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## Inferring the effects of past climate fluctuations on the distribution pattern of *Iranolacerta* (Reptilia, Lacertidae): Evidence from mitochondrial DNA and species distribution models

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### ABSTRACT

A variety of different methods are available for investigating the effects of past climate fluctuation on biota. Among them, molecular phylogeography and species distribution modeling approaches have been shown to be especially useful tools to trace past climate induced modifications of species' geographic distributions. The results of both analytical approaches are here combined to better understand the influences of past climate changes on the fragmented distribution pattern of the lizard genus *Iranolacerta* that currently occurs in the northwest and central Zagros Mountains of Iran. While *Iranolacerta zagrosica* is restricted to few localities and cannot be modeled, the potential distribution of *Iranolacerta brandtii* suggested a large region of unfavorable habitat between northwest and the central Zagros Mountains populations. From the phylogenetic perspective, both species display deep genetic separation likely pre-dating the Pleistocene. However, limited genetic diversity across this divided range of *I. brandtii* implies that the current separation is recent. Deeper genetic divergences were, nevertheless, uncovered within the northwest population. Since no recent geological events explain these results, the most likely explanation for such a distribution pattern is due to past climate fluctuations. Both sources of evidence suggest that during the early Holocene the northwest and Zagros populations of *I. brandtii* were connected, and the current pattern was shaped with their disconnection in the early Holocene. Further studies may identify potential glacial refugia for other species in this biodiverse region.

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### 1. Introduction

During the Pleistocene, global temperatures underwent extreme fluctuations producing progressively larger ice sheets across Eurasia and North America (Webb and Bartlein, 1992). In the northern latitudes, glaciations, or periods with low temperatures left distinctive marks on the genetic diversity patterns of many taxa and have shaped their current genetic structures (Avise, 2000; Hewitt, 2000). Similarly, repeated cycles of range restriction to refugia during glacial periods and outward expansion during interglacials have shaped the current distribution pattern of many plant and animal species in Europe (Hewitt, 1999; Lesbarreres, 2009). On the other hand, currently arid regions such as much of

North Africa and the Middle East experienced an alteration of dry and humid periods, which would have allowed lower or greater connectivity than currently for many mesic species (Schuster et al., 2006). The most recent glaciation, termed the Riss-Würm, reached its maximum about 18,000–21,000 years ago and in the Sahara and Middle East corresponded to a period of higher aridity than present (Ehlers and Gibbard, 2004). In contrast, after the end of the glaciations, conditions in this region during the Holocene Climatic Optimum (HGO) 9000–5000 years ago were much more humid than present (Kaufman et al., 2004).

Different approaches, comprising molecular phylogeography and species distribution modeling techniques (SDMs), are available to investigate the effects of such massive, often rapid changes in climatic conditions on species distributions. With the development of molecular methods it is now possible to investigate the geographical variation using multiple markers, and to deduce intraspecific phylogeographic structures (Taberlet et al., 1998; Avise, 2000). On the other hand, SDMs are now widely used to assess the potential distribution of species (Guisan and Thuiller, 2005; Jeschke and

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Strayer, 2008). Using SDMs, it can be assessed how a set of environmental factors influences the distribution patterns of a species. The models are based on the ecological niche concept, which assumes that the environmental (i.e. the Grinnellian) niche of a species determines its large scale distribution, which is ultimately determined through its fundamental niche breadth and position. The latter is defined as the range of biotic and abiotic conditions in a multidimensional space, which allows the species to persist without immigration (Hutchinson, 1957; Soberón and Peterson, 2005; Soberón, 2007; Sillero, 2011). In practice, information derived from a number of environmental parameters is combined with known locations of presences and a model is fitted to describe the requirements of the target species (Anderson et al., 2003). A special case of SDMs are climate envelope models (CEMs) which focus only on the climatic niche of the species (Elith and Leathwick, 2009). SDMs are widely used to predict the potential distribution of rare species (Peterson and Vieglais, 2001; Pearson and Dawson, 2003; Pearson et al., 2007; Jiménez-Valverde et al., 2011).

Comparisons of the independent results of SDMs and molecular phylogeographic analyses extends the range of evolutionary biology studies even further by assessing geographic patterns of ecological and genetic variation in evolutionary processes (Cushman et al., 2006; Knouft et al., 2006; Chan et al., 2011). Comparing results from phylogeographic and SDM methods that utilize a heterogeneous set of quantitative and qualitative data obtained from a wide variety of sources with differing data types improves our understanding of evolutionary processes. In fact, it allows comparisons among results derived from independent, but complimentary methods. If they are coherent, the evolutionary hypotheses are then better supported (Kidd and Ritchie, 2006; Rödder et al., 2010).

