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Molecular phylogeny and taxonomic evaluation of the genus *Asaccus* Dixon and Anderson, 1973 (Reptilia: Phyllodactylidae) in Iran

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The Iranian species of the phyllodactylid geckos of the genus *Asaccus* are found only in the valleys of the Zagros Mountains, a region which represents an important area of endemism in western Iran. Recently, many relict species have been described from the central and southern parts of the Zagros Mountains, which were previously known as *A. elisae*. The recent descriptions of species within this complex suggest that diversity within the genus may be higher than expected and that its taxonomy and systematics should be revised. In the present study, phylogenetic relationships within the genus *Asaccus* were evaluated using two mitochondrial and one nuclear gene. Genetically, the genus shows high levels of variability. The molecular phylogeny of the genus suggests the presence of three main clades along the Zagros Mountains with the southern population (from the Hormozgan province) and one clade (*A. sp8* and *A. sp9*) being sister taxon to *A. montanus* from UAE. The remaining samples are separated into two reciprocally monophyletic groups: the northern (Kurdistan, Kermanshah and Ilam provinces) and the central (Lorestan, Khuzestan, Kohgiluyeh-Bouyer Ahmad and Fars provinces) Zagros groups. The results of the present study suggest that populations attributed to *A. elisae* in Iran correspond to distinct lineages with high genetic distances. In brief, our results suggest that the genus needs a major taxonomical revision. The Arabian origin of the genus has not been confirmed, because two populations from Zagros were located within the *A. montanus*, *A. gallagheri* and *A. platyrhynchus* clade. Further morphological analyses are needed to systematically define each genetic lineage as a new taxon.

Keywords: *Asaccus*, genetic variability, Iran, Phyllodactylidae, species diversity, Zagros Mountains

INTRODUCTION

The high diversity and endemism in Iran may be found in the Zagros Mountain region (Šmíd et al., 2014). The old geological history of this region, its geographical location between two different zoogeographical realms, the existence of many peaks and canyons distributed across more than 1,600 km in a northwest-southeast direction, and its many local microclimates are the main reasons for high diversity and endemism of the local fauna and flora (Mandaville, 1977). The uplifting of the Zagros Mountains was initiated by the northward collision of the Arabian plate with the Eurasian landmass that took place from the Oligocene to the Miocene (35 – 20 million years ago (MYA)) (Mouthereau, 2011). Major uplift of the Zagros Mountains was initiated from 12.4 MYA (Khadiji, 2010). Most species present in this region are endemic to the area and adapted to the local

ecological conditions (Eskandarzadeh et al., 2018). There are many species of reptiles, with representatives from the Viperidae, Colubridae, Lacertidae, Gekkonidae and Phyllodactylidae families being the main inhabitants of the Zagros Mountains (Šmíd et al., 2014; Eskandarzadeh et al., 2018; Rajabizadeh, 2018).

The genus *Asaccus* Dixon and Anderson, 1973 corresponds to a group of geckos distributed in the Middle East (Carranza et al., 2016). Traditionally, the genus consists of only three species (two from the Zagros Mountains and one from the Hajar Mountains), but recent taxonomic revisions in Iran, UAE and Oman increased the species number to 19, ten from Iran of which nine are endemic to the country (Werner, 1895; Arnold, 1972; Dixon & Anderson, 1973; Arnold & Gardner, 1994; Gardner, 1994; Rastegar-Pouyani, 1996; Rastegar-Pouyani et al., 2006; Werner, 2006; Afrasiab & Mohamad, 2009; Torki, 2010; Torki et al., 2011;

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Carranza et al., 2016; Nasrabadi et al., 2017; Uetz, 2019). These geckos were placed previously within the family Gekkonidae, but were recently extracted from this family based on both morphological and molecular approaches and were placed within the Phyllodactylidae (Gamble et al., 2008). The Hajar Mountains in Oman and the United Arab Emirates (UAE) is one of the richest areas for the genus *Asaccus* and many local populations were elevated to new species recently thanks to the combination of molecular and morphological data (Carranza et al., 2016). Based on these results, Carranza et al. (2016) suggested that the Zagros Mountains most probably also included many undescribed species of *Asaccus* (Carranza et al., 2016). Simó-Riudalbas et al. (2017, 2018) provided more evidence for the high level of diversity and endemism in the Hajar Mountains and suggested that this mountain range presented higher levels of reptile endemism than the southernmost part of the Zagros Mountains. Rastegar-Pouyani et al. (2006) proposed that the Zagros Mountains are the center of origin of the genus *Asaccus*. However, the most recent molecular studies (Papenfuss et al., 2010; Carranza et al., 2016; Tamar et al., 2019) placed *Asaccus montanus* (one of the endemic species from the Hajar Mountains) as the sister taxon to all the other species, reinterpreting the history of this genus.

