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Taxonomic revision of the spider geckos of the genus *Agamura* senso lato Blanford, 1874 (Sauria: Gekkonidae) in the Iranian Plateau

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In this study, we present an integrative systematic revision of the spider gecko, *Agamura* senso lato, in Iran. We sampled 56 geckos of this complex from its distributional range in Iran and western Pakistan and sequenced these for two mitochondrial markers, cytochrome b and 12S ribosomal RNA, and one nuclear marker, melano-cortin 1 receptor. We combined our molecular data with species distribution modelling and morphological examinations to clarify *Agamura persica* systematics and biogeography. Due to a lack of published data, we used only our data to investigate the spatial and temporal origin of spider geckos within a complete geographic and phylogenetic context. The phylogenetic analyses confirm the monophyly of *Agamura*. Among spider geckos, *Rhinogekko* diverged around the early-mid Miocene (17 Mya) from the Lut Block, and then *Cyrtopodion* diverged from the *Agamura* clade about 15 Mya in the mid-Miocene as a result of the uplifting of the Zagros Mountains. Subsequent radiation across the Iranian Plateau took place during the mid-Pliocene. *Agamura kermanensis* exhibits deep divergence from two other species of *Agamura* (*A. persica* and *A. cruralis*), whereas no geographical substructure was observed on the Iranian Plateau for *A. persica* and *A. cruralis*. Our findings reveal that diversification is consistent with a biogeographical model explained by different dispersal waves and vicariant events on the Iranian Plateau during the last 18 Mya. The divergence times between clades are compatible with orogenic events in southern Iran that resulted from the collision with Arabia. According to the genetic differentiation of both mtDNA genes (12S and cytochrome b), the systematic status of *A. cruralis* is confirmed, the new clade was distinguished from the genus *Agamura*, monophyly of Rhinogekko was confirmed and the allocation of *Cyrtopodion gastrophole* to the *Cyrtopodion* clade was confirmed.

Key words: Agamura, *agamuroides* group, divergence time, Iranian Plateau, *Rhinogekko*, spider geckos, vicariance

INTRODUCTION

The genus *Agamura* has a wide distribution range in the Iranian Plateau and occupies different habitat types (Anderson, 1999). However, due to the different habitat types, a high degree of geographic variation and genetic divergence exist. The Iranian Plateau is located in south-western Asia and is comprised of various ecoregions (e.g., both mountains and dry deserts), surrounded by several mountain ranges such as the Zagros in the west, Alborz in the north, Kopet Dagh in the north-east and Hindokush and Soleiman in the east and the south-east (Macey et al., 1998). These mountain ranges create a rain shadow, preventing high precipitation on the plateau, creating hot dry deserts in this central region (Ahmadzadeh et al., 2012). The Iranian Plateau has a high number of endemic lizards, especially within the arid clades of geckos (Šmid et al., 2014). This high endemicity may have been facilitated by the various habitat types in the region such as mesic, alpine and xeric, which are restricted by mountain chains (Farahmand & Nazari, 2015). The formation of the plateau started ca.40 million years ago (Mya) as a result of the merger/collision among Arabian, Eurasian and Indian plates (Mouthereau, 2011). The formation and the geological history of the Iranian Plateau has had a fundamental influence in shaping the distribution patterns of the reptilian biota and the speciation processes by both dispersal and vicariance events (Macey et al., 2000).

Iran is an interesting region to study biogeographical patterns because it is located near the junction of the Eurasian, Arabian and Indian continental plates (Macey et al., 1998). Collisions between these plates created different mountain chains that affected the herpetofaunal biodiversity in the region (Van Hinsbergen et al., 2012; Ahmadzadeh et al., 2012). In south-western Asia, several phylogenetic studies have been conducted to gain insights on the biogeographical patterns and speciation events among geckos (Bauer et al., 2013; Šmíd

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et al., 2013; de Pous et al., 2015). One such example is the study of Šmíd et al. (2013) on the genus *Hemidactylus* and the following descriptions of several new species. Bauer et al. (2013) presented the phylogeny of naked-toed geckos suggesting that most speciation events in the western Palearctic took placed during the Miocene by vicariance due to different uplift events in the Iranian Plateau. There are more than 15 genera of the naked-toed gecko and one of them is *Cyrtopodion* that was later divided into several genera (Szczerbak & Golubev, 1996; Bauer et al., 2013).

