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ORIGINAL ARTICLE

Speciation in the Iranian plateau: Molecular phylogeny and evolutionary history of the Persian long-tailed desert lizard

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Abstract

Mesalina watsonana is a well-known species of small lacertid lizards with an extensive species distribution that exhibits high genetic diversity. The species has a wide distribution range in Iran, some parts of Turkmenistan, Afghanistan, Pakistan, and northwest India. This study aims to generate phylogenetic and phylogeographic evidence to derive taxonomic recommendations supporting. Furthermore, this species can be used as a model for examining Iranian Platuea's historical biogeography. We conducted a very detailed sampling of its distribution and used genetic approaches. Phylogenetic analyses were done implementing two mitochondrial (Cyt*b* and 16S) and one nuclear (C-mos) gene fragments. Combination of these results indicated that seven well-supported distinct clades exist within this species complex, i.e. Kerman clade, Esfarayen clade, Halil clade, Ardestan clade, *M. watsonana* clade, Bardaskan Clade, and Khuzestan Clade in Iran. Also, our results revealed that several distinct clades diverged due to geologic events when the Dasht-e-Kavir and the Zagros Mountains were formed. It seems that the ancestor of *M. watsonana* spread to Iranian Plateau before the formation of the Zagros Mountains. Orogenic activities of the Zagros Mountains and the formation of deserts have influenced the separation of these lineages from the Late Miocene by allopatric speciation. Generally, our findings suggest that each of the seven clades corresponding to distinct geographic regions deserves to be elevated to the species level.

KEYWORDS

allopatric speciation, Dasht-e-Kavir, *Mesalina watsonana*, Zagros Mountains

1 | INTRODUCTION

Geological and climatic changes are the main driving forces of speciation and diversification processes particularly in small vertebrates (Aghbolaghi et al., 2019; Hua et al., 2013; Wu et al., 2023). Geological events, e.g. mountain uplifting may form barriers to gene flow between populations through habitat discontinuity (Gonçalves et al., 2012; Macey et al., 1998). Additionally, species response to climatic fluctuations may also have an important role in speciation. These oscillations isolate populations in disjunct areas, leading to genetic divergence and ultimately to speciation (Ahmadzadeh et al., 2020; Amiri et al., 2021; Hewitt, 1996; Tarkhnishvili et al., 2013). Evolutionary diversification rate, extinction, and speciation shape global patterns of biodiversity in different regions (Schluter & Pennell, 2017).

The Iranian Plateau is an upland area that is delimited in the west by the high mountain ranges of the Zagros, in the north by Alborz and Kopet Dagh, in the east by the lofty summits of the Hindu Kush in Afghanistan, and in the south by the Makran and Sulaiman mountain ranges and east of Pakistan (Fisher, 1968; Khan, 1980; Macey et al., 1998). The plateau is an area of highly complex landscape and incredibly diverse habitats. Its topography and climatic diversity have created a particular biogeographical area with unique biodiversity. Despite the extensive literature regarding tectonics and palaeogeography (Ahmadzadeh et al., 2017 and references therein), systematic and speciation studies in this region are lacking, and our understanding of the historical processes that shaped its biodiversity remains limited.

The Iranian plateau is a region of biogeographic interest, having rich and diverse reptile fauna, which have occupied various habitats and microhabitats due to their very ancient natural history (Kafash et al., 2020; Šmíd et al., 2014). This region with dominantly arid and semi-arid habitats provides suitable home for reptiles in particular widely distributed lizards of the tribe Eremidini (e.g. *Acantodactlus*, *Eremias*, *Mesalina*, and *Ophisops*). Several studies in the plateau and neighbouring areas have shown that the lizards are an excellent model species for studying speciation processes, and the effects of geological events and climate fluctuations on their genetics (Ahmadzadeh et al., 2011; Ghaedi et al., 2021; Ghane-Ameleh et al., 2021; Kapli et al., 2015; Rahimian et al., 2015; Rastegar Pouyani et al., 2010; Saberi-Pirooz et al., 2018, 2021). To understand how the multiple diversity patterns of the plateau were shaped, we studied the historical biogeography and speciation process of its most diverse lacertid lizard species complex, *Mesalina watsonana* (Stoliczka, 1872).

