Wiley
Journal of Zoological Systematics and Evolutionary Research
Volume 2025, Article ID 4086871, 11 pages
https://doi.org/10.1155/jzs/4086871

WILEY

Research Article

Assessing Genetic Diversity and Habitat Suitability in Endemic Iranian *Darevskia* (Reptilia: Lacertidae) Under Climate Change

Seyyed Saeed Hosseinian Yousefkhani [1,2] **and Hossein Nabizadeh** [3]

[1]*Department of Animal Science, School of Biology, Damghan University, Damghan, Iran*
[2]*Institute of Biological Science, Damghan University, Damghan, Iran*
[3]*Department of Biology, Faculty of Sciences, University of Qom, Qom, Iran*

Correspondence should be addressed to Seyyed Saeed Hosseinian Yousefkhani; s.hosseinian@du.ac.ir

Received 6 June 2025; Revised 23 July 2025; Accepted 22 August 2025

Academic Editor: Savel Daniels

The Iranian Plateau, a biogeographic hotspot shaped by Pleistocene refugia and topographic complexity, hosts six endemic *Darevskia* lizard species (*D. caspica*, *D. defilippii*, *D. schaekeli*, *D. kamii*, *D. steineri*, and *D. kopetdaghica*), ideal for studying evolutionary responses to environmental changes. These species, characterized by cryptic diversity and narrow ecological niches, face threats from habitat loss and climate change. We integrated population genetics and ecological niche modeling (ENM) to assess their genetic diversity, population structure, and future habitat suitability. Using cytochrome b and *MC1R* gene sequences (1248 bp, 75 sequences), we found high haplotype diversity (e.g., *D. schaekeli*, Hd = 0.985) and evidence of historical population expansion (raggedness = 0.0052, $\tau = 34.204$), suggesting refugial persistence in the Hyrcanian forests and Alborz Mountains. *Darevskia steineri* showed low diversity and high gene flow indicating demographic stability. Phylogenetic analyses confirmed species distinctiveness but revealed intra-lineage structuring in the *Persiodarevskia* group. Maxent-based ENM, using WorldClim data, project significant habitat contractions and elevational shifts by 2070 across four climate scenarios, with *D. kopetdaghica* and *D. kamii* most vulnerable due to restricted ranges. Temperature extremes and precipitation seasonality emerged as key climate drivers. By combining genetic distinctiveness with ENM, we identify conservation priorities, emphasizing microrefugia preservation and range-edge monitoring. These findings underscores the role of historical biogeographic processes in shaping *Darevskia* diversity and highlight their vulnerability to climate-driven habitat loss, urging target conservation to safeguard their evolutionary legacy.

Keywords: cryptic diversity; habitat contraction; *Hyrcanodarevskia*; intralineage structuring; *Persiodarevskia*

1. Introduction

Understanding how biodiversity responds to environmental change is a central focus in evolutionary biology and conservation science [1]. Reptiles, particularly lizards, are highly sensitive to environmental fluctuations, making them valuable indicators of habitat health and climate-driven impacts on biodiversity [2]. The mountainous and ecologically diverse regions of the Iranian Plateau host a rich reptilian fauna, with several taxa exhibiting endemism and fine-scale habitat specialization. Among these, the genus *Darevskia* is of particular interest due to its restricted ranges, cryptic diversity, and sensitivity to climatic and anthropogenic pressures [3, 4].

The genus *Darevskia*, distributed across the Caucasus and Iranian Plateau, represents a unique model for studying biogeography, cryptic speciation, and climate-driven habitat shifts. In Iran, six endemic species including *D. caspica*, *D. defilippii*, *D. schaekeli*, *D. kami*, *D. steineri*, and *D. kopetdaghica*, inhabit fragile ecosystems like the Hyrcanian forests, where microhabitat specificity and anthropogenic pressures (e.g., tourism and infrastructure) threaten their survival [5, 6]. The six endemic Iranian species of the genus *Darevskia* exhibit subtle

morphological differences, making systematic identification challenging [4]. These lizards are generally small to medium-sized, with overlapping scalation patterns and coloration, though some species show distinct ecological preferences and microhabitat use. *D. defilippii* and its close relatives form a cryptic species complex (*Persiodarevskia* group), with genetic studies revealing several highly divergent clades despite their morphological similarity [4, 7]. *D. steineri* closely resembles *D. chlorogaster* in coloration and often shares forest habitats in northeastern Iran. *D. caspica* and *D. kopetdaghica* are restricted to specific regions, with the former inhabiting the Caspian coastal forests and the latter found in the Kopet Dagh Mountains. *D. kami* and *D. schaekeli* also display limited distributions and subtle morphological distinctions, such as differences in body proportions and scalation [8].

Despite their ecological and evolutionary significance, the population genetics and conservation status of Iranian *Darevskia* species remain poorly understood. Molecular data are essential for uncovering hidden diversity, resolving taxonomic ambiguities, and guiding conservation planning. Recent advances in genomic tools have allowed for more precise assessments of gene flow, population structure, historical demography, and key parameters in evaluating species resilience and adaptive potential in the face of environmental change.

Climate change is projected to significantly alter the distribution of suitable habitats for montane and forest-dwelling reptiles. Species with narrow ecological niches, such as the Iranian *Darevskia*, are particularly vulnerable to habitat loss, fragmentation, and elevational range shifts. These shifts may lead to genetic bottlenecks, reduced connectivity among populations, and increased extinction risk. Integrating population genetic data with climate modeling provides a powerful framework for assessing the future viability of these lizards under various climate scenarios [9, 10].