Within Gekkonidae, the spider gecko, *Agamura*, is a monotypic genus, phylogenetically close to *Cyrtopodion*, *Rhinogekko*, *Bunopus* and *Crossobamon* (Bauer et al., 2013), which ranges across Iran, Pakistan and Afghanistan. In Iran, it is known throughout most of the Iranian Plateau, east of the Zagros Mountains and south of the Alborz and Kopet Dagh mountain ranges, but is absent from the Kavir and Lut central deserts. *Agamura* occupies stony and rocky habitats, including hillsides and barren plains, with sparse shrubby vegetation (Anderson, 1999, Sindaco & Jeremčenko, 2008). To date, neither morphological investigations nor molecular studies on this monotypic genus have been carried out across its distribution range. *Agamura* has previously been used as an outgroup in other studies (Červenka et al., 2010; Bauer et al., 2013; de Pous et al., 2015); however, these studies did not investigate the phylogenetic relationships within the genus, or its population structure and diversity. *Agamura* previously included four taxa (i.e., *A. persica*, *A. gastropholis*, *A. misonnei* and *A. femoralis*; (Szczerbak & Golubev, 1996), all of which were endemic to the Iranian Plateau. Later studies examined the genus and excluded the three latter species from *Agamura*: *A. misonnei* and *A. femoralis* were allocated to the genus *Rhinogekko*, and *A. gastropholis* to the genus *Cyrtopodion* (Anderson, 1999; Krysko et al., 2007). Thus, currently the genus *Agamura* includes a single species, *A. persica*, which exhibits differences among populations in colour pattern and morphological characteristics (Szczerbak & Golubev, 1996; Anderson, 1999). The subspecies *A. p. persica* and *A. p. cruralis* represent western and eastern populations, respectively (Anderson, 1999; Szczerbak & Golubev, 1996). Differences between these two subspecies are apparent in the shape of the rostral scale, the number of the dorsal scales, and the sharper shape of the tubercles in the eastern populations (Anderson, 1999). *Rhinogekko misonnei* is another species that is endemic to Iran and only distributed around the Lut Desert and its phylogenetic status among other gekkonid species requires verification, as there has been no study on the species (Šmid et al., 2014).

In this study, we conduct an integrative revision of the monotypic genus *Agamura* senso lato in Iran. We provide the first comprehensive morphological and molecular analyses throughout its wide range in the Iranian Plateau with the aim of elucidating the evolutionary and biogeographic history of this enigmatic genus. For this purpose, we sampled different taxa throughout the Iranian Plateau including *A. persica* (different populations of the species from whole distribution range in Iranian Plateau) and its close relatives, *Rhinogekko misonnei* (providing the first genetic data for this genus), *C. gastrophole*, *C. persepolense*, and *C. agamuroides*. We used nuclear and mitochondrial markers to revise the systematics of *Agamura*. We used species distribution modelling and phylogeographic analyses to explore the phylogeographic structure of *Agamura persica* on the Iranian Plateau, and climate suitability that can affect the divergence between species in Iranian Plateau.

MATERIALS AND METHODS

We used two criteria to assess the species limits; first, the identification of lineages based on mitochondrial and nuclear markers and, second, the presence of diagnostic morphological characters.

Sampling, DNA extraction and amplification

A total of 56 individuals were collected from the Iranian Plateau during field trips from 2014 to 2016. We used two recently described species of the genus *Cyrtopodion* that is clearly situated in the main clade of *Cyrtopodion* (Nazarov et al., 2009; Nazarov et al., 2012). The dataset contains seven recognised species: *Agamura persica*, *A. cruralis*, *A. kermanensis*, *Rhinogekko misonnei*, *Cyrtopodion gastrophole*, *C. persepolense*, *C. sistanense* and *C. agamuroides* (Hosseinian Yousefkhani et al., 2018). Localities and coordinates for each sample are presented in the Supplementary Materials, Table S1. Specimens were deposited in the Sabzevar University Herpetological Collection (SUHC). Sequences of *Hemidactylus turcicus* were retrieved from GenBank and used as outgroup (Table S1).