The three mountain ranges, the Azerbaijan in the northwest, the Alborz in the north and the Zagros in the west of Iran are well-known barriers for the distribution of lacertid lizards, tribe Lacertini, to the west (Anderson, 1999; Arnold et al., 2007; Ahmadzadeh et al., 2008). In this area and neighbouring regions as well, the species of the small Lacertini often display fragmented ranges (Arnold, 1973; Nilson et al., 2003; Pavlicev and Mayer, 2009). The fragmented distribution pattern of *Iranolacerta* species offers an interesting model that can be examined with such integrated approaches. *Iranolacerta* is a small genus comprising *Iranolacerta brandtii* De Filippi, 1863 and *Iranolacerta zagrosica* (Rastegar-Pouyani and Nilson, 1998), both of which are native Iranian species with a limited distribution (Anderson, 1999; Arnold et al., 2007; Ahmadzadeh et al., 2008). *I. brandtii* is present in northwestern Iran in Azerbaijan Provinces and discontinuously in Esfahan Province. Two subspecies have been described, the nominate subspecies and *I. b. esfahanica* that was reported by Nilson et al. (2003) from the Zagros Mountains in the Esfahan province of Iran, although there are also doubtful records in the Caucasus (In den Bosch, 1996; Anderson, 1999; Nilson et al., 2003; Rezazadeh et al., 2010). *Iranolacerta zagrosica* is known only from around the type locality also in the Zagros Mountains (Rastegar-Pouyani and Nilson, 1998). The habitat type is rocky and vertical slopes. It lives in sympatry but not syntopic with *I. b. esfahanica* (Rastegar-Pouyani and Nilson, 1998; Anderson, 1999; Nilson et al., 2003; Arnold et al., 2007).

The influences of past climate fluctuations on the geographical distribution of the genetic diversity in this area is still poorly known. Therefore, in this study, we investigated the evolutionary history of *I. brandtii* and *I. zagrosica* in the light of past climate change, by linking the independent results of two different analytical approaches, i.e. SDMs and phylogeography. Specific questions were: (1) What shaped the current biogeographic pattern? (2) Which is the level of genetic variability between the two species and the two subspecies of *I. brandtii*?, and (3) Can this group be used as a model

to examine the influences of the Northwestern and Zagros mountains on biogeographical patterns in the light of climate change and post-glacial events?

## 2. Materials and methods

### 2.1. Specimens and species records

Specimens used in this study and respective locations are listed in Table 1. A comprehensive sampling was conducted across the whole distribution areas of *I. brandtii* and *I. zagrosica* from the Azerbaijan provinces and the central Zagros Mountains in Iran (Fig. 1). The samples are classified in three main groups; (i) Esfahan–Central Zagros including four *I. zagrosica* and two *I. b. esfahanica* specimens, (ii) Azerbaijan–Tabriz comprising the specimens from Sahand Mountains and Verzeghan and (iii) Azerbaijan–Ardabil, with additional samples from the North. All specimens from (ii) and (iii) areas are considered *I. b. brandtii*. All known geographic records of *I. brandtii* ( $n = 10$ ) were used for SDM development (see below).

### 2.2. Phylogenetic analyses

Total genomic DNA from each specimen was extracted using standard saline methods (Sambrook et al., 1989). Polymerase chain reaction (PCR) amplifications were performed for fragments of three different mitochondrial DNA (cytochrome *b*, 16S and 12S ribosomal RNA) genes. The amplification and sequencing of three mtDNA gene fragments, was performed using the primers Cytb1/cb2R, 16SL/16SH, and 12Sa/12Sb (Kocher et al., 1989; Hedges et al., 1991). PCR reactions were carried out in a 25  $\mu$ l total volume, containing 2.5  $\mu$ l reaction Buffer, 1.5 mM of  $MgCl_2$ , 0.5 mM each