Given their endemic status, restricted distributions, and cryptic diversity, the six Iranian *Darevskia* species present an ideal system for investigating the interplay between evolutionary processes and environmental threats. This study aims to (1) assess the genetic diversity and population structure of these species using molecular markers; (2) evaluate the impacts of projected climate change on their potential habitat distributions; (3) identify conservation priorities based on genetic distinctiveness and vulnerability. By combining population genetics, bioclimatic modeling, and ecological context, we aim to contribute to the long-term conservation of this unique lizard fauna in Iran.

2. Materials and Methods

2.1. Study Area and Sampling. Field surveys were conducted across the known distribution ranges of the six endemic *Darevskia* species in Iran: *D. caspica*, *D. defilippii*, *D. schaekeli*, *D. kamii*, *D. steineri*, and *D. kopetdaghica*. Sampling sites covered diverse habitats including the Hyrcanian forests, the Alburz and Kopet Dagh mountain ranges, and isolated mid-elevation woodlands (Figure 1). Specimens were captured by hand during active periods (spring and summer, 2024–2025),

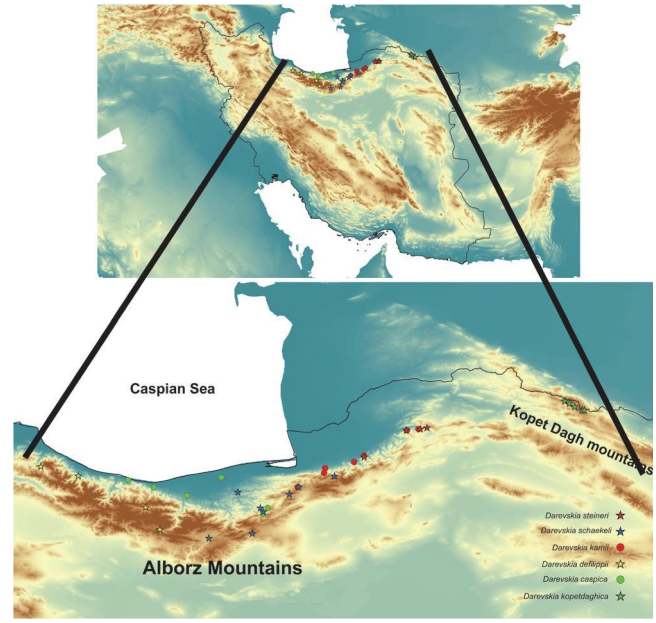

FIGURE 1: Iran and the study area in the present study. The distribution points of the six species are shown on the map with different symbols and have been visited.

TABLE 1: Estimated genetic diversity parameters of the partial mtDNA fragment (*cytb*) for six species of the genus *Darevskia* in Iran.

Species	N	H	Hd	Pi (π)
Darevskia caspica	12	10	0.970	0.0291
Darevskia kamii	6	6	1.000	0.0119
Darevskia kopetdaghica	4	3	0.833	0.0084
Darevskia steineri	13	5	0.756	0.0059
Darevskia schaekeli	20	14	0.953	0.0332
Darevskia defilippii	8	7	0.964	0.0187

and geographic coordinates were recorded using handheld GPS devices.

Tissue samples (tail clips) were collected from different populations and moved to the laboratory (Table S1). Samples were preserved in 96% ethanol and stored at –20°C until DNA extraction. All procedures followed ethical guidelines and were approved by relevant institutional and environmental authorities at Damghan University under permission number IR.DU.REC.1403.008.

2.2. DNA Extraction, Amplification, and Sequencing. Genomic DNA was extracted using a standard salt method protocol [11]. DNA quality was assessed via agarose gel electrophoresis and quantified with a Nanodrop spectrophotometer. A fragment of the cytochrome b gene (612 bp) was amplified using standard primers: L14724 (5'-GACCTGCGGTCCGAAAAACCA-3') and H16064 (5'-CTTTGGTTTACAAGAACAATGCTTTA-3') [12] and Ei700r (5'-GGGGTGAAAGGGGATTTTRTC-3') [13]. PCR conditions included an initial denaturation at 94°C (4 min), followed by 35 cycles of denaturation (94°C, 40 s), annealing (51–49°C, 45 s), and extension