DNA was extracted from tissue samples using the salt method (Kabir et al., 2006). The quality of extracted DNA was measured using 1% agarose gels stained by 0.5 µl GreenViewer 6X and visualised under ultraviolet light. We amplified two mitochondrial genes 12S rRNA (12S) using the primers 12SL (5′-AAACTGGGATTAGATACCCCACTAT-3′) and 12SH (5′-GAGGGTGACGGGCGGTGTGT-3′) (Kocher et al., 1989), and Cytochrome b (Cytb) with the primers L14724 (5′-GACCTGCGGTCCGAAAAACCA-3′) and H16064 (5′-CTTTGGTTTACAAGAACAATGCTTTA-3′) (Burbrink et al., 2000) and L14919 (5′-AACCACCGTTGTTATTCAACT-3′) and Ei700r (5′-GGGGTGAAA GGGGATTTTRTC-3′) (Rastegar-Pouyani et al., 2010) and one nuclear gene Melano-cortin 1 receptor (MC1R) with the primers MC1R-F (5′-AGGCNGCCATYGTCAAGAACCGGAACC-3′) and MC1R-R (5′-CTCCGRAAGGCRTAAATGATGGGGTCCAC-3′) (Eskandani et al., 2010).

Phylogenetic analyses and haplotype network construction

We used Clustal W as implemented in Bioedit alignment editor v. 7.0 (Hall, 1999) to align sequences with default parameters. Protein coding sequences (Cytb and MC1R) were translated into amino acids with Mega v.6.0 (Tamura et al., 2013) and no stop codons were observed. Uncorrected genetic distances (*p*-distance) were calculated using Mega v.6.0 and ExcaliBAR (Aliabadian et al., 2014) for 12S and Cytb gene fragments independently.

The best-fit models of nucleotide evolution were assessed using ModelTest 3.7 (Posada & Crandall, 1998) and the best fit models of evolution according to the Akaike Information Criterion (AIC) were: 12S-GTR+I+G; cyt b-TIM+I+G; mc1r-TrN+I+G.

The phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods and for this purpose, all gene alignments were combined into a single alignment totalling 1670 bp (389 bp of 12S; 625 bp of cytb; 656 bp of mc1r). We also considered *Hemidactylus* as outgroup in the analyses (Pyron et al., 2013). We used 50 cytb sequences from Genbank to clarify the phylogenetic structure of the genera.

Maximum Likelihood analyses were conducted with RaxML 7.4.2 (Stamatakis, 2006) as implemented in RaxmlGUI 1.3 (Silvestro & Michalak, 2012) with a GTR+I+G model. The analyses were run in heuristic search and the nodal support was obtained by bootstrap analysis with 1000 replicates (Felsenstein, 1985). MrBayes 3.2.1 (Ronquist et al., 2012) was used for the BI analyses and the best-fit models were specified above for concatenated dataset. The analyses were run for 10^7 generations with a sample frequency of every 1000 generations. Some parameters, like the number of runs and the number of chains, were kept as default and a sufficient number of generations were evaluated by the log likelihood value (lnL) and split frequency lower than 0.01. We conservatively discarded the first 25% of trees as burn-in (Condamine et al., 2015). To reconstruct the ancestral area Bayesian Binary MCMC (BBM; Ali et al., 2012), we employed Reconstruct Ancestral State in Phylogenies (RASP) (Yu et al., 2012) using all spider gecko sequences. MrBayes was used to prepare the input tree file. Six areas were designated based on zoogeographical regions for reconstruction as: Central Plateau, East Iran, South Iran, South-west Pakistan, South-west Iran, and Zagros Mountains. We chose these areas to identify the direction of dispersal within the Iranian Plateau spider geckos.

