, S., Salvi, D., 2016. Evaluating the phylogenetic signal limit from mitogenomes, slow evolving nuclear genes, and the concatenation approach. New insights into the Lacertini radiation using fast evolving nuclear genes and species trees. Mol. Phylogenet. Evol. 100, 254–267, http://dx.doi.org/10.1016/j.ympev.2016.04.016.

Mouthereau, F., Lacombe, O., Verges, J., 2012. Building the Zagros collisional orogen: timing, strain distribution and the dynamics of Arabia/Eurasia plate convergence. Tectonophysics 532-535, 27–60, http://dx.doi.org/10.1016/j.tecto.2012.01.022.

Popov, S.V., Rögl, F., Rozanov, A.Y., Steininger, F.F., Shcherba, I.G., Kovac, M., 2004. Lithological-paleogeographic maps of the paratethys: late eocene to pliocene. Cour. Forschungsinstitut Senckenb. 250, 1–46.

Pouyani, E.R., Pouyani, N.R., Noureini, S.K., Joger, U., Wink, M., 2010. Molecular phylogeny of the Eremias persica complex of the Iranian plateau (Reptilia: lacertidae), based on mtDNA sequences. Zool. J. Linn. Soc. 158, 641–660, http://dx.doi.org/10.1111/j.1096-3642.2009.00553.x.

Pyron, R., Burbrink, F.T., Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. 13, 93, http://dx.doi.org/10.1186/1471-2148-13-93.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Šmíd, J., Frynta, D., 2012. Genetic variability of *Mesalina watsonana* (Reptilia: Lacertidae) on the Iranian plateau and its phylogenetic and biogeographic affinities as inferred from mtDNA sequences. Acta Herpetol. 7, 139–153.

Sborshchikov, I.M., Savostin, L.A., Zonenshain, L.P., 1981. Present plate tectonics between Turkey and Tibet. Tectonophysics 79, 45–73.

Schleich, H.H., Kästle, W., Kabisch, K., 1996. Amphibians and Reptiles of North Africa. Koeltz, Koenigstein, pp. 627.

Sindaco, R., Jeremčenko, V.K., 2008. The Reptiles of the Western Palearctic, Monografie Della Societas Herpetologica Italica. Edizioni Belvedere, Latina.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.

Stoliczka, F., 1872. Notes on reptiles collected by surgeon F. Day in Sind. Proc. Asiat. Soc. Bengal 1872, 85–92.

Tang, C.Q., Humphreys, A.M., Fontaneto, D., Barraclough, T.G., 2014. Effects of phylogenetic reconstruction method on the robustness of species delimitation using single-locus data. Methods Ecol. Evol. 5, 1086–1094.

Van Dam, J.A., 2006. Geographic and temporal patterns in the late Neogene (12-3 Ma) aridification of Europe: the use of small mammals as paleoprecipitation proxies. Palaeogeogr. Palaeoclimatol. Palaeoecol. 238, 190–218.

Van Hinsbergen, D.J.J., Lippert, P.C., Dupont-Nivet, G., McQuarrie, N., Doubrovine, P.V., Spakman, W., Torsvik, T.H., 2012. Greater India Basin hypothesis and a two-stage Cenozoic collision between India and Asia. Proc. Natl. Acad. Sci. 109, 7659–7664, http://dx.doi.org/10.1073/pnas.1117262109.

Yousefkhani, S.S.H., Rastegar-Pouyani, E., Rastegar-Pouyani, N., 2013. Geographic variation in Mesalina watsonana (Sauria: lacertidae) along a latitudinal cline on the Iranian Plateau. Salamandra 49, 171–176.

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876.