In this study, we aimed at resolving the systematics and evolution of *Asaccus* in Iran, through analyses of populations from north Zagros in Kurdistan and Kermanshah provinces to south Zagros in Khuzestan. Our specific aims were as follows: a) to comprehensively sample across the Zagros Mountains, from western to southern Iran to clarify the systematics and biogeography of *Asaccus*; and b) to test the previous hypothesis of the center of origin of the genus by adding many newly found populations of *Asaccus* from Iran.

METHODS

Sampling, DNA extraction and amplification

A total of 97 tissue samples of *Asaccus* were collected from the Iranian Plateau during field work from 2002 to 2018 (Fig. 1). We used the following GenBank sequences to complete the study: 20 sequences belonging to *A. montanus* (1 sequence), *A. nasrullahi* (1), *A. griseonotus* (1), *A. gallagheri* (1), *A. platyrhynchus* (1), *A. margaritae* (4), *A. caudivolvulus* (5), *A. gardneri* (5) and *Haemodracon riebeckii* (1) as outgroup (Simó-Riudalbas et al., 2018). Our dataset contains all described species of the genus *Asaccus* in Iran from their type localities and different unknown populations. Localities and coordinates for each sample are presented in the online supplementary materials, Table S1. All voucher specimens were deposited in the Sabzevar University Herpetological Collection (SUHC), Iran.

DNA was extracted from tissue samples using the high salt SDS method described in Kabir et al. (2006). The obtained DNA was quantified by 1 % agarose gels and Nanodrop 1000. We amplified three genes: two mitochondrial genes, 12S (12S) and Cytochrome *b* (*Cytb*), and one nuclear gene, Melanocortin 1 receptor

(*MC1R*), because we aimed to combine our data with the previous published datasets (Carranza et al., 2016; Simó-Riudalbas et al., 2018; Tamar et al., 2018). These genes were amplified using four pairs of primers (primer information and PCR conditions are presented in Table S2 of the online supplementary materials).

Phylogenetic analyses and divergence time estimation

We used ClustalW within BioEdit v.7.0 (Hall, 1999) to align sequences. We used Mega v.6.0 (Tamura et al., 2013) to translate protein coding sequences (*Cytb* and *MC1R*) into amino acids and no stop codons were observed. Uncorrected genetic distances (*p*-distances) were calculated using Mega v.6.0 for the two mitochondrial genes separately.

Phylogenetic analyses were carried out to resolve the evolutionary history of the genus in Iran. For this purpose, a concatenated alignment was prepared and the phylogenetic trees were calculated under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria. We used Modeltest 3.7 (Posada & Crandall, 1998) to find the best-fit models of nucleotide evolution. The evolutionary models obtained were as follows: 12S = GTR+I+G; *Cytb* = TrN+I+G; *MC1R* = TrN+I+G. Two methods were used for phylogenetic analyses: Maximum Likelihood (ML) and Bayesian Inference (BI). For this purpose, all gene sequences were aligned and combined to reach a final concatenated alignment totaling 1,857 bp (*Cytb*: 937 bp; 12S: 392 bp; *MC1R*: 528 bp). RaxML 7.4.2 (Stamatakis, 2006), as implemented in RaxmlGUI 1.3 (Silvestro & Michalak, 2012), was used for ML analysis with the GTR+G+I model. The ML analysis was run in heuristic search method and node support was obtained using bootstrapping with 1000 replicates (Felsenstein, 1985). The BI analysis was conducted with MrBayes 3.2.1 (Ronquist et al., 2012) and the best fit evolutionary models indicated above. The analyses were run for 107 generations with a sample frequency of every 1000 generations. The adequacy of the runs was evaluated using variation in log Likelihoods (lnL) and the requirement of a split frequency lower than 0.01. The first 25 % of all trees was discarded as burn-in (Condamine et al., 2015).