Mesalina watsonana is found in a wide range of areas including Iran, Turkmenistan, Afghanistan, Pakistan, and north-west India (Anderson, 1999; Hosseinian Yousefkhani et al., 2013, b; Kapli et al., 2015; Khan, 2006). Šmíd and Frynta (2012) have identified four different clades using the mtDNA cytochrome b gene within the species that are geographically isolated from each other. In that study, morphological and meristic analyses have shown that *M. watsonana* can be divided into two large groups. Phylogenetic studies suggest that *M. watsonana* diverged from other *Mesalina* clades and was placed as a sister to all other members of its genus. During the Miocene period the species complex found in lowlands and mountain hills of arid and semi-arid habitats. The objective of this research is to examine the process of speciation in the Iranian Plateau and nearby regions, using the *M. watsonana* complex as a model. Additionally, the study aims to analyse historical biogeography to explain current biodiversity patterns in the area. Despite previous studies on *M. watsonana*, there is limited knowledge about its phylogenetic relationships and evolutionary history within the species complex. Therefore, this study seeks to clarify these relationships by utilizing a comprehensive dataset from the entire range of the complex in Iran, employing genetic methods. In addition, this study aims to generate phylogenetic and phylogeographic evidence to derive taxonomic recommendations eventually confirming morphological variation.

2 | MATERIALS AND METHODS

2.1 | Genetic sampling

In the study, 65 tissue samples were collected during extensive field studies between October 2019 and April 2021 (Figure 1; Table S1). We selected 25 samples based on geographic distribution range (we did not include repeated samples from each location in our study) for molecular analysis. Tissue samples were taken from the specimens by removing a small section of tail tips. The lizards were then released into their habitat. The tissues were preserved in 96% ethanol and stored in a freezer at −20°C for long-term maintenance.

2.2 | Laboratory procedures

Total genomic DNA was extracted with standard protocols of high-salt and phenol-chloroform methods (Sambrook et al., 1989). Three partial genes including two mtDNA markers Cytochrome b (Cyt*b*) and 16S ribosomal RNA (16S) and one nuclear gene Oocyte maturation factor Mos (C-mos) were used. Primer pairs used for Cyt*b* and 16S included GluDg/Peil (Engström et al., 2007; Palumbi et al., 1991) and 16Sa/16Sb (Palumbi, 1996), respectively. The nuclear primer pairs were L1zmos and Hcmos1 for C-mos (Pavlicev & Mayer, 2006). 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 µmol.µl), 10.5 µL ddH$_2$O, and 1 µL of template DNA (50–100 ng). PCRs amplification cycling conditions and details of primers are presented in Table S1. PCR products were visualized on 1% agarose gel. The successfully amplified samples were then sent to Niagenenoor company (Niagenenoor, Tehran, Iran) for sequencing. Sequences were edited using Geneious Prime program 2021.1.1.0 (Biomatters Ltd, http://www.geneious.com/). All sequences were submitted to GenBank (Table S2).