Relationships among lineages and species were assessed with allele network of the *mc1r* nuclear marker. The nuclear alignments were imported into TCS 1.21 (Clement et al., 2000) using a parsimony method to obtain the haplotype network.

Estimation of divergence time

Because of the absence of internal calibration points for spider geckos and their relatives, we applied direct estimations obtained from other groups of lizards. The substitution rate of the same mitochondrial genes (12S and Cyt*b*) that 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) were used to estimate the divergence time. These substitution rates have already been used for divergence time estimates for different taxa including *Hemidactylus*, *Bunopus*, *Asaccus* and etc. (Carranza & Arnold, 2012; Sindaco et al., 2012; Šmíd et al., 2013). BEAST 1.8 (Heled & Drummond, 2010) was used to estimate divergence time among the spider geckos and the models and priors were applied as follows (otherwise by default): evolutionary models were set for each gene separately; random starting tree; clock models were set as lognormal relaxed clock with unlinked status; tree priors were set as coalescent and constant size. Finally, divergence times were assessed by the mean rate of molecular evolution for the ucld. priors for 12S (mean: 0.00755, stdev: 0.00247) and Cyt*b* (mean: 0.0228, stdev: 0.00806) gene fragments (Carranza & Arnold, 2012) independently.

Species distribution modelling

A total of 189 presence records from the examined species and clades were obtained from the literature, museum records and our direct filed surveys (Supplementary Materials, Table S2). Climatic layers were downloaded from the worldclim website (www.worldclim.org) in 30 arc second (Hijmans et al., 2005) and extracted using ArcGIS 10.3 (ESRI) only for Iran (Table S3).

Correlations between climatic layers were calculated using ENMTools 1.3 (Warren et al., 2010) and the correlative layers (>0.7) were removed from the analyses. Maxent 3.3.3e (Phillips et al., 2006) was employed to predict the potential distribution area using only presence records. The final set of variables with lower correlation than 0.7 used for all species distribution modelling consisted of 12 bioclimatic variables (Table 2). All models were run for 10 replicates under a crossvalidate model and 10000 background points, with a convergence threshold of 0.00001, and maximum number of iterations as 500. The model accuracy was evaluated using area under the curve (AUC) criterion that ranges between 0 and 1 (Fielding & Bell, 1997). Model visualisations were done by ArcGIS 10.3 (ESRI) and we exported relevant maps as the species distribution prediction.

Morphological analyses

Populations of *Agamura* were examined using 27 morphological characters (12 metric and 15 meristic characters; Supplementary Materials, Table S4) on the samples that were sequenced for phylogenetic analyses. Metric characters were measured using digital callipers (rounding to nearest 0.1 mm) and meristic characters were examined using an Olympus loupe. Operational taxonomic units (OTUs) were classified according to the cluster analysis and zoogeographic regions on the Iranian plateau (Anderson, 1999). Three OTUs were defined as western, eastern and southern clades. Analysis of variance (ANOVA) performed on the OTUs and the significant variables ($P< 0.05$) were excluded to run principal component analysis (PCA) and canonical variate analysis (CVA) and to visualise the morphological variation by these analyses.

RESULTS

Taxon sampling and sequence data

Our dataset included mitochondrial fragments of 12S (389bp; *V* = 182; *Pi* = 136) and Cyt*b* (625 bp; *V* = 297; *Pi* = 239) and a nuclear gene fragment MC1R (656 bp; V = 94; Pi = 63) totalling 1670 bp. Thirty-six unique