Estimating divergence time

There are no internal calibration points for the Iranian *Asaccus* and their relatives, so we applied direct estimations as substitution rates for two mitochondrial genes. These substitution rates were calculated for three lizard families from the Canary Islands: *Tarentola* (Phyllodactylidae) (Carranza et al., 2000), *Gallotia* (Lacertidae) (Cox et al., 2010) and *Chalcides* (Scincidae) (Brown & Pestano, 1998). Many studies have used these substitution rates to estimate divergence times (Carranza & Arnold, 2012; Sindaco et al., 2012; Šmíd et al., 2013; Hosseini Yousefkhani et al., 2019), although we are aware that this approach is far from ideal and could be an additional factor, along with use of partitions, that potentially leads to poor estimation of posteriors on divergence times (Jin & Brown, 2018). We used BEAST v. 1.8 (Heled & Drummond, 2010; Drummond et al.,

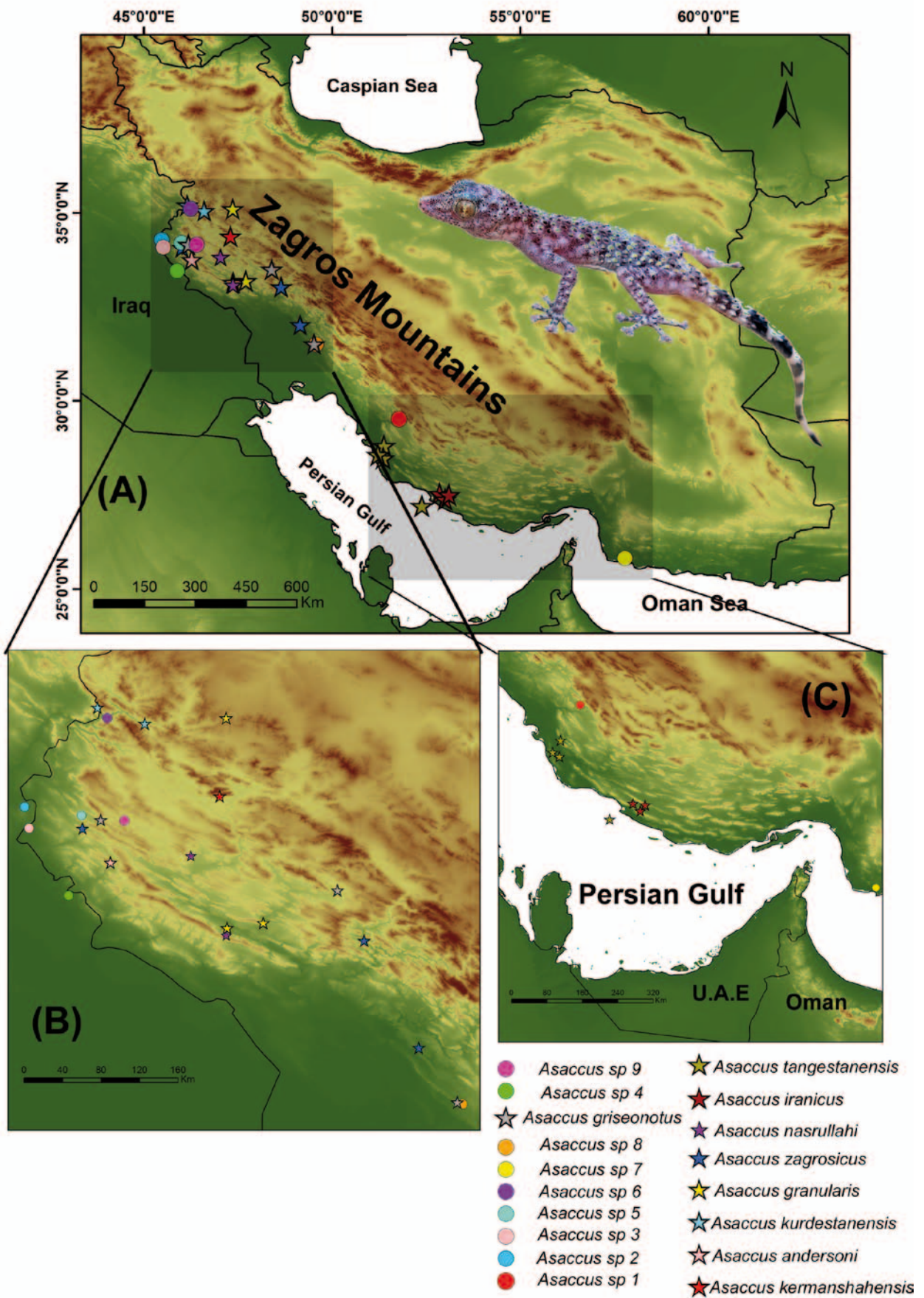


Figure 1. A) Sampling localities for all geckos used in the study. B) Western Iran and species in that regions. C) Sampling localities in south of Iran for the genus *Asaccus*.

2012) to estimate divergence times, among all species and populations of the genus *Asaccus*. The models and priors were set as follows: separate evolutionary models for each gene; random starting tree; clock models were set as lognormal relaxed clock; tree priors were set as coalescent and constant size. UclD priors were set for 12S (mean: 0.00755, stdev: 0.00247) and *Cytb* (mean: 0.0228, stdev: 0.00806) separately (Carranza & Arnold, 2012).

RESULTS

Sequences and phylogenetic analyses