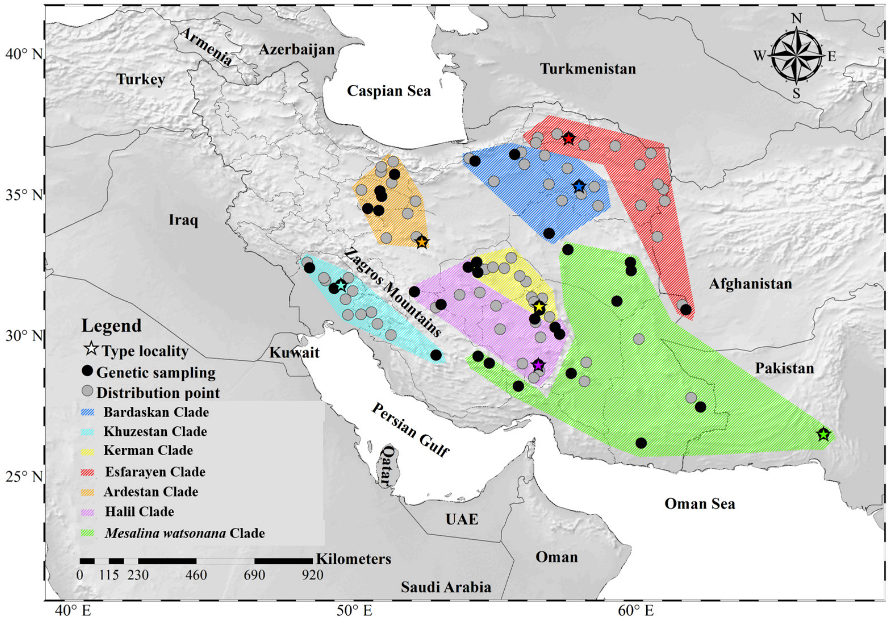


FIGURE 1 The geographical distribution range of the seven main clades of the *M. watsonana* complex. Grey circles represent the distribution points of species, and black circles represent the locations of samples used in the genetic analyses. (These points were collected from reliable sources such as Anderson, 1999; Šmíd & Frynta, 2012; Hosseinian Yousefkhani, Rastegar-Pouyani, & Rastegar-Pouyani, 2013; Hosseinian Yousefkhani, Rastegar-Pouyani, Rastegar-Pouyani, Masroor, & Šmíd, 2013; Šmíd et al., 2014; Kapli et al., 2015; Sindaco et al., 2018; Simó-Riudalbas et al., 2019 and this study).

TABLE 1 Summary of nucleotide substitution models for three partitions for *Mesalina* dataset.

Subset	Best model	Partition names
1	TIM2 + F + I + G4	16S
2	F81 + F + I	Cyt*b*_pos (1–2)
3	TPM2 + F + I + G4	C-mos/Cyt*b*_pos 3

2.3 | Phylogenetic study

To assess the phylogenetic relationship of the *M. watsonana* complex, other species of Lacertidae were retrieved from previous studies and were then added to our dataset (Kapli et al., 2015; Simó-Riudalbas et al., 2019; Sindaco et al., 2018; Šmíd & Frynta, 2012) (see Table S2). The datasets of each gene were aligned with MAFFT v.6 (Katoh et al., 2017) (https://mafft.cbrc.jp/; algorithm: Auto; scoring matrix: 200Pam/k = 2; Gap open penalty: 1.53), separately and were then combined, resulting in a final 1214 bp alignment (Cyt*b*: 472 bp, 16S: 381 bp, and C-mos: 361 bp). The best-fit partitioning scheme with the nucleotide substitution models for each partition was estimated by PartitionFinder v. 2 (Lanfear et al., 2016) under Akaike's Information Criterion (Akaike, 1974). The best scheme was three partitions, a summary of models for each partition is shown in Table 1.

The maximum likelihood (ML) inference was conducted for the combined genes (three genes) using IQ-Tree v.1.6.12 (Nguyen et al., 2015) by three partition schemes (Table 1), and the confidence of branch supports was evaluated from 1000 pseudoreplicates using the ultra-fast Bootstrap (UFB) (Hoang et al., 2018).

The Bayesian Inference (BI) analysis was performed for the combined genes using MrBayes v.3.2 (Huelsenbeck & Ronquist, 2001). The best-fit models were applied for each partition. The analysis was done using two independent runs with four chains for 2 million generations. Subsampling trees and parameters were saved every 100 iterations. Finally, 25% 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 0.0013. The convergence to the stationary distribution was evaluated using Tracer v.1.6 (Rambaut & Drummond, 2009).

Uncorrected genetic distances (*p*-distance) were calculated using MEGA X (Kumar et al., 2018) between the main clades for mtDNA genes (Cyt*b* and 16S), separately.

2.4 | Estimation of divergence times

Divergence times within *Mesalina* were estimated with BEAST v.1.7.2 (Drummond & Rambaut, 2007) using the combined dataset (Three gene fragments). In order to date the phylogeny, two secondary calibration points were used: (a) the diversification of the genus *Mesalina* [c. 23 million years ago (henceforth Mya) high posterior density interval (HPD): 8.1284–31.4332] and (b) the radiation of *M. watsonana* (c). 6.6 Mya (HPD: 10–22) (Kapli et al., 2015). The calibration point was applied to *Mesalina* (normal distribution, M: 22.7, S: 8.0) and *M. watsonana* nodes (normal distribution, M: 6.6, S: 0.7). A lognormal relaxed clock (uncorrelated) was used for all markers with the Yule model for the speciation prior. The analysis was run for 4×10^7 generations and sampling every 1000 generations. Convergence diagnostics for the MCMC analyses were assessed using Tracer v. 1.6.1.

Lineage through time plotting (LTT) for *M. watsonana* was performed using Tracer v.1.6 with the combined dataset.

2.5 | mtDNA species delimitation

The *M. watsonana* species 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 16S). 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' (http://r-forge.r-project.org/projects/splits/). An ultrametric tree obtained by BEAST was employed for the analysis. The bPTP infers species boundaries via the number of substitutions. A phylogenetic tree (ML) was applied as the input file. The analysis was run on the bPTP online web server (http://species.h-its.org/ptp/) with 5×10^5 MCMC generations, with a thinning of 100 and a burn-in of 10%.