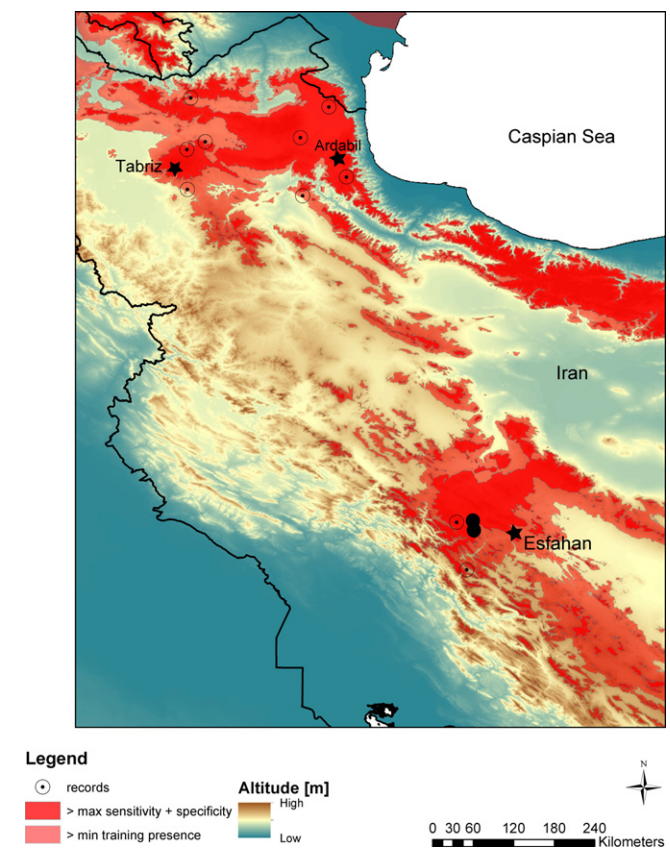


Fig. 1. The map showing the sampling sites for specimens sequenced in this study (dots: *I. brandtii*; black circles: *I. zagrosica*) and also the current estimated potential distribution of *I. brandtii* as estimated by Maxent models.

**Table 1**

List of samples analyzed, sampling localities, origin of the samples and GenBank accession numbers for the samples included in this study.

Species	Code	Locality	Reference	GenBank accession numbers (12S, 16S, cyt b.)		
<i>I. brandtii</i>	DB5144	Iran, Esfahan, Central Zagros	This study	JX082203	JX082221	JX082239
<i>I. brandtii</i>	DB5145	Iran, Ardabil, Samian	This study	JX082204	JX082222	JX082240
<i>I. brandtii</i>	DB5146	Iran, Ardabil, Samian	This study	JX082205	JX082223	JX082241
<i>I. brandtii</i>	DB5147	Iran, Ardabil, Samian	This study	JX082206	JX082224	JX082242
<i>I. brandtii</i>	DB6098	Iran, Tabriz, Sahand	This study	JX082207	JX082225	JX082243
<i>I. brandtii</i>	DB6099	Iran, Tabriz, Varzeghan	This study	JX082208	JX082226	JX082244
<i>I. brandtii</i>	DB6100	Iran, Tabriz, Varzeghan	This study	JX082209	JX082227	JX082245
<i>I. brandtii</i>	DB6101	Iran, Tabriz, Varzeghan	This study	JX082210	JX082228	JX082246
<i>I. brandtii</i>	DB6102	Iran, Tabriz, Varzeghan	This study	JX082211	JX082229	JX082247
<i>I. brandtii</i>	DB6103	Iran, Tabriz, Varzeghan	This study	JX082212	JX082230	JX082248
<i>I. brandtii</i>	DB6104	Iran, Tabriz, Varzeghan	This study	JX082213	JX082231	JX082249
<i>I. brandtii</i>	DB6108	Iran, Ardabil, Samian	This study	JX082214	JX082232	JX082250
<i>I. brandtii</i>	DB6109	Iran, Ardabil, Razey	This study	JX082215	JX082233	JX082251
<i>I. brandtii</i>	DB6110	Iran, Ardabil, Talesh	This study	JX082216	JX082234	JX082252
<i>I. brandtii</i>	<i>l.b.e-K</i>	Iran, Esfahan, Central Zagros	Harris et al. (1998)	AF080319	AF080321	AF080320
<i>I. brandtii</i>	<i>l.b.b-p</i>	Iran, Tabriz, Sahand	Pavlicev and Mayer (2009)	GQ142084	GQ142107	DQ461765
<i>I. zagrosica</i>	DB6105	Iran, Esfahan, Central Zagros	This study	JX082217	JX082235	JX082253
<i>I. zagrosica</i>	DB6106	Iran, Esfahan, Central Zagros	This study	JX082218	JX082236	JX082254
<i>I. zagrosica</i>	DB6107	Iran, Esfahan, Central Zagros	This study	JX082219	JX082237	JX082255
<i>I. zagrosica</i>	DB5143	Iran, Esfahan, Central Zagros	This study	JX082220	JX082238	JX082256

dNTP, 0.5 mM each primer, 0.1 U Taq DNA polymerase and approximately 100 ng of template DNA with the following conditions: an initial cycle of 92° for 2 min, followed by 35 cycles of 92 °C for 30 s, 50° for 40 s and 72° for 45 s, and a final cycle of 72° for 5 min. All amplified fragments were sequenced by a commercial company (Macrogen®, Korea).