The dataset included 117 samples containing two mitochondrial fragments 12S (366 bp; $V = 173$, $Pi = 141$) and *Cytb* (657 bp; $V = 412$, $Pi = 339$) and one nuclear gene fragment *MC1R* (629 bp; $V = 152$, $Pi = 79$) that provided 1,652 bp in total. Protein coding sequences were checked using MEGA 6.0 to ensure they did not contain stop codons. All accession numbers of new and retrieved sequences are provided in Supplementary Table S1.

Because of the similar topology of both ML and BI trees, we show only the BI tree (Fig. 2). Un-corrected genetic distances (p -distances) were calculated among clades based on the phylogenetic tree and are shown

in Table 1. Genetic divergence for the *Cytb* gene among clades is relatively high and in most cases above 20 % (Table 1).

The results of this study uncovered high levels of hidden diversity in the Zagros Mountains and highlights the importance of this unique mountain chain as a hotspot of reptile diversity that needs priority for wildlife conservation management. The genus *Asaccus* shows high differentiation in Iran and at least 18 genetic lineages can be distinguished (Fig. 2). A clade formed by *A. gallagheri*, *A. platyrhynchus*, *A. montanus*, *A. sp. 9* and *A. sp. 8* originates from the earliest node within the tree and therefore forms a sister clade to the clade that includes all other *Asaccus* species included in the analyses (Fig. 2). It is perhaps surprising that the two distinct genetic lineages *A. sp8* and *A. sp9*, are nested among the Arabian species of *Asaccus*. These two unknown lineages were collected from Izeh, Khuzestan and Islamabad Gharb, Kermanshah provinces, respectively.

Asaccus elisae is a species complex in Iran and comprises many local populations that are defined morphologically as *A. elisae*, but our study shows high divergence among them. For example, node support is high for the branch leading to the sample from Kazeroun, in Fars province indicating a new genetic