2.6 | Biogeographic analysis

Statistical dispersal vicariance (S-DIVA) and Bayesian binary MCMC (BBM) analyses were conducted using RASP 2.1 beta (Yu et al., 2015) for Cyt*b*, as there were sufficient samples and a higher mutation rate for reconstructing *M. watsonana* complex ancestral ranges. For S-DIVA, 20,000 trees were generated from the Mr Bayes tree to account for phylogenetic uncertainty. The BBM analysis was run for 5×10^6 generations under ten MCMC, and the sampling frequency was every 100 generations. We analysed BBM using the fixed Jukes–Cantor model with equal rate variation among sites.

TCS networks were constructed in PopART v1.7 (Leigh & Bryant, 2015) for Cyt*b* and C-mos genes, respectively.

3 | RESULTS

3.1 | Molecular analysis

3.1.1 | Phylogenetic tree

Both ML and BI approaches generated similar topologies. The results of the combined genes indicated that *M. watsonana* forms a monophyletic group regarding other *Mesalina* sp. with several genetic structures with high support values (Figure S1). Also, the trees indicated that the species complex is a sister clade to the rest of *Mesalina* species. At the intraspecific level, seven distinct clades were recovered and were separated from each other with high support values. The phylogenetic trees of *M. watsonana* from the eastern and western Dasht-e-Kavir create two distinct clades. The Western Dasht-e-Kavir clade (Ardestan clade) is a sister clade to the Central clade (Halil clade) of Iran. The center of Iran comprises two clades that are far from each other (Halil clade and Kerman clade). The western (Khuzestan clade) and eastern (Bardaskan clade) Zagros Mountains formed separate clades (Figure S1).

Uncorrected genetic distances for Cyt*b* and 16S were approximately 6%–13% and 3%–5% between the main clades of *M. watsonana* complex, respectively (Table 2).

TABLE 2 Uncorrected genetic *p*-distances between *Mesalina watsonana* clades based on Cyt*b* (below matrix) and 16S (above matrix).

		1	2	3	4	5	6	7
1	Kerman clade		0.0432	0.0514	0.0520	0.0357	0.0280	0.0415
2	Esfarayen clade	0.0936		0.0496	0.0485	0.0364	0.0303	0.0523
3	Halil clade	0.1115	0.1248		0.0321	0.0427	0.0463	0.0448
4	Ardestan clade	0.1130	0.1339	0.0611		0.0455	0.0457	0.0475
5	*M. watsonana* clade	0.0856	0.0815	0.1238	0.1180		0.0300	0.0432
6	Bardaskan clade	0.0815	0.0943	0.0956	0.0978	0.0808		
7	Khuzestan clade	0.0854	0.1064	0.0956	0.0927	0.1127	0.0883	