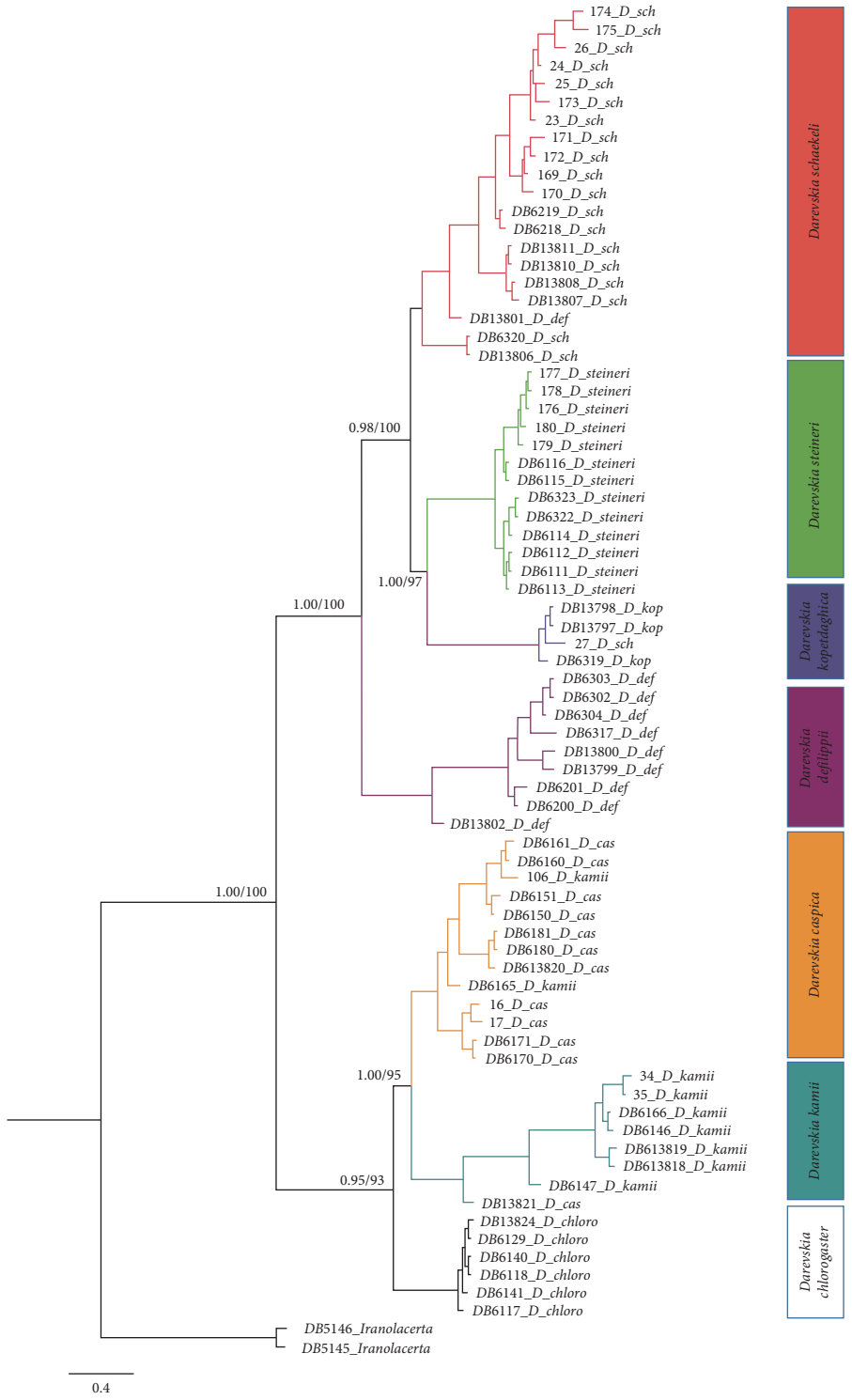

FIGURE 2: Molecular phylogenetic tree for the genus *Darevskia* that endemic in Iran based on mitochondrial and nuclear gene fragments (*cytb*, *MC1R*).

(72°C, 1 min), with a final extension at 72°C (8 min). Also, the gene fragment of Melanocortin 1 receptor (*MC1R;* 636 bp) was amplified using following primers: MC1R-F (5'-AGGCNGCCATYGTCAAGAACCGGAACC-3') and MC1R-R (5'-CTCCGRAAGGCRTAAATGATGGGGTCCAC-3') [14]. PCR products were purified and sequenced in forward direction using Sanger sequencing by Momgene Company, Tehran, Iran.

2.3. Molecular Phylogenetic and Population Genetic Analyses. To reconstruct the evolutionary relationships among the six endemic *Darevskia* species, phylogenetic analyses were

conducted using both Bayesian inference (BI) and maximum likelihood (ML) approaches. Sequence alignments of the mitochondrial cytochrome b gene were checked in Bioedit v. 7.0 [15]. The best-fitting model of nucleotide substitution was selected based on the Akaike Information Criterion (AIC) using ModelTest v3.7 [16], which identified the GTR + I + G model as optimal. Bayesian phylogenetic inference was performed in MrBayes v3.2.7 [17], with two independent runs of four Markov chain Monte Carlo (MCMC) chains each, run for 10 million generations and sampled every 1000 generations. The first 25% of trees set as burn-in. ML analysis was run by RaxML 7.4.2 [18] implemented in RaxMLGUI 1.3 [19] using the same substitution model, with nodal support assessed via 1000 bootstrap replicates [20].

Comparison mean genetic distances (uncorrected p-distances) among species and populations were calculated using MEGA X [21] to assess levels of divergence. Phylogenetic trees were visualized and edited in FigTree v1.4.4.

To evaluate genetic diversity and structure among populations of the six endemic *Darevskia* species, sequence data (*Cytb*) were analyzed using DnaSP V.5.0 [22] for each species, standard indices of genetic diversity—including the number of haplotypes (*H*), haplotype diversity (Hd), nucleotide diversity (π)—were calculated in DnaSP. The mismatch distribution analysis (Population size changes) were done using DnaSP. Additionally, haplotype networks were constructed in PopART v1.7 [23] using the TCS algorithm to visualize relationships among haplotypes and identify potential population structure or cryptic lineages.

2.4. Ecological Niche Modeling (ENM). Species distribution models (SDMs) were generated using Maxent v3.3.3e [24] to predict current and future habitat suitability under different climate scenarios. Occurrence records were compiled from field surveys and verified museum/locality data, cleaned for spatial autocorrelation using a 1 km^2 thinning threshold.