Table 1. Genetic distance between clades in Figure 1. Lower-diagonal entries are genetic distances for *Cytb* and upper-diagonal entries are 12S. 1) *Asaccus kermanshahensis*; 2) *A. sp2*; 3) *A. griseonotus_type*; 4) *A. kurdestanensis_type*; 5) *A. sp8*; 6) *A. sp9*; 7) *A. sp3*; 8) *A. sp5*; 9) *A. zagrosicus*; 10) *A. andersoni*; 11) *A. sp1*; 12) *A. granularis*; 13) *A. sp4*; 14) *A. iranica*; 15) *A. kurdestanensis_A*; 16) *A. nasrullahi*; 17) *A. sp7*; 18) *A. tangestanensis*; 19) *A. sp6*; 20) *A. montanus*; 21) *A. gallagheri*; 22) *A. platyrhynchus*; 23) *A. margaritae*; 24) *A. caudivolvulus*; 25) *A. gardneri* (the names of these groups correspond with those in Figure 1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1		13.2	07.5	07.5	11.2	11.2	06.8	07.8	12.1	10.0	12.5	11.4	12.5	12.0	07.5	06.9	13.2	11.9	06.4	14.9	11.2	13.2	12.2	11.5	10.1
2	23.5		13.9	12.3	15.6	15.6	11.9	12.2	11.4	14.1	10.8	09.6	09.5	11.4	12.3	14.4	16.6	11.2	12.2	18.6	14.6	17.3	15.9	17.6	16.5
3	20.8	24.2		08.0	10.8	10.8	08.7	07.9	11.7	10.7	12.2	11.0	12.5	12.6	08.1	09.8	12.3	12.4	06.7	15.1	10.2	12.8	11.2	11.1	09.8
4	16.5	27.4	25.0		09.4	09.4	08.9	04.5	12.4	12.2	12.4	11.6	12.7	12.9	00.3	09.5	15.8	12.7	03.7	15.3	11.4	13.8	11.4	12.7	10.3
5	25.0	26.8	26.9	28.7		00.0	10.2	11.5	12.2	11.0	12.5	10.6	14.9	13.1	09.4	10.9	15.6	12.9	09.8	07.1	11.9	13.6	13.9	12.2	10.4
6	23.7	29.6	26.7	25.5	23.9		10.5	11.5	12.2	11.0	12.5	10.6	14.9	13.1	09.4	10.9	15.6	12.9	09.8	07.1	11.9	13.6	13.9	12.2	10.4
7	25.4	17.6	24.0	25.2	23.2	24.1		08.1	08.8	10.0	10.2	08.2	11.5	11.7	09.2	05.0	10.8	11.6	07.8	14.9	11.9	11.9	09.8	09.8	09.1
8	-	-	-	-	-	-	-		13.3	10.0	13.6	11.9	11.2	13.1	04.8	09.4	14.9	12.9	02.4	15.9	11.2	14.6	12.2	11.9	10.4
9	26.1	17.3	23.2	26.7	24.6	25.8	04.6	-		14.4	11.0	05.9	11.4	11.0	12.6	11.9	14.0	10.8	13.2	16.0	13.0	14.2	13.9	15.0	13.5