Sequences were imported to BioEdit (Hall, 1999) where they were first aligned using ClustalW with default parameters and then adjusted by hand. Sequences from all fragments were concatenated giving a total alignment of 1018 bp. Conflict between partitions was assessed using the qualitative evaluation of Wiens (1998). Since there was no strong conflict between gene regions, concatenated analyses were performed. From GenBank, two previously published sets of the same genes from *I. brandtii* (Harris et al., 1998; Pavlicev and Mayer, 2009) were included in our analysis (Table 1). New sequences have been submitted to GenBank (accession numbers XXXX to XXXX – to be added upon acceptance). Following Arnold et al. (2007) and Pavlicev and Mayer (2009), *Anatololacerta danfordi* was included as an outgroup in the phylogenetic analyses.

Bayesian analysis was implemented using Mr. Bayes v.3.1 (Huelsenbeck and Bollback, 2001) with parameters estimated as part of the analysis. The analysis was run for  $1 \times 10^6$  generations, saving one tree in each 100 generations. The log likelihood values of the sample points were plotted against the generation time, and all the trees prior to reaching stationarity (10%) were discarded, ensuring that burn-in samples were not retained. Remaining trees were combined in a 50% majority consensus tree. MP and ML analyses were carried out in PAUP 4.0b10 (Swofford, 2002). MP analysis was carried out using heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 100 replicates. Bootstrap support (Felsenstein, 1985) was estimated for MP and ML with 1000 and 100 replicates, respectively. The appropriate models of evolution were determined through jModeltest (Posada, 2008). Additionally, concatenated sequences without the outgroup were assessed with haplotype networks constructed using statistical parsimony (Templeton et al., 1992) implemented in the program TCSv 1.21 (Clement et al., 2000) to better visualize the distribution of the different haplotypes across the area.

Divergence times were estimated using a relaxed molecular clock approach with the program BEAST 1.5.3 (Drummond and Rambaut, 2007). Following other studies of lacertids (e.g. Ahmadzadeh et al., 2012; Paulo et al., 2008) a mutation rate of 2% for cytochrome *b* was used. The analysis was run for 10 million

generations, sampling every 1000, with the first 10% discarded as burn-in.

### 2.3. Climate data and variable selection

*I. brandtii* has been recorded between 1000 and 3200 m a.s.l. in Iran. The species occurs within gullies and on slopes and hill-sides, in overgrazed steppe and shrub land habitat on volcanic tuff, or amongst boulders (Anderson, 1999; Rezazadeh et al., 2010). Because *I. zagrosica* is only known from the type locality and one other locality very close to this, SDMs could not be computed for this species. All records were mapped using DIVA-GIS 7.4 (Hijmans et al., 2005a, available through <http://www.diva-gis.org>) for visual inspection, and coordinates were checked to spot possible errors. We obtained climate data from WorldClim, v.1.4 (Hijmans et al., 2005b, <http://www.worldclim.org>) with a grid cell resolution of 30 arc s, which represents monthly precipitation and temperature data as averages of the period 1950–2000. Data from a global network of weather stations were interpolated for the whole land surface of the globe with latitude, longitude and elevation as independent variables (Hijmans et al., 2005a). Monthly temperature and precipitation data were summarized into 19 so-called bioclimatic variables (Busby, 1991; Beaumont et al., 2005). In order to reduce possible negative effects of multicollinearity of predictors (Heikkinen et al., 2006), we selected a subset of the 19 bioclimatic variables based on the species ecological requirements, and a pair-wise correlation matrix using squared Pearson's *r* scores. For SDM computation, we selected only seven variables with  $R^2 < 0.75$  (BIO1 = annual mean temperature; BIO4 = temperature seasonality; BIO5 = max temperature of warmest month; BIO7 = temperature annual range; BIO8 = mean temperature of wettest quarter; BIO12 = annual precipitation; BIO15 = precipitation seasonality) as predictor variables.

### 2.4. Species distribution modeling

MAXENT v.3.3.3a (Phillips et al., 2004, 2006, 2009, available through <http://www.cs.princeton.edu/schapiro/maxent/>) was used to assess the potential distribution of *I. brandtii*. Maxent is a grid-based machine-learning algorithm following the principles of maximum entropy (Jaynes, 1957; Phillips et al., 2004), which derives the potential distribution of a species from presence information compared to a randomly selected set of pseudo-absences.