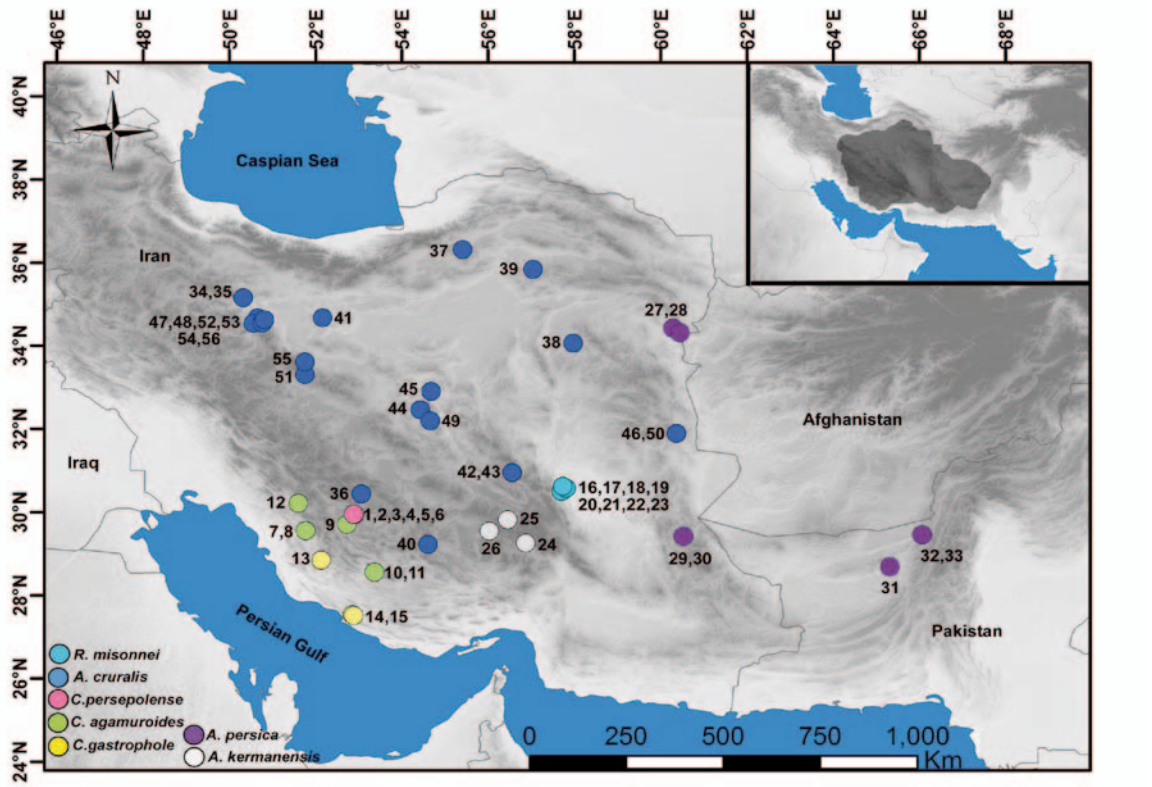

Figure 1. Sampling localities of spider geckos including all species. Numbers correspond to specimens listed in Table S1 and colours to specimens in Fig. 2 and 3. The black region in the right-top map indicates the sampling region in the Iranian Plateau.

Table 1. Uncorrected genetic variation (*p*-distance) among different species of angular-toed geckos in the Iranian Plateau. Above the diagonal represents the variation in 12S and below the diagonal refers to Cyt*b* diversity.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) *A. cruralis*		3.6	3.7	14.7	16.7	14.0	19.5
(2) *A. persica*	8.3		4.2	14.4	15.8	13.2	19.1
(3) *A. kermanensis*	13.2	11.7		14.0	15.7	14.2	18.5
(4) *C. agamuroides*	20.1	18.8	19.6		4.4	9.3	20.1
(5) *C. persepolense*	19.4	18.1	19.3	4.0		9.6	21.7
(6) *C. gastrophole*	17.9	17.4	19.3	14.1	13.7		21.1
(7) *R. misonnei*	19.1	18.5	21.5	18.5	18.5	18.7	

Table 2. Percentage contribution of climate variables under the Maxent modelling conducted in the present study. Definitions of variables are presented in Supplementary Materials, Table S3. Bold values refer to the most contributed variable in each species distribution modelling.