10	25.7	25.4	24.7	26.8	28.3	28.1	22.1	-	22.6		13.4	12.1	12.7	12.6	12.5	08.0	13.4	12.2	10.0	14.1	12.4	14.7	12.0	12.0	13.7
11	25.0	23.5	21.8	24.3	29.0	27.0	20.6	-	20.7	22.4		09.0	11.9	11.8	12.6	11.9	14.2	11.6	11.5	16.9	13.2	14.9	13.9	15.6	14.5
12	24.5	17.4	21.0	26.6	23.8	24.4	16.5	-	16.2	24.8	22.5		09.9	08.8	11.6	10.4	13.3	08.6	10.7	15.0	13.6	14.7	13.7	13.1	12.3
13	26.2	14.5	21.9	29.4	27.3	26.0	18.1	-	17.0	23.7	21.8	17.3		09.7	13.0	13.8	15.3	09.4	11.5	16.3	15.3	16.3	16.9	16.6	15.3
14	24.3	19.9	20.8	27.2	29.4	27.5	18.8	-	17.9	24.7	18.4	17.4	16.0		13.2	12.2	13.1	00.5	12.0	15.5	13.4	15.8	14.5	15.5	14.8
15	26.1	16.5	23.6	25.7	23.5	24.8	01.5	-	03.4	22.4	20.2	16.2	17.0	18.4		09.6	16.0	13.0	03.8	15.2	11.4	13.9	11.6	12.8	10.4
16	25.5	27.4	21.5	24.9	28.6	30.1	22.5	-	22.9	16.4	23.9	22.2	23.8	26.4	22.5		11.9	12.1	08.5	13.7	12.2	12.5	11.2	09.9	09.9
17	31.0	33.2	28.1	28.1	31.1	29.0	27.9	-	29.1	29.4	29.7	29.2	31.0	30.3	28.3	30.4		12.9	14.6	17.6	15.3	14.9	12.5	11.5	13.5
18	24.2	20.8	21.5	26.9	27.5	26.2	20.5	-	19.5	25.0	20.8	18.9	17.2	08.6	19.9	27.5	29.4		11.9	15.1	13.3	15.6	14.3	15.3	14.6
19	18.0	25.7	21.8	13.7	27.9	23.7	23.5	-	23.8	23.5	22.1	24.5	26.6	24.4	23.5	23.9	25.7	24.3		15.6	11.2	13.9	11.5	11.2	15.2
20	27.9	31.6	26.7	30.9	27.2	29.2	27.9	-	28.9	29.8	28.3	29.8	29.2	29.1	28.7	30.4	34.1	29.3	27.9		12.9	14.9	16.3	16.3	15.2
21	26.1	25.4	24.0	29.9	27.2	22.1	24.3	-	25.3	29.0	25.0	25.9	27.3	27.3	24.6	28.6	31.3	28.2	26.1	30.5		11.9	11.9	12.9	11.8
22	22.8	29.0	26.8	24.6	24.6	02.4	24.6	-	26.2	27.6	25.7	23.8	24.8	26.9	25.4	29.9	28.6	25.8	23.2	29.0	22.4		08.8	11.9	12.1
23	24.2	26.7	24.4	25.6	27.8	25.6	26.7	26.7	-	26.9	26.9	24.9	23.3	23.3	22.4	27.1	25.3	23.6	23.1	29.0	27.1	25.6		09.8	09.8
24	23.5	27.9	24.7	24.6	27.6	24.1	25.4	25.4	-	24.9	24.3	25.7	23.9	23.9	24.0	25.7	24.6	23.8	21.7	30.1	26.1	23.2	24.5		04.7
25	21.4	23.4	23.3	23.3	26.0	24.7	21.2	21.2	-	21.4	22.8	25.5	21.0	21.0	21.6	21.5	24.5	22.2	20.5	29.9	24.2	24.9	23.1	14.1	