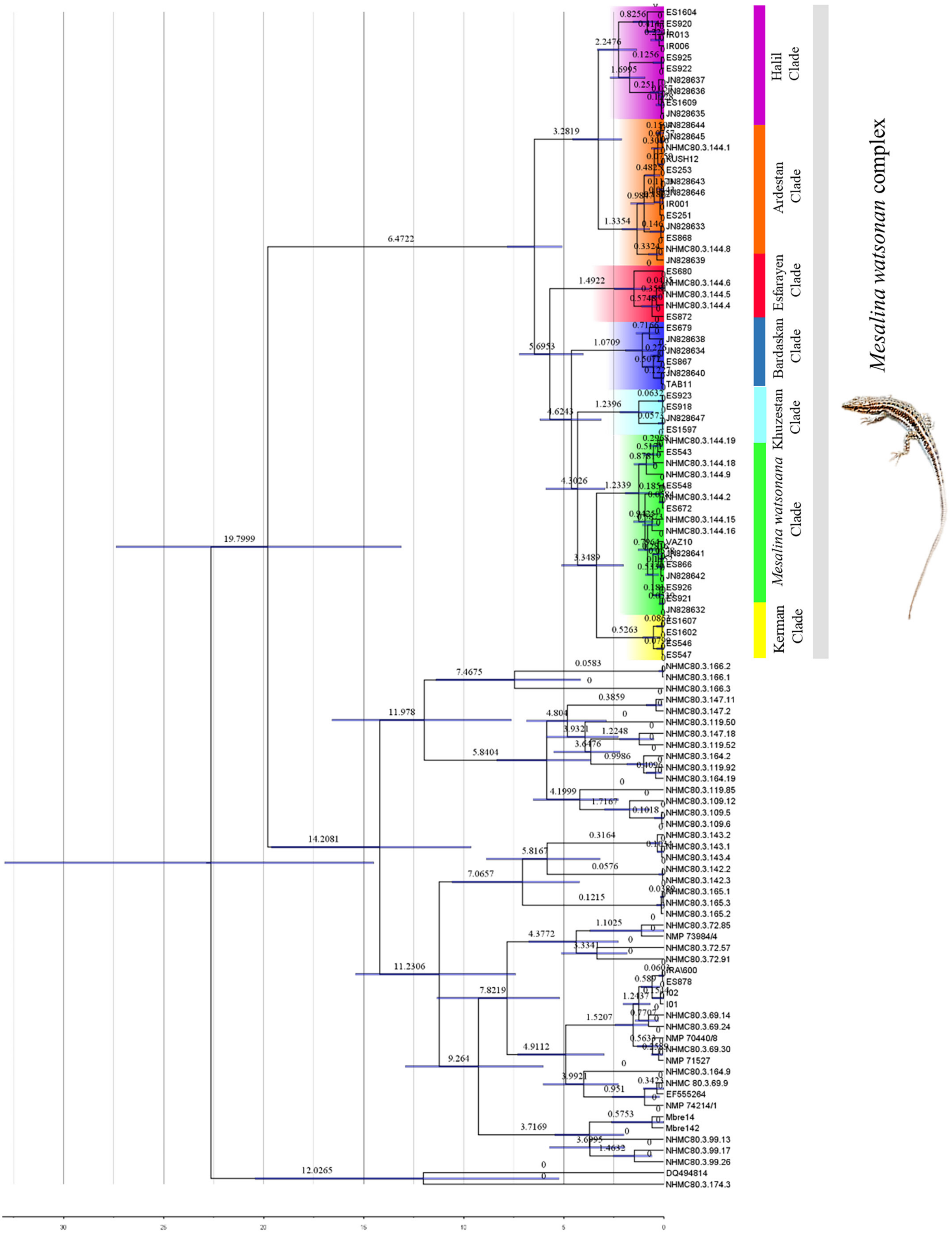


FIGURE 2 The dated phylogenetic trees using the combined dataset (mtDNA and nuDNA), within *Mesalina*. Blue bars show 95% highest posterior density intervals of the estimated node ages; numbers under the branches are mean node ages (Mya).

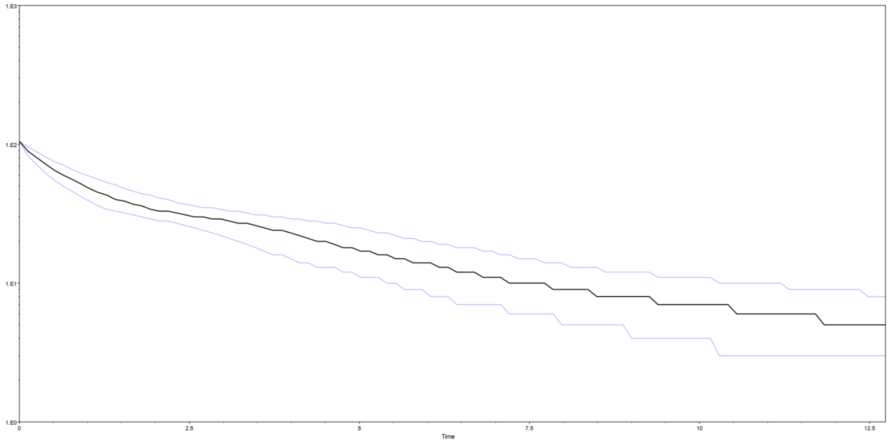


FIGURE 3 Lineage through time plot within *Mesalina*. The time axis indicates millions of years.

3.1.2 | Estimating divergence time

The results of the time calibrated tree showed that *M. watsonana* was separated earlier from the rest of *Mesalina* species around samples at 19.79 Mya (95% HPD: 12.8–25.7 Mya). The diversification within *M. watsonana* has happened 6.47 Mya (95% HPD: 4.9–7.6 Mya). The Halil clade diversified at 2.24 Mya (95% HPD: 1.3–3.2 Mya), and Ardestan clade separated from the Halil clade around 3.28 Mya (95% HPD: 2.04–4.44 Mya). The remaining clades were separated from each other at around 5.69 Mya (95% HPD: 3.9–7.05 Mya, Figure 2).