Nineteen bioclimatic variables were extracted from the WorldClim v2.1 database at 30 arc-second resolution. Highly correlated variables ($r > 0.75$) were excluded via pairwise Pearson's correlation analysis. Climate projection for 2070 was obtained under four representative concentration pathways (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5).

Model performance was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) plot. Habitat suitability maps were reclassified using threshold values that maximize training sensitivity and specificity.

To ensure ecological realism in our Maxent models, we defined the accessible area ("M") as the geographic region potentially accessible to *Darevskia* species based on its dispersal capabilities and biogeographic constraints. We delineated "M" by applying a 50 km buffer around all known occurrence points, reflecting the species' documented dispersal distance of approximately 20–70 km per generation [25]. Additionally, we excluded areas beyond major biogeographic barriers, such as the Zagros Mountains and Central Iranian Desert, which limit the species' range based on ecological studies [4]. This definition of "M" ensures that background points used in Maxent reflect environmentally relevant areas, improving the model's accuracy for both current and future climate scenarios

Table 2: Uncorrected genetic distance (%) between different species of the genus *Darevskia*.

Species name	1	2	3	4	5	6	7
D. schaekeli	—	0.3	1.4	1.4	0.5	0.2	1.2
D. kopetdaghica	8.7	—	1.5	1.6	0.5	0.3	1.4
D. caspica	13.3	13.3	—	0.1	1.2	1.3	0.4
D. kamii	14.2	14.9	8.8	—	1.2	1.3	0.5
D. steineri	6.6	8.9	14.1	15.1	—	0.5	1.0
D. defilippii	10.1	10.2	12.1	14.3	10.5	—	1.1
D. chlorogaster	14.4	14.2	6.3	8.5	14.0	13.6	

Note: The values above the matrix correspond to the *MC1R* gene and the values below the matrix correspond to the *Cytb* gene.

3. Results

3.1. Molecular Phylogenetic and Population Genetic Analyses. Tissue samples ($n = 22$) comprising tail tip specimens were collected from six species across their distribution ranges. To ensure comprehensive spatial coverage, field sampling sites were strategically selected through a dual approach: 1) critical evaluation of gaps in existing distribution data from published literature, and 2) analysis of specimen records in the Global Biodiversity Information Facility (GBIF). This integrative methodology allowed synergistic utilization of newly acquired samples with publicly available genetic data from the National Center for Biotechnology Information (NCBI) database. Another 51 sequences from different species of the genus *Darevskia* were obtained from the GenBank and used in this study (Table S1). Two other sequences from the genus *Iranolacerta* were also obtained from the GenBank and used as outgroups. Totally, we used 75 sequences in the molecular analyses.

The concatenated dataset consists of 1248 bp belong two gene fragments as: *cytb* (612 bp; variable sites (V) = 193, parsimony informative (Pi) = 200) and *MC1R* (636 bp; variable sites (V) = 28, parsimony informative (Pi) = 26). The *cytb* gene fragment shows the higher variability than the *MC1R* and *cytb* show high Hd (0.985). The separate nucleotide diversity of each species presented in Table 1. The diversity among populations of *D. schaekeli* is higher than other species and the *D. steineri* show the lowest diversity. Generally, the mismatch distribution analysis of 68 *Cyt b* sequences (612 bp) revealed a high level of genetic diversity, with an average of 60.399 pairwise differences (k), and 193 polymorphic sites. The low raggedness statistic ($r = 0.0052$) and moderate R2 statistic (0.1554) suggest a unimodal mismatch distribution, indicative of a historical population expansion. The estimated time since expansion ($\tau = 34.204$) points to a relatively ancient demographic event, though precise timing requires the species-specific mutation rate. The observed variance of k (746.5297) aligns closely with the expected variance under a no-recombination model (696.607), but not with free recombination (20.734), confirming negligible recombination, as expected for mitochondrial DNA. These findings collectively support a population that has undergone a significant