In the past, it has frequently outperformed other competing algorithms (Elith et al., 2006; Heikkinen et al., 2006), in particular, when the number of available species records is limited (Hernandez et al., 2006; Wisz et al., 2008), as in the case of *I. brandtii*. In this situation, the potential distribution suggested by the SDM can be interpreted as a summary of what is currently known about the species' ecological tolerance. The resulting maps can be used as guidance for further field surveys, but do not necessarily represent a comprehensive assessment of the species' niche (Pearson et al., 2007). Hence, given the intrinsically limited number of known populations of *I. brandtii*, we prefer to avoid projections of the SDM through time.

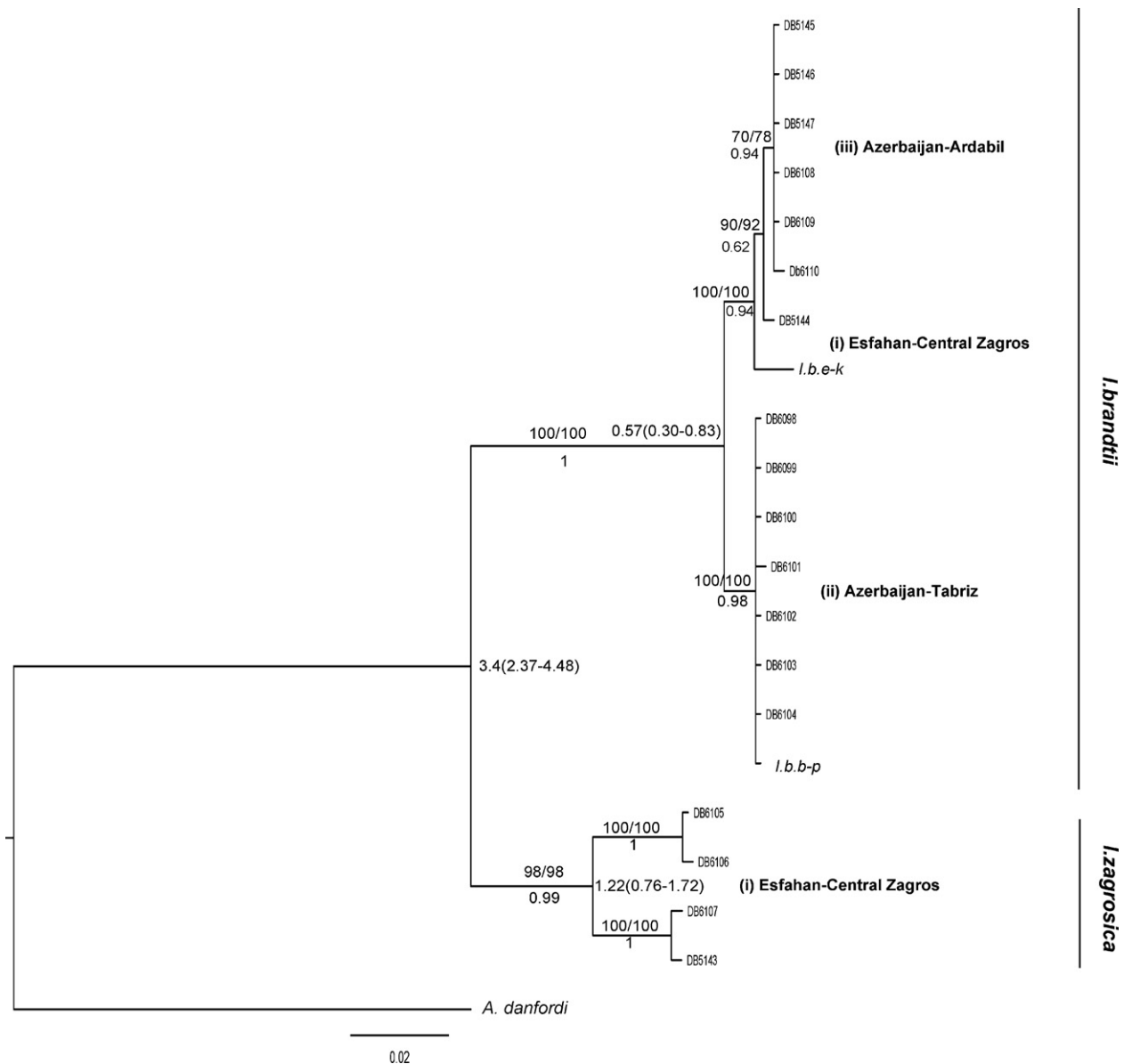
In order to assess model performance and to reduce uncertainties, we applied an ensemble modeling approach as suggested by Araújo and New (2007) by computing 100 SDMs each trained with all except one species record, which was used for model evaluation via the Area Under the receiver operating Curve (AUC) (Swets, 1988). The average of all models was used for further processing, and results were imported into DIVA-GIS (Hijmans et al.,