The plot of LTT showed a gradual exponential increase in speciation over time (Figure 3).

3.1.3 | mtDNA species delimitation

Eight genetic clusters were obtained using the GMYC model. The bPTP model for species delimitation predicted 12 lineages as putative species within *M. watsonana* complex.

3.1.4 | Biogeographic analysis

SDIVA and BBM results showed similar results. All main clades demonstrated dispersal and vicariance events at their ancestral nodes (Figure 4). The formation of new clades within the *M. watsonana* complex is influenced by allopatric speciation.

TCS networks showed seven major clades within the complex species for both mitochondrial and nuclear genes (Figure S3).

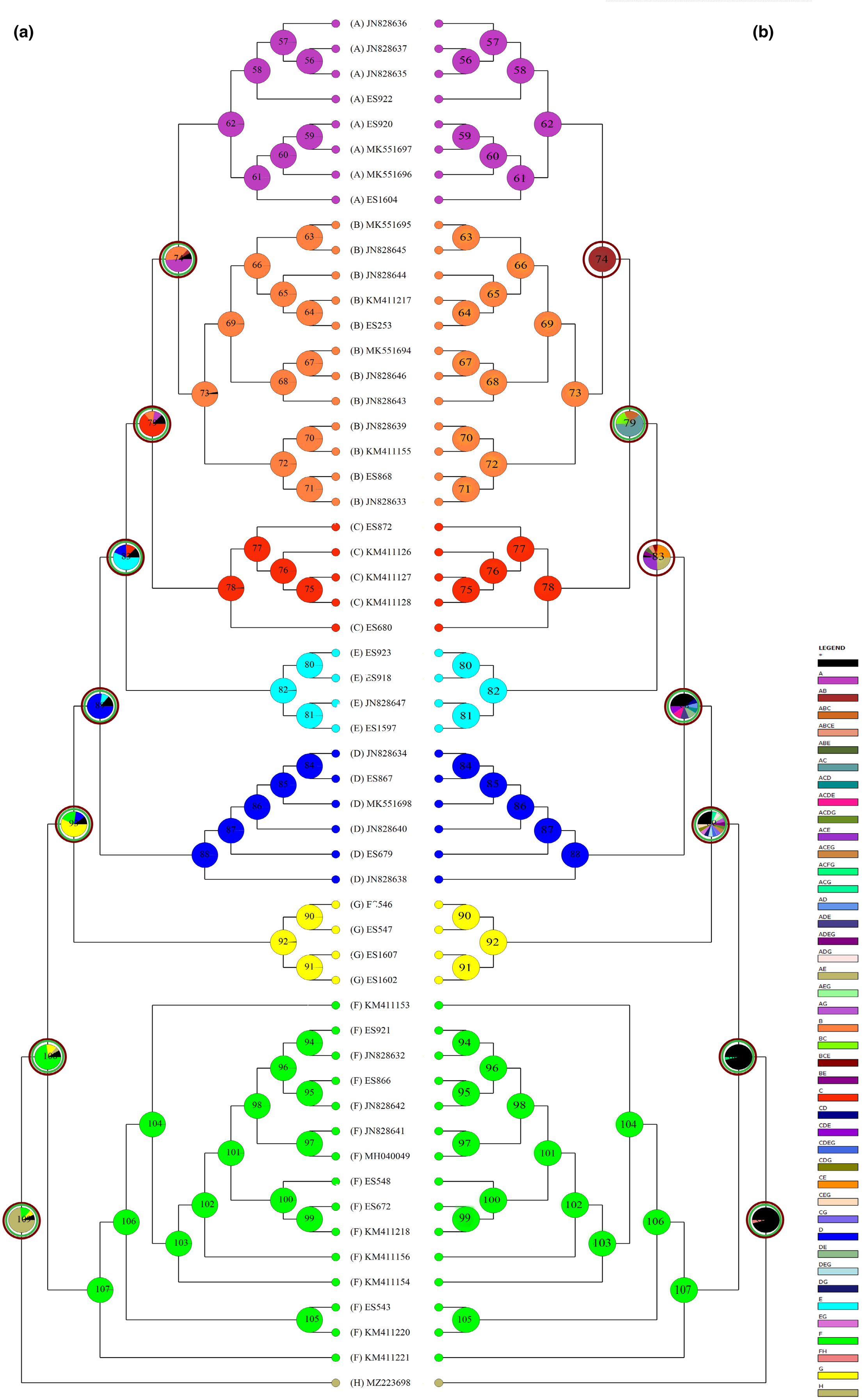
4 | DISCUSSION

In the current study, to reconstruct the evolutionary history of *M. watsonana* and assess species diversity, we conducted an extensive study with genetic approaches. The results showed that several clades may belong to different species. Although at first glance within the species of the *M. watsonana* complex, the external morphology is believed to be similar, it has nevertheless been established that there is a high degree of genetic diversity which highlights speciation processes and diversification within the Iranian lateau.

4.1 | Phylogenetic relationships

According to the assembled dataset, *Mesalina* is a monophyletic genus and *M. watsonana* is divided into seven well-established monophyletic clades (Figure S1 and Figure S3). Samples from eastern Dasht-e-Kavir and western Dasht-e-Kavir form two distinct clades. Specimens of the Ardestan Clade are more closely related to Iran's Central clade. There are two clades in Iran's central region that are very far apart from one another (Halil and Kerman clades). This study attempted to cover the entire range of the species in

FIGURE 4 The biogeographic analysis of *Mesalina watsonana* complex using S-DIVA (a) and BBM (b) based on Cyt*b* sequences. For these analyses, seven regions were considered (A: purple nodes) distribution of Halil clade, (B: orange nodes) distribution of Ardestan clade, (C: red nodes) distribution of Esfarayen clade; (D: dark blue nodes) distribution of Bardaskan clade, and (E: light blue nodes) distribution of Khuzestan clade, (F: green nodes) distribution of *M. watsonana* clade, and (G: yellow nodes) distribution of Kerman clade. The brown and green circles around the nodes show dispersal and vicariance events, respectively.


(a)
(b)
(A) JN828636
(A) JN828637
(A) JN828635
(A) ES922
(A) ES920