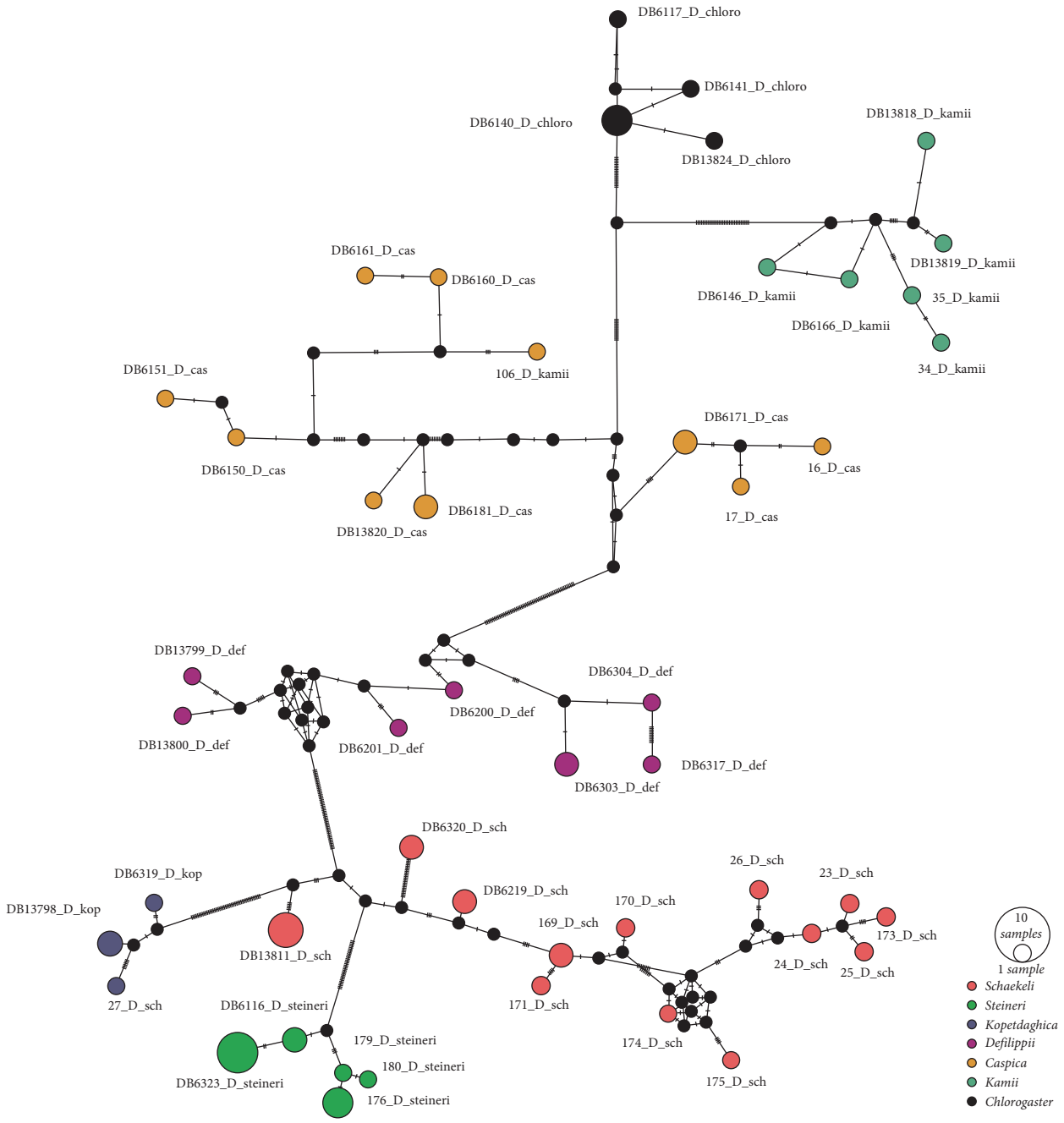

FIGURE 3: Haplotype network for *Darevskia* endemic species, generated with the median-Joining algorithm in Popart 1.7 using the *cytb* gene fragment. Circles indicate distinct haplotypes, their sizes reflecting haplotype frequency. Connecting lines represent mutational steps, revealing genetic relationships, and population structure.

historical expansion, with high genetic diversity and no evidence of a stable, constant population size.

A molecular phylogenetic tree was drawn for six endemic species of the genus *Darevskia* in Iran, in which all described species retained their stipules (Figure 2). However, some species that were less studied showed significant diversity. For example, in the *Persiodarevskia* group, the species *D. schaekeli* has a high diversity in its populations and is located in the last branch of the tree. The species *D. steineri*, as another member of this group, represents two separate groups known as the eastern clade (Tangrah and Loveh) and the western clade (Shirabad). However, the genetic diversity and differences between these two populations are very low and gene flow is high (Table 2). However, one of the findings of this study is the westernmost record of the presence of this species in the Shirabad region. *D. kopetdaghica* is known as the easternmost species of this genus in the world, which is located in the north of North Khorasan and Razavi Khorasan provinces and has two known populations. Other endemic species including *D. caspica*, *D. kami*, and *D. defilippii* show acceptable genetic