2005b). The continuous Maxent probabilities in the logistic output format were transformed into presence/absence maps applying the minimum training presence and maximum training sensitivity plus specific logistic thresholds. These are non-fixed thresholds, as recommended by Liu et al. (2005), wherein the later balances commission and omission errors. Variable response was estimated using the implemented jackknife approach of Maxent (e.g. Yost et al., 2008). Area calculations of global potential distribution were performed with ArcMap 10 (ESRI, Redlands, CA, USA).

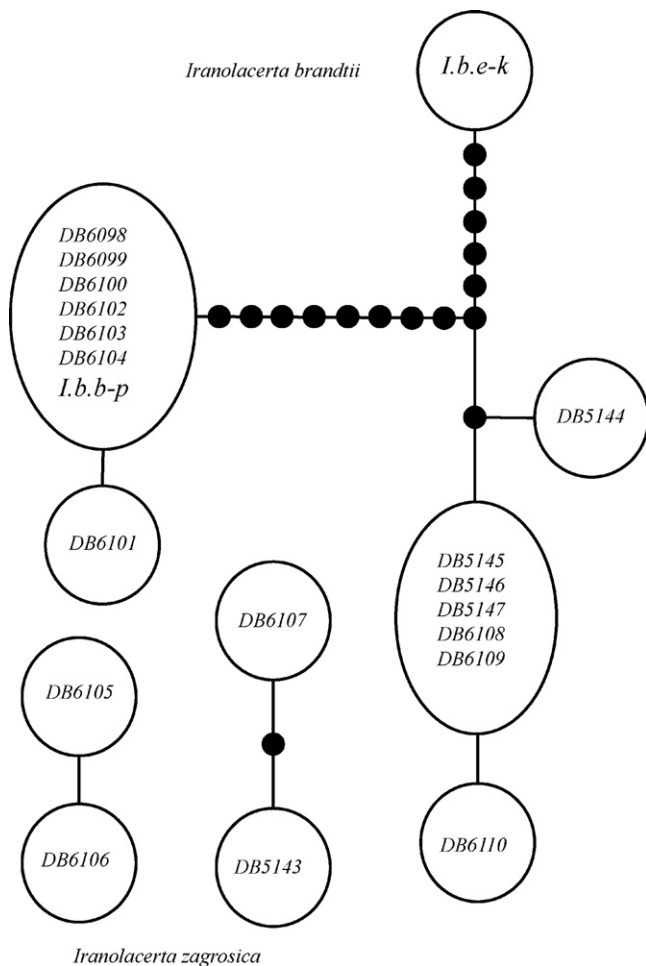
### 3. Results

#### 3.1. Genetic variability

A total of 1018 bp of mtDNA, corresponding to 292 bp for cytb, 333 bp for 12S rRNA and 383 for the 16S rRNA were analyzed. In total 122 positions (excluding the outgroup) were variable. With minor discrepancies, all the methodologies produced the same



**Fig. 2.** Phylogenetic tree derived from the 1018 bp mtDNA fragments (Cytb, 12S and 16S rRNA concatenated). As Bayesian, ML and MP analyses show similar tree topologies; only the ML tree is presented. Numbers under the branch are posterior probability values for Bayesian and bootstrap support values for ML/MP (above) analyses. Dates for time to most recent common ancestor (in million years) for *I. brandtii*, *I. zagrosica*, and the two species as sister taxa as estimated with a relaxed molecular clock are given above the nodes.



**Fig. 3.** Statistical parsimony networks showing the relationships of the *Iranolacerta* species and populations (*I. brandtii* and *I. zagrosica*) in Iran, inferred from a concatenated fragment of Cytb, 12S and 16S rRNA of 1018 bp. Black dots indicate missing haplotypes. Code sample details are shown in Table 1.