(A) MK551697
(A) MK551696
(A) ES1604
(B) MK551695
(B) JN828645
(B) JN828644
(B) KM411217
(B) ES253
(B) MK551694
(B) JN828646
(B) JN828643
(B) JN828639
(B) KM411155
(B) ES868
(B) JN828633
(C) ES872
(C) KM411126
(C) KM411127
(C) KM411128
(C) ES680
(E) ES923
(E) ES918
(E) JN828647
(E) ES1597
(D) JN828634
(D) ES867
(D) MK551698
(D) JN828640
(D) ES679
(D) JN828638
(G) ES546
(G) ES547
(G) ES1607
(G) ES1602
(F) KM411153
(F) ES921
(F) JN828632
(F) ES866
(F) JN828642
(F) JN828641
(F) MH040049
(F) ES548
(F) ES672
(F) KM411218
(F) KM411156
(F) KM411154
(F) ES543
(F) KM411220
(F) KM411221
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Iran with comprehensive samples, the rest of the studies focused on some parts of the species' range (Kapli et al., 2015; Šmíd & Frynta, 2012). Also, we were only able to obtain two samples from Pakistan, specifically NHMC80.3.144.18 and NHMC80.3.144.19. These samples were found within the Iranian samples in the *Mesalina watsonana* clade. Consequently, we cannot ascertain the situation in other regions without additional samples. Nevertheless, given the substantial variation observed within this complex, it appears to exhibit greater diversity. Šmíd and Frynta (2012) showed four clades within the species with Cyt*b* including the southern lineage (three samples from localities Gahkom and Vazireh), the central lineage (one specimen from Anjireh), the northern lineage (Bardeskan, Tabas and Mayamey), and the western lineage (samples from Izadkhast, Ardestan, Salafchegan, and Kushk-e Nosrat). They mentioned that a broader sampling across the entire distribution, as well as more genes (such as nuclear) involved, might help to a deeper understanding of the phylogeny and phylogeography of these lizards. Kapli et al. (2015) investigated the evolutionary and historical biogeography of the genus *Mesalina* using mitochondrial and nuclear markers (Cyt*b*, 16S, and B-fib). According to them, *Mesalina* may be divided into three geographical groups based on the origin and distribution of species or species complexes. *Mesalina watsonana* from Iran, Afghanistan, and Pakistan were the main geographical groups of *M. watsonana*. There were only a few samples from Iran included in the study. Therefore, they were unable to establish phylogenetic relationships with species complexes (Kapli et al., 2015). The studies have all confirmed that the species complex contains substantial geographic structure, despite the fact that there are only a few samples, so they are unable to correctly establish this diversity.