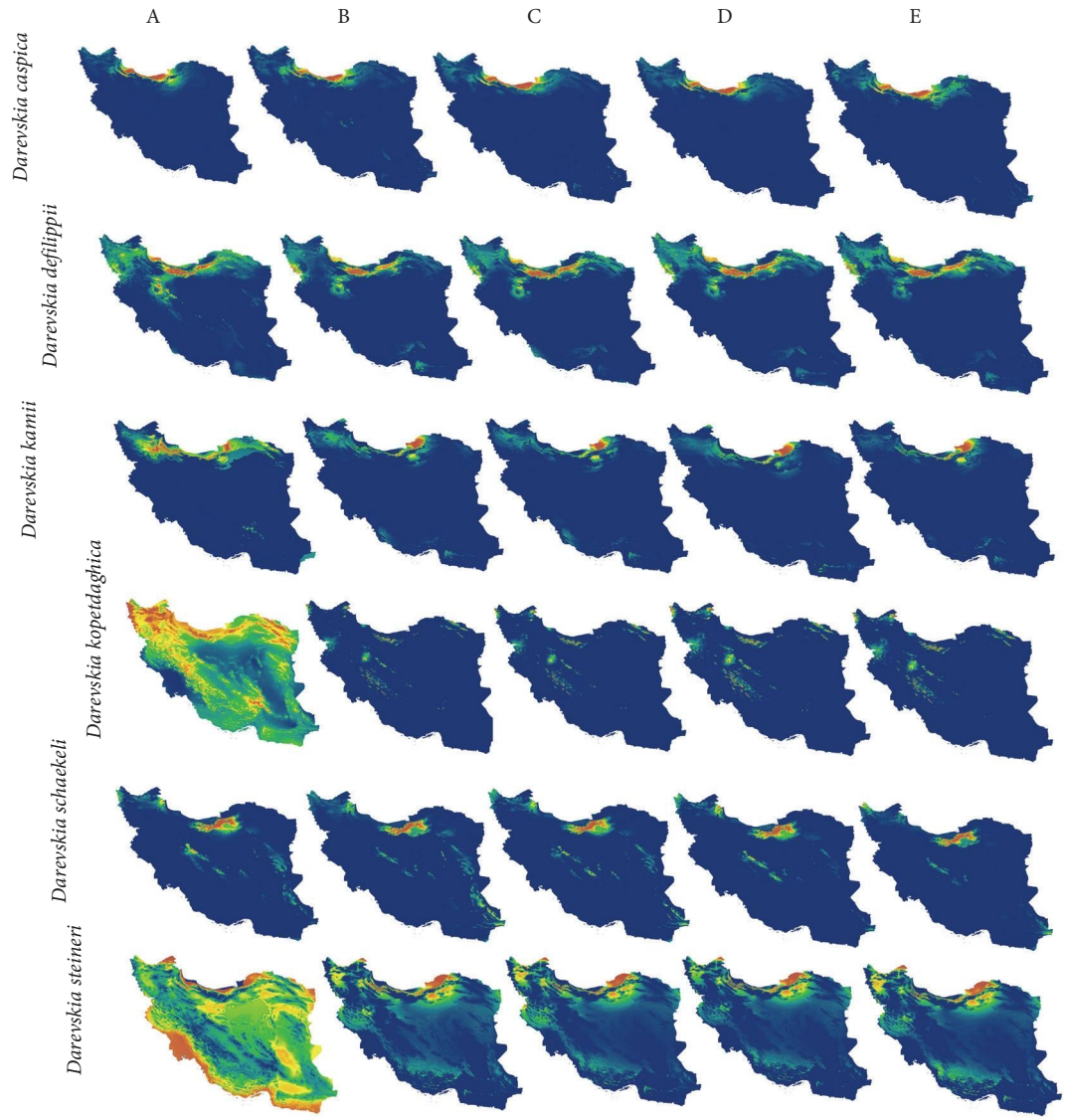

Figure 4: Distribution potential of *Darevskia* species in Iran (A) present and future 2070 scenario 2.6 (B), scenario 4.5 (C), scenario 6.0 (D), and scenario 8.5 (E).

diversity within their populations. According to the haplotype network drawn, it clearly shows that Hd between populations and species under study is very high (Figure 3).

3.2. ENM. In this study, using the maximum entropy (maxent) method, five different models were performed in the present and future time periods (2070 with four different scenarios; Figure 4), and all models were performed in 10 replicates with the crossvalidation method. As can be seen in Figure 4, the distribution status of species of the genus *Darevskia* native to Iran changes in different time periods. Also, a table of the contribution of climatic layers to the presence of species is presented (Tables 3 and 4). The AUC value was obtained for all models with a relatively good range, which is summarized in Tables 3 and 4 (Figure 4). The most important climatic layers and their most influential for each time period are shown in bold in Tables 3 and 4.

According to the results, in the current period, BIO18 is (precipitation rate in the warmest season of the year), BIO 16 (precipitation rate in the wettest season of the year), BIO5 (maximum temperature in the warmest month of the year), BIO14 (precipitation rate in the driest month of the year), BIO11 (average temperature in the coldest season of the year), and BIO15 (standard deviation in seasonal precipitation) for the species *D. steineri*, *D. schaekeli*, *D. kopetdaghica*, *D. kamii*, *D. defilippii*, and *D. caspica*, respectively. In future period (2070), the BIO15 (standard deviation in seasonal precipitation), BIO3 (isothermality), and BIO5 (maximum temperature in the warmest month of the year); BIO14 (precipitation rate in the driest month of the year), BIO7 (annual average temperature), and BIO5 (maximum temperature in the warmest month of the year); BIO9 (average temperature in the driest season of the year) and BIO16 (precipitation rate in the wettest season of the year); BIO6 (minimum

Table 3: Layer contribution for all species in current period.

Bioclimatic layer	*Darevskia caspica*	*Darevskia defilippii*	*Darevskia kamii*	*Darevskia kopetdaghica*	*Darevskia schaekeli*	*Darevskia steineri*
BIO1	—	—	—	—	—	—
BIO2	—	1.3	—	—	—	—
BIO3	5	22.4	14.8	—	—	—
BIO5	2	4.9	—	**93.2**	28.6	—
BIO6	—	—	—	—	—	4
BIO7	—	—	2.2	—	—	—
BIO8	25.1	—	1	—	—	—
BIO9	—	—	—	—	0.4	—
BIO10	7.4	—	—	—	—	—
BIO11	—	**43**	—	—	—	6.6
BIO12	—	—	—	6.8	—	11.9
BIO14	—	—	**82**	—	—	—
BIO15	**60.5**	1.7	—	—	22.2	—
BIO16	—	26.7	—	—	**48.8**	—
BIO17	—	—	—	—	—	—
BIO18	—	—	—	—	—	**77.6**
AUC	0.968	0.917	0.979	0.977	0.978	0.817

Note: Bold values refer to the high contributed layer.

temperature in the coldest month of the year) were identified as the most effective climate variables for the species *D. caspica*, *D. defilippii*, *D. kamii*, *D. kopetdaghica*, *D. schaekeli*, and *D. steineri*, respectively (Tables 3 and 4).