topologies, with a TPM2uf model of evolution fitting each gene partition best. As seen in Fig. 2, the two *Iranolacerta* species are evident, with one clade containing *I. zagrosica* and other containing the samples of *I. brandtii*. The second clade also shows two distinct mtDNA lineages, separated by 1.5% uncorrected divergence for the Cytochrome *b* gene. One lineage is represented by Sahand and Varzeghan populations (Azerbaijan–Tabriz), whereas the other lineage comprises the westernmost (Azerbaijan–Ardabil) and southernmost (Esfahan–Central Zagros) populations, including both *I. brandtii esfahanica* and *I. b. brandtii*. A total of 10 haplotypes were identified among the twenty samples in the network, four of them belonging to *I. zagrosica* and 6 to *I. brandtii* (Fig. 3). Under statistical parsimony, three independent networks were produced, one for *I. brandtii* and two for the two sampled populations of *I. zagrosica*. Again, the separation of the Tabriz population is clear, but not that of the isolated Esfahan population. *Iberolacerta brandtii* and *I. zagrosica* were estimated to have shared a common ancestor 3.4 million years ago (2.37–4.48 mya). The major division within *I. zagrosica* was dated to 1.22 mya, and the subdivision within *I. brandtii* was dated to 0.57 mya (Fig. 2).

### 3.2. Current potential distribution

The current potential distribution map is seen in Fig. 1. The AUC value (AUC<sub>Test</sub> = 0.71) supported the discriminative power of the models, despite the few localities known for the species.

Analysis of the variable contributions to the final model revealed high scores for the annual precipitation (43.9%), annual mean temperature (34.9%), and precipitation seasonality (8.3%), respectively. The maximum sensitivity and specific logistic threshold was 0.48. *I. brandtii* has a quite restricted potential distribution range in the northwestern and western mountains of Iran. Although the current potential distribution map is more similar to the known distribution area, some new regions were detected providing potentially suitable climatic conditions. These included the Alborz Mountain range in northern Iran, Karabak in the Southern Caucasus and Khoy–Chaldoran mountain ranges in the administrative borders of Iran and Turkey (not shown in the map). As evident in the model, areas suitable for the species are mainly those at high altitudes. However, the SDM did not predict suitable habitats in terms of current climate in the intermediate area between the ranges of the two subspecies, where the mountain ranges are much lower.

### 4. Discussion

By using the independent results derived from a genetic marker system and a SDM approach as two parallel methods we were able to assess the potential effects of past climate change on the distribution pattern of *Iranolacerta* species throughout its range.

Following Arnold et al. (2007), our results indicating high phylogenetic divergence between both species (14–16%, uncorrected cytochrome *b*) support the currently accepted two species which also display highly divergent morphology (Arnold et al., 2007). However, the subspecies arrangement of northern and southern populations of *I. brandtii* (Nilson et al., 2003) should be revised. Namely, our results indicate that the uncorrected genetic distance based on cytochrome *b*, between the populations of *I. brandtii* is low (maximum 3.5%) and, surprisingly, the genetic distance between *I. b. esfahanica* and the Azerbaijan–Ardabil population (1%) is smaller than that between the two main groups within the nominate subspecies (Azerbaijan–Ardabil and Azerbaijan–Tabriz – 2.8%). Low genetic differentiation between populations from Ardabil and the Zagros Mountains indicates that the separation of these populations is relatively recent. However, at least in the last five million years no remarkable geological events (e.g. mountain uplifting) have occurred in the area (Girdler, 1984; Macey et al., 1998), implying that the separation is likely due to climate fluctuations. It is then suggested that the two groups were connected during the early Holocene. During the glaciations the populations of this species would be expected to shift to lower altitudes. However, the aridification resulting from the Last Glacial Maximum (Ehlers and Gibbard, 2004) would have prevented them from being connected. During the early Holocene, the increase of humidity would have favored connection while the increase temperature and aridification during the late Holocene would have allowed them to return to higher altitudes. In this way, the lowlands, during more humid Holocene climatic optimum (Kaufman et al., 2004), would have served as refuge for the species. Several lines of evidence make it likely that the last glacial in northern Iran and the Zagros mountains was a period of dry and more or less cold climatic conditions (Wright, 1962; Kehl, 2009). As a result present distribution patterns may be very recent, explaining the low genetic distance. This idea is supported by the Wick et al. (2003) finding that present day climatic conditions in eastern Turkey in the Van Lake area probably started only around 8200 years ago.

On the other hand, based on the current SDM, those areas with climatically suitable conditions at present were identified. Expectedly, the model also showed a gap between Azerbaijan and the Central Zagros populations. It therefore appears that not much suitable habitat connects both populations. Ideally the current distribution model could be projected to the past climate scenarios in

order to infer the historical biogeography of the species (Waltari et al., 2007; Elith and Leathwick, 2009). In practice, the lack of sufficient number of records makes a projection of the SDM onto past conditions highly unreliable.