Variable	*A. cruralis*	*A. persica*	*A. kermanensis*	*C. agamuroides*	*C. gastrophole*	*C. persepolense*	*R. misonnei*
BIO2	5.9				8.7	15.7	
BIO4	8.5	**39.4**	**44.5**				
BIO6		34.3		**21.3**	**51.2**	16.4	**46**
BIO9	19			5.5			
BIO11	**23.6**	28	40.6	9.8		9.6	
BIO12	20.3		5.2			**19.9**	42.9
BIO13					12.2	15.7	
BIO14	6.6					5.4	
BIO15				13.8	6.1		
BIO16				16.6			
BIO17				15.9	6.4		
Slope			7.6				2.8

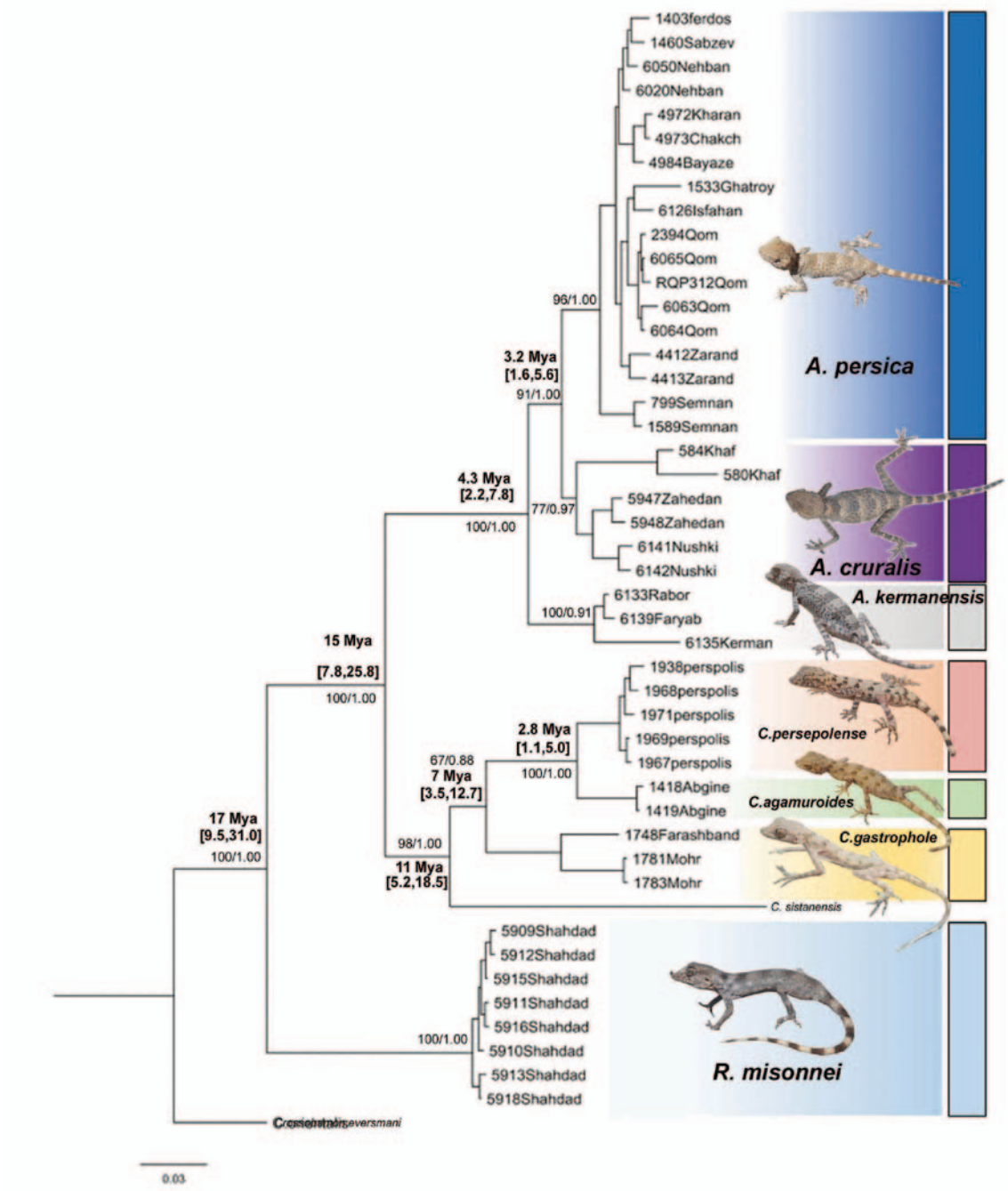

Figure 2. Bayesian Inference (BI) gene tree of spider geckos inferred from 1670 bp of mitochondrial (12S and Cyt*b*) and nuclear (MC1R) gene fragments. ML bootstrap support and posterior probability of Bayesian analyses are presented next to the nodes, respectively. Age estimated based on the substitution rates are denoted near the relevant nodes and include the mean and, between brackets, the HPD 95% confidence interval.

Table 3. Mean ± SD and range for significant characters of seven metric, meristic and ratio characters measured in *Agamura* population from Iranian Plateau. The right column refers to the significant values among populations.

Character	*A. cruralis* (n = 18)		*A. persica* (n = 5)		*A. kermanensis* (n = 7)		*P* value
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
HH	12.65±1.44	9.31-17.97	8.86±0.39	7.20-12.89	9.35±0.48	7.25-11.33	0.002
SL	5.03±0.19	4.49-5.58	4.87±0.15	3.81-6.14	4.17±0.22	3.34-5.11	0.030
IO	10.11±0.41	8.81-11.40	8.79±0.29	6.60-11.22	10.85±0.68	8.59-13.63	0.005
HLL	46.96±0.96	43.71-49.44	40.87±1.09	33.72-49.53	41.66±3.36	30.03-56.67	0.042
FLL/SVL	0.52±0.02	0.46-0.58	0.51±0.01	0.44-0.59	0.54±0.01	0.52-0.61	0.049
NSA	28±0.94	25-30	37.11±1.01	31-47	27.14±1.77	21.-36	0.000
NPV	53.20±2.10	46-59	54±0.77	49-59	48.85±1.07	46-54	0.014