SDMs project significant alterations in the potential ranges of *Darevskia* species within the next five decades (2070). For instance, the suitable habitat for *D. caspica* is anticipated to contract, shifting from the eastern and southern Caspian Sea regions toward the Alborz slopes and narrowing closer to the Caspian coastline (Figure 4). Similarly, *D. defilippii*, which is currently confined to the central and eastern Alborz and highland areas in the western Caspian region, is expected to lose substantial portions of its favorable habitat in both the eastern and western central Alborz under future scenarios.

The distribution of *D. schaekeli* has already excluded the eastern Alborz from its viable range, with current models (Figure 4) reflecting only theoretical projections rather than observed distributions. *D. kamii* is predicted to experience a marked reduction in its geographical extent, with future suitable areas restricted mainly to the eastern Gorgan Gulf and certain valleys within the Hyrcanian forests of southern Golestan and southeastern Mazandaran provinces (Figure 4).

Modeling for *D. kopetdaghica* presently identifies many mountainous regions across Iran as suitable, despite the species' actual distribution being limited to northeastern Iran and the Turkmenistan border (Figure 4). Projections across all scenarios indicate a dramatic contraction of its range, rendering the species highly threatened due to its confinement to a small area in the northeast.

The current distribution of *D. schaekeli* is already limited to the highlands of eastern Alborz, constrained by local water flows and springs (Figure 4). Future projections suggest an even greater restriction, with suitable habitats shifting to higher elevations under all modeled scenarios. Finally, *D. steineri*, like to *D. kopetdaghica*, is expected to undergo a pronounced reduction in suitable habitat (Figure 4), becoming confined to the forested regions of eastern Hyrcania and Golestan Province, where it is still observed today.

4. Discussion

This study provides one of the most comprehensive molecular and ecological assessments of the six endemic *Darevskia* species in Iran, offering valuable insights into their genetic diversity, evolutionary relationships, and potential vulnerability to climate change. The high haplotype and nucleotide diversity detected in most species, particularly *D. schaekeli*, highlights previously unrecognized intraspecific variation, suggesting the presence of cryptic lineages within some taxa [8]. Conversely, the low diversity and high gene flow observed in *D. steineri* indicate potential demographic stability across its fragmented populations, albeit within a narrow ecological range [4, 26].

Phylogenetic analyses confirmed the distinctiveness of all six species, yet also exposed substantial intra-lineage structuring, especially in *D. steineri* and the *Persiodarevskia* group, supporting previous findings of complex evolutionary histories within the genus [4, 7]. The discovery of new distributional records, such as the westernmost population of *D. steineri* in Shirabad, underscores the need for continued field exploration to refine range boundaries and conservation priorities [27].

Ecological niche models revealed marked shifts in future habitat suitability under all climate scenarios, with projected contractions and elevational displacements for all species [28, 29]. Particularly at risk are *D. kopetdaghica* and *D. kamii*, whose already narrow distributions [4, 30] are expected to diminish further, likely due to increased temperature extremes and precipitation variability. These changes could exacerbate population fragmentation, reduce gene flow, and heighten

Table 4: Layer contribution for all species in future period (2070) under four scenarios.

	Darevskia caspica				*Darevskia defilippii*				*Darevskia kamii*				*Darevskia kopetdaghica*				*Darevskia schaekeli*				*Darevskia steineri*			
Bioclimatic layer	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5	RCP 2.6	RCP 4.5	RCP 6.0	RCP 8.5
BIO1	14.2	10.9	2.4	12.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BIO2	—	—	—	—	4.2	5.2	7.7	6.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BIO3	9.1	7.6	9.8	9.6	**37.3**	**39.8**	33.9	33.8	5.1	6.2	4.6	7.3	—	—	—	—	—	—	—	—	—	—	—	—
BIO5	4.4	9.5	2.3	6.5	33.1	34.4	**36.7**	**36.1**	—	—	—	—	39.2	**44.6**	**45.7**	**45.5**	18.3	15.4	28.5	18.2	—	—	—	—
BIO6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**41.2**	**40.5**	**43.1**	**43.8**
BIO7	—	—	—	—	—	—	—	—	24.1	24.7	24.1	25.0	**44.9**	41.4	41.4	40.5	—	—	—	—	—	—	—	—
BIO8	0.5	0.8	5.9	0.8	—	—	—	—	8.1	9.1	4.1	7.0	—	—	—	—	—	—	—	—	—	—	—	—
BIO9	1.0	2.0	0	5.2	—	—	—	—	—	—	—	—	—	—	—	—	26.3	**33.5**	12.9	19.1	—	—	—	—
BIO10	5.8	3.9	4.5	1.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BIO11	—	—	—	—	2.1	2.1	2.5	2.4	—	—	—	—	—	—	—	—	—	—	—	—	19.2	14.0	20.2	25.0
BIO12	—	—	—	—	—	—	—	—	—	—	—	—	4.7	3.4	2.6	3.1	—	—	—	—	10.6	14.8	4.7	5.3
BIO14	—	—	—	—	—	—	—	—	**62.7**	**60.0**	**67.3**	**60.6**	—	—	—	—	—	—	—	—	—	—	—	—
BIO15	**65.0**	**65.3**	**75.0**	**63.2**	9.1	7.6	5.1	8.1	—	—	—	—	—	—	—	—	21.9	20.9	23.6	27.2	—	—	—	—
BIO16	—	—	—	—	14.1	10.9	14.2	13	—	—	—	—	—	—	—	—	**33.5**	30.2	**35.1**	**35.5**	—	—	—	—
BIO17	—	—	—	—	—	—	—	—	—	—	—	—	11.2	10.6	10.3	10.9	—	—	—	—	—	—	—	—
BIO18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29.0	30.7	32.0	26.0
AUC	0.970	0.964	0.964	0.971	0.946	0.951	0.947	0.948	0.957	0.964	0.971	0.982	0.997	0.997	0.995	0.996	0.953	0.957	0.957	0.955	0.923	0.926	0.931	0.934

Note: Bold values refer to the high contributed layer.

extinction risk, especially in montane specialists with low dispersal capacity [31].