It needs to be noted that there are differences between a species' fundamental niche in terms of macroclimatic conditions and its realized niche (Hutchinson, 1957), wherein the latter can be considerably smaller than the former due to limited accessibility of some areas or other biological and ecological requirements (Heikkinen et al., 2006; Sillero, 2011). However, the model predicted some other environmentally suitable habitats that are ecologically very similar to those currently known (In den Bosch, 1996; Anderson, 1999; Nilson et al., 2003). Why these areas are currently not occupied may be explained either by dispersal limitations (Lomolino et al., 2006; Krebs, 2009), by local extinctions (e.g. by human modification) from the area or by competitive interactions with other ground-dwelling lacertids (i.e. *Eremias* sp., *Ophisops elegans*, Anderson, 1999). However, low occurrence probabilities across the range are indicative of its highly contracted ecological niche, being mostly found in the mountainous areas (Rezazadeh et al., 2010).

Most of the taxa that belong to the Lacertini diversified rapidly and often have fragmented ranges which may be indicative of the reduction of total range and relictual distribution for some of them (Arnold, 1989; Harris et al., 1998; Fu, 2000; Carranza et al., 2004; Arnold et al., 2007; Pavlicev and Mayer, 2009). Based on cytochrome *b* and 12S mtDNA calibrations, the two species of *Iranolacerta* are estimated to have diverge around 8.9 million years ago (Arnold et al., 2007) likely in some place in western Iran, although our estimate of divergence is considerably more recent than this (2.37–4.48 mya), highlighting the uncertainties implicit in these kind of time estimates. Bearing the type of microhabitat and the substrate used by the both species in the mind, it seems when the ancestor species separated, one adapted to be ground dwelling and the other to live in rocky areas (In den Bosch, 1996; Rastegar-Pouyani and Nilson, 1998; Arnold et al., 2006; Rezazadeh et al., 2010). *Iranolacerta zagrosica* is restricted to a few known localities in high elevations (3200 m a.s.l.) while *I. brandtii* has a comparatively wider, but fragmented, range (Nilson et al., 2003). Surprisingly, despite this tiny distribution high genetic diversity (8.1% for cytochrome *b*) was recovered within the four specimens of *I. zagrosica* that form two separate groups under statistical parsimony analysis. Clearly, this deserves further investigation of diversity across the entire geographic range. Another question that remains unanswered is the relationship between two genetically separated Azerbaijan populations, where the geographic distribution distance between the two groups is ten times less than that between Ardabil and Esfahan. However, the Sabalan Mountain Range separates the two Azerbaijan populations, and the only connection between them is a narrow corridor between the Sabalan and Bozghosh mountain ranges. Possibly during the last glacial period, the Ardabil-Arabil population was connected to Zagros more than to Azerbaijan–Tabriz, and this could be considered as one possible interpretation for the genetic differences.

Rastegar-Pouyani and Nilson (1998) suggested that *I. zagrosica* is either (1) a relict of widely distributed lacertids which invaded the area of Zagros from the Mediterranean and northern Iran, or (2) it is one of the northernmost isolated and fragmented populations of an ancient group of lacertids that once were continuously distributed throughout southern parts of Iran. They also mentioned that occurrence of *Omanosaura* in the mountains of Oman could support this last pattern. However, it is now known that *Omanosaura* is not related to the Lacertini, and Arnold et al. (2007), based on molecular and morphological approaches, showed that *I. zagrosica* is the sister species of *I. brandtii*. Unfortunately, it is not clear which taxa are most closely related to *Iranolacerta*, which is related to an unresolved polytomy within the Lacertini (Arnold et al., 2007). Both

species appear to have found a refuge in the Zagros corridor, and thus both species could be considered as relict species, although it is likely that saxicolous species such as *I. zagrosica* are more prone to become isolated, fragmented and eventually extinct, than ground-dwellers such as *I. brandtii* (Arnold, 1987).

In general, the possible influences of climate change during the Pleistocene climate fluctuations and subsequently the glaciations on Iranian biota have not been investigated. The area is poorly studied despite the high biodiversity and endemic species (Azerbaijan and Zagros Mountains), and the potential of having acted as a post-glacial refuge. Seddon et al. (2002) showed that lower latitudes and varied topography make the southern Caucasus, like southern Europe and northern Turkey, suitable places for multiple glacial refugia where genetic lineages may have diverged over several ice ages. Because of its rich biodiversity, the studied area is also a good candidate to be such a refuge as evidenced by the results of this study and some others (e.g. Djamali et al., 2008). Certainly, further investigations will shed new light onto the impact of past climate changes on the biota of the Northwest and Zagros Mountains of Iran.

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