The current study showed that the genetic distances between clades (Table 2) are comparable to genetic distances of other valid Lacertidae species (e.g. within the genera *Apathya*, *Dareveskia*, and *Eremias* species) (see Ahmadzadeh et al., 2013; Kafimola et al., 2022; Mozaffari et al., 2020). The mitochondrial lineages, delimited using the bPTP, GMYC models, and a 6% nucleotide sequence threshold for species delimitation, demonstrated the existence of seven putative species belonging to *M. watsonana*.

Overall, we revisited the relationships between clades of *M. watsonana* complex and demonstrated that the species diversity was substantially underestimated. According to our molecular findings, each of the seven clades deserves to be promoted to species status.

4.2 | Evolutionary history of *Mesalina watsonana*

Based on our dataset, it has been determined that the ancestor of *Mesalina* diverged from other lineages during the Miocene period, approximately 19.79 million years ago (95% HPD: 12.83–26.76; Figure 2). This separation is believed to have been influenced by the movement of the Arabian tectonic plate and associated climatic changes, resulting in the geographic differentiation of the *Mesalina* genus in North Africa and the Middle East during the Miocene (Kapli et al., 2015). It is presumed that *M. watsonana* diverged from its ancestral species prior to its morphological differentiation. Subsequently, gene flow between *M. watsonana* and other species within the genus ceased. The population that colonized the Iranian plateau around 6.5 million years ago underwent subsequent diversification, giving rise to four distinct lineages (Šmíd & Frynta, 2012). Geographical barriers such as the Zagros Mountains in the west, the Alborz Mountains in the north, and the extensive sandy basins of the eastern boundary, including the Hamun basin, Dasht-e-Lut, Dasht-e-Kavir, Dasht-e Namak, and Namak Lake, likely prevented the dispersion of species within the *M. watsonana* complex (Šmíd & Frynta, 2012). The Zagros Mountains played a significant role in isolating *M. watsonana* from other lineages, as these mountains were uplifted due to the collision of the Arabian lithospheric plate with the Eurasian landmass (Mouthereau, 2011).

Our study confirmed through SDIVA and BBM analyses that allopatric speciation played a crucial role in the emergence of new clades within the *M. watsonana* complex (Figure 4). During the Miocene, stable oxygen and carbon isotopes in northern Iran indicate three distinct climatic phases: (1) a period of increased aridity, particularly between 17.5 and 13.2 million years ago; (2) a phase of increased precipitation resulting in semi-arid conditions between 13.2 and 10.3 million years ago (Böhme et al., 2008; Van Dam, 2006); and (3) a subsequent decrease in aridity from approximately 9.6 to 7.6 million years ago, coinciding with similar climate changes across Asia and India (Ballato et al., 2010). These climatic events contributed to the formation of unique environmental conditions on the elevated Iranian plateau, leading to the isolation of populations in the highlands from those in the Mesopotamian lowlands and facilitating the specific differentiation of numerous reptile and amphibian species (Feldman & Parham, 2004; Hrbek et al., 2006; Rastegar-Pouyani et al., 2006; Wischuf & Fritz, 1996). Other species complexes, such as *Eremias persica* and *Phrynocephalus scutellatus*, also occur in the same region and have existed on the Iranian Plateau since the upper Miocene. Evidence from both species suggests that a combination of climatic changes and orogenic events on the plateau contributed to their diversification (Ahmadzadeh et al., 2017; Rahimian et al., 2015).

5 | CONCLUSION

The study showed, there are seven distinct clades within *M. watsonana* complex species. They include Kerman clade, Esfarayen clade, Halil clade, Ardestan clade, *M. watsonana* clade, Bardaskan clade, and Khuzestan clade. The results revealed that several distinct clades diverged in the area because of geological events when the Dasht-e-Kavir and the Zagros Mountains formed. It appears that the ancestral line of *M. watsonana* spread to Iran before the Zagros Mountains orogeny. As a result, orogeny activities in the Zagros Mountains coincidence climatic conditions and the formation of deserts contributed to the separation of these lineages from the Late Miocene. Taking into account our findings, each of the seven clades corresponding to distinct geographic regions should be elevated to species status.

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CONFLICT OF INTEREST STATEMENT

None.

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SUPPORTING INFORMATION

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