Key climatic variables—such as BIO5 (maximum temperature of warmest month) and BIO15 (precipitation seasonality)—were consistently influential, reflecting the lizards' sensitivity to thermal and hydric regimes [32]. The shift in dominant climatic predictors between current and future models suggests altered ecological pressures that may challenge species' adaptive thresholds.

Integrating genetic data with ENM has enabled the identification of both evolutionarily distinct and environmentally vulnerable populations, providing a basis for prioritizing conservation actions. Management strategies should emphasize habitat preservation in climatically stable microrefugia and enhance monitoring in range-edge populations [33–35]. Given their ecological specificity, high endemism, and projected habitat loss, Iranian *Darevskia* lizards warrant urgent conservation focus, including the incorporation of genetic units into protected area planning and climate adaptation frameworks.

4.1. Biogeographic Context of Iranian Darevskia Species. The Iranian Plateau, encompassing the Hyrcanian forests, Alborz, and Kopet Dagh mountains, represents a critical biogeographic region for understanding the evolutionary history of the endemic *Darevskia* species. This region, part of the broader Near East, has served as a hotspot for diversification and refugial persistence during the Pleistocene, shaping the genetic and distributional patterns of numerous taxa, including reptiles, birds, and mammals. The high genetic diversity and cryptic lineage structuring observed in *Darevskia*, particularly in species like *D. schaekeli* and *D. steineri*, align with patterns of mitochondrial differentiation documented in other regional species, such as the green woodpecker complex (*Picus viridis*) and great spotted woodpecker (*Dendrocopos major*) [36–38]. These studies highlight the role of the Hyrcanian forests and adjacent montane systems as Pleistocene refugia, where populations persisted through climatic oscillations, fostering genetic divergence and speciation.

The Hyrcanian forests, a temperate relict ecosystem along the Caspian Sea, have been a stable refuge since the Pliocene, maintaining mesic conditions that supported isolated populations during glacial maxima [39]. For *Darevskia*, this refugial stability likely facilitated the persistence of divergent lineages, as seen in the high Hd and unimodal mismatch distribution indicative of historical population expansions (e.g., *D. schaekeli*). The Alborz and Kopet Dagh Mountains further contributed to diversification by acting as barriers to gene flow, promoting allopatric divergence among populations of *D. caspica*, *D. kopetdaghica*, and others. Similar patterns are observed in the rock nuthatch (*Sitta tephronota*), where mitochondrial differentiation reflects isolation in montane refugia across the Iranian Plateau [39]. The complex topography of the region, coupled with climatic fluctuations, likely drove cycles of fragmentation and secondary contact, as evidenced by the distinct eastern and western clades of *D. steineri* and the high intraspecific diversity in the *Persiodarevskia* group.

Pleistocene climatic oscillations also influenced range dynamics, with refugial populations in the Hyrcanian forests and Alborz expanding during interglacial periods, as suggested by the ancient demographic expansion ($\tau = 34.204$) inferred from cytochrome b data. Comparative studies on woodpeckers indicate that such expansions across the Near East were common, with postglacial recolonization shaping current distributions [36, 38]. For *D. kopetdaghica*, restricted to the Kopet Dagh, limited dispersal and habitat specificity mirror patterns in other montane specialists, where isolated ranges amplify genetic drift and divergence [37]. These biogeographic processes underscore the vulnerability of *Darevskia* species to ongoing climate change, as projected habitat contractions in our ecological niche models align with reduced refugial stability under warming scenarios. By integrating these regional evolutionary insights, our study contextualizes the genetic and ecological patterns of *Darevskia* within the broader biogeographic framework of the Iranian Plateau, emphasizing the interplay of historical refugia, topographic barriers, and climatic shifts in shaping their diversity and conservation needs.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information section of this article.

Ethics Statement

The research sampling was conducted following ethical guidelines under the Institutional Animal Care and Use Committee (IACUC) Number IR.DU.REC. 1403.008 from the Damghan University.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

The study conception and design were done by Seyyed Saeed Hosseinian Yousefkhani. Sample collection was performed by Seyyed Saeed Hosseinian Yousefkhani and Hossein Nabizadeh. Data collection and analysis were performed by Seyyed Saeed Hosseinian Yousefkhani. The first draft of the manuscript was written by Seyyed Saeed Hosseinian Yousefkhani and Hossein Nabizadeh.

Funding

This work is based upon research funded by Iran National Science Foundation (INSF) (Grant 4029229).

Acknowledgments

We thank the Damghan University authority for supporting lab work and instruments in the School of Biology.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)
Table S1. Details of samples of six endemic species of the genus *Darevskia* in Iran, along with outgroup sequences of

Iranolacerta brandtii obtained from GenBank, including their respective GenBank accession numbers for the Cytb and MC1R gene fragments.

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