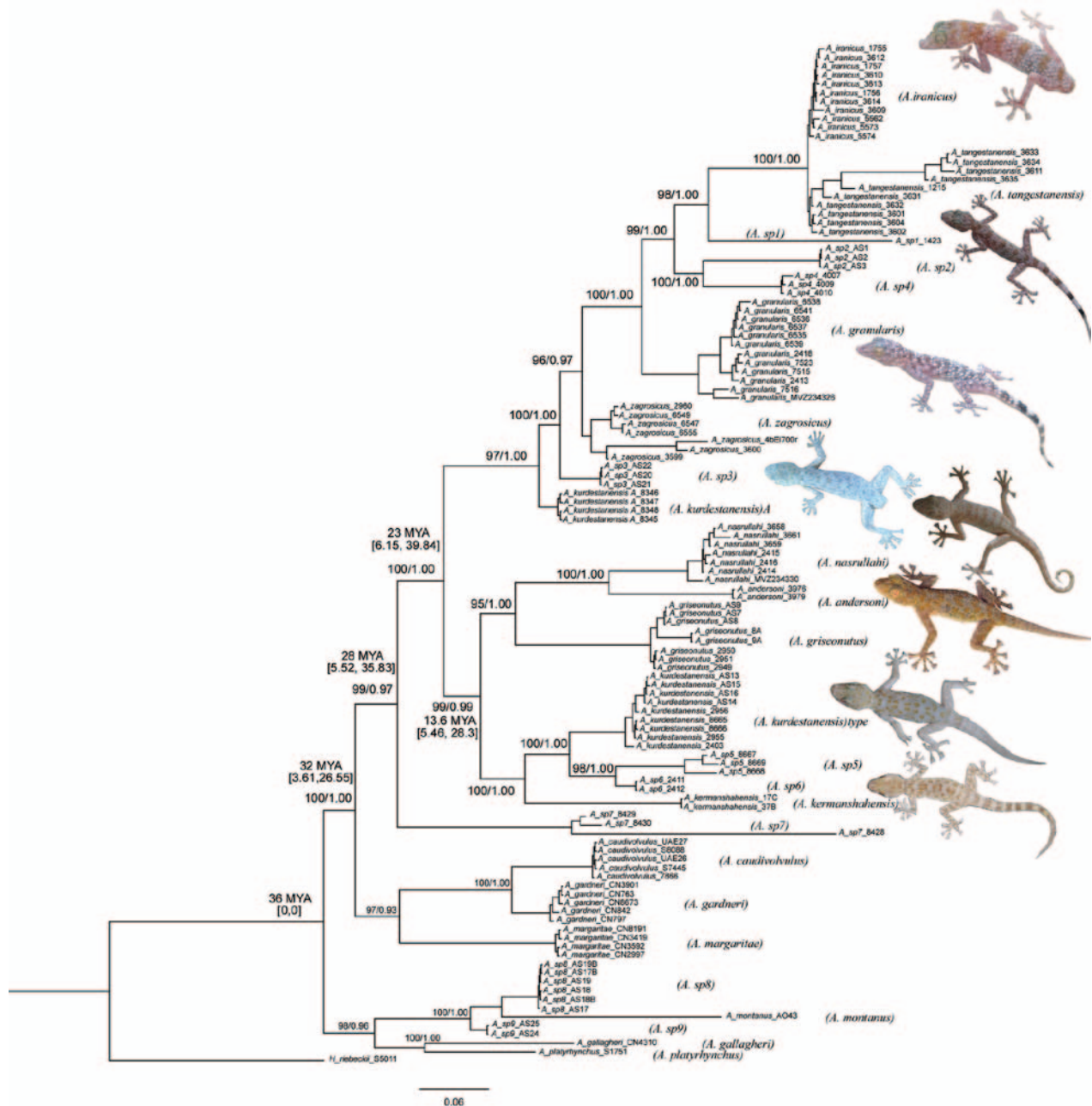


Figure 2. Bayesian Inference (BI) gene tree of spider geckos inferred from 1857 bp of mitochondrial (*12S* and *Cytb*) and nuclear (*MC_{1R}*) gene fragments. ML bootstrap support and posterior probability of Bayesian analyses are presented next to the nodes, respectively. Age estimates based on the substitution rates are denoted near the relevant nodes and include the mean and, in parentheses, the HPD 95 % confidence interval.

lineage (*A. sp1*). Two distinct genetic lineages from Gilan-e Gharb, Kermanshah province appear in distinct clades of our tree (*A. sp2*, *A. sp3*). One genetic lineage in the phylogenetic tree (*A. sp4*) is from Bina & Bijar, Ilam province which appears as a sister taxon to *A. sp2*. *Asaccus sp5* and *A. sp6* from Patagh, Sarpol-e Zahab and Nosud area respectively, in Kermanshah province form sister lineages. These two lineages form a clade that is sister to one of the clades containing *A. kurdestanensis* samples (namely, the one comprising the holotype) and these three form a sister clade to the clade that includes *A. kermanshahensis*. Surprisingly, the samples of *A. kurdestanensis* included in the present study represent

two highly divergent lineages, the one described above containing the holotype of the species and another which includes all four samples from Sarv abad- Qaleji village. In addition a geographically distant population of *Asaccus*, found near Jask in southern Iran, originates from quite an old node within the tree (*A. sp7*).

Asaccus iranica and *A. tangestanensis* are two species that were recently described from south Iran (Torki et al., 2011), although they show only 8.6 % divergence (*p*-distance for *Cytb*) and are the most closely related species (Fig. 2). *Asaccus granularis* was known only from the type locality (near Pol Dokhtar, Lorestan province) and samples from Darreshahr in Ilam province

clustered with type samples of *A. granularis*. One sample from the Museum of Vertebrate Zoology (MVZ 234326) that was deposited in GenBank as *A. griseonotus* from 99 km SW Khorram Abad clustered with our *A. granularis* clade, which suggests a possible error in this record.

Asaccus zagrosicus, represents a distinct genetic lineage that is sister to the clade containing *A. granularis*, *A. iranicus*, *A. tangestanensis*, *A. sp2* and *A. sp4*. *Asaccus andersoni* and *A. nasrullahi* are two closely related species that clustered with *A. griseonotus* (Fig. 2). The genetic distance between *A. andersoni* and *A. nasrullahi* is very high (19.4 % for *Cytb*) and indicates their deep history of divergence. *Asaccus kermanshahensis* from the type locality, 32 km north-east of Kermanshah city, is another species that is clearly distinct from other groups. This taxon has high genetic distance from other clades (more than 16 % for *Cytb*), with *A. kurdestanensis* showing the lowest distance from it, 16 % in the *Cytb* gene fragment.

Divergence time estimation

Ages obtained from the phylogeny are shown in Figure 2 and show that diversification within the genus *Asaccus* started about 36 MYA. Based on our estimation time of divergence, the majority of the Iranian populations of the genus *Asaccus* began diversification about 28 MYA (95 % HPD = 5.5-35.8), when they divided into southern and western clades.

DISCUSSION

Using a molecular phylogenetic approach, we have presented the diversification patterns for a clade of phyllodactylid geckos from the Zagros Mountains, which is a major geographical system in western Iran that separates the Central Iranian Plateau in the east from the Mesopotamian plain in the west. Zagros runs in a north-west to south-east direction from West Azarbaijan province to Hormozgan province in south Iran in a 1600 km long line (Falcon, 1974). It is part of the Alpine-Himalayan mountain system that borders the Arabian shield and is very interesting for herpetologists because of the large numbers of deep valleys in the foothills (Falcon, 1974). In addition to the tectonic events in the Zagros formation, several paleoclimatic fluctuations played important role in the Middle East biodiversity (Zachos et al., 2001; Fathinia et al., 2018). For example, climatic conditions in the Zagros Mountains, led to the ancient immigration of mesic adapted species from the Mediterranean basin (García-Antón et al., 2002).

The genus *Asaccus* currently has ten described species in Iran (Šmíd et al., 2014), but specimens from several taxa, e.g., *A. elisae* and *A. kurdestanensis*, appear in distinct lineages along with several unnamed ones. On the other hand, distinct species appear genetically much more closely-related than previously considered (e.g., *A. iranicus* with *A. tangestanensis*). Our study highlights the high level of variation and local isolation of populations within the genus in the Zagros Mountains (Fig. 1). The results of our study support the importance of the Zagros Mountains due to the high level of endemism in reptile

and amphibian species found there (Hosseinzadeh et al., 2014). Based on the literature, there are many endemic species and isolated populations of reptiles in Iran (about 21.09 % of all Iranian reptiles are endemic) (Hosseinzadeh et al., 2014), most of which are situated in the east of Iran and the Zagros Mountains. These results emphasise the need for more investigation in the area to understand its biodiversity.

The central part of the Zagros Mountains in Chahmahal Bakhtiari and Lorestan provinces have many deep and hardly accessible valleys that are suitable as microendemic areas. Among several local species of the genus *Asaccus*, the status of *A. elisae*, with a wide distribution range, is controversial and needs serious revision. The molecular phylogenetic tree, surprisingly demonstrates the huge genetic diversity among local populations within this species.

Our analysis reveals 9 distinct unnamed lineages of *Asaccus* in Iran that each of which has the potential to be considered as full species (based on the molecular data). Our study focused on the unknown populations from the northern to the southern parts of the Zagros Mountain region, so our findings illustrate hidden genetic diversity within the populations traditionally attributed to *Asaccus elisae*. Many isolated populations of the genus *Asaccus* were added into the analyses and phylogenetically differentiated from other species and populations. Combining these findings with further morphological and ecological information will help to provide more clarification. Carranza et al. (2016) referred to Arabia as the origin of the genus *Asaccus*. In our study, *A. sp9*, *A. sp. 8*, *A. montanus*, *A. platyrhynchus* and *A. gallagheri* originate from one of the earliest lineages within the tree, which challenges the view provided by Carranza et al. (2016).

Our molecular phylogenetic tree was produced based on all available populations of the species from Iran and GenBank samples from Arabia. We have demonstrated at least nine new genetic lineages with divergences comparable to the divergences found between well-recognised species. Further investigations including morphological and ecological studies are needed to describe these new species properly, and such a study has already been already started. The present results highlight the need for future conservation programs for the Zagros Mountains as one of the most important hotspots of endemism in Iran.

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