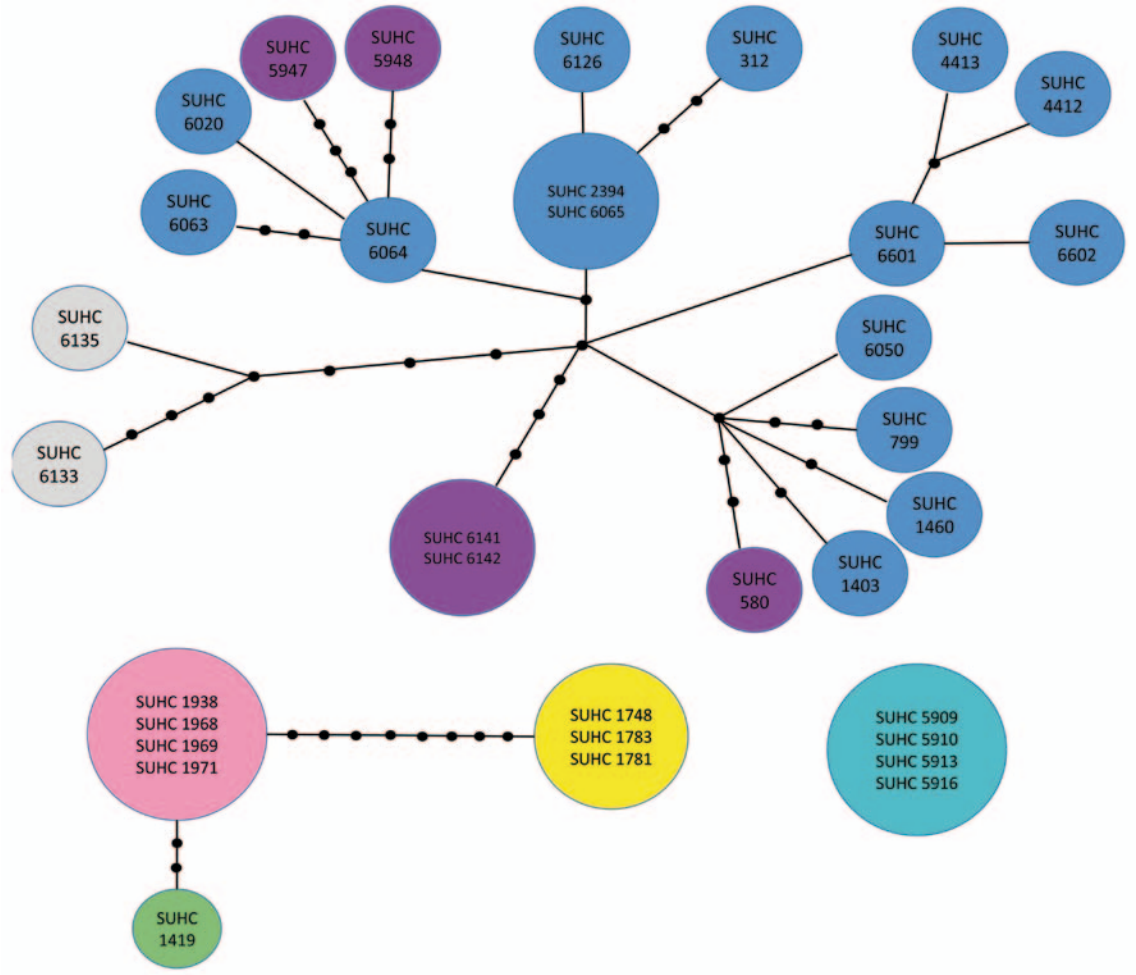

Figure 3. Unrooted haplotype network of MC1R as nuclear marker. Circle size is proportional to the number of samples pre-haplotype, with colours corresponding to species in Fig. 2. Codes correspond to the specimens presented in Table S1.

Table 4. Factor loadings of the first three principal components (PCs). The characters are defined in Table S4.

Characters	PC1	PC2	PC3
HH	0.891	-0.079	0.172
SL	0.936	-0.005	0.034
IO	0.857	0.298	0.110
HLL	0.892	0.312	0.156
NSA	-0.342	0.772	0.430
FLLSVL	-0.636	0.620	-0.056
NPV	0.410	0.487	-0.748
Eigenvalues	3.892	1.410	0.815
Accumulated percent of trace	55.606	75.747	87.389

Table 5. Factor loadings of the first three canonical variates (CVs). Characters were defined in Table S4.

Characters	CV1	CV2
FLLSVL	-0.269	0.314
NSA	0.701	-0.323
NPV	0.110	0.209
HH	-0.320	0.980
SL	0.922	-0.048
IO	-1.149	-1.103
HLL	0.492	0.706
Eigenvalue	4.744	1.028
Accumulated percent of trace	82.2	100.0

haplotypes were distinguished within the concatenated mitochondrial dataset and the nuclear MC1R marker included 32 unique haplotypes.

Phylogenetic analyses and network construction

The Bayesian and Maximum Likelihood trees showed identical topologies with high Bayesian posterior probabilities and bootstrap values (Figs. 2, S1). According to the phylogenetic analyses, *Agamura* is monophyletic (Fig. 2). *Agamura* is divided into three clades (Fig. 2) consisting of *A. cruralis* (the previously known species from eastern Iran), *A. persica* as a western clade (as mentioned by Szczerbak & Golubev, 1996) and *A. kermanensis*. Genetic distances (*p*-distance) of the two mitochondrial markers between clades reveals high diversity among the three lineages (i.e., 12S: 3.6-4.2%; Cyt*b*: 8.3-13.2%). Variation within each clade is very low (12S: 0-0.8%; Cyt*b*: 0.8-1.4%) especially within *A. persica*. The *Cyrtopodion* clade consists of four species that are distinctly separated from *Agamura* and each species is delimited from others with high bootstrap values and posterior probabilities support, but based on the Cyt*b* tree (Supplementary Materials, Fig. S3) the genus was paraphyletic and *Cyrtopodion gastrophole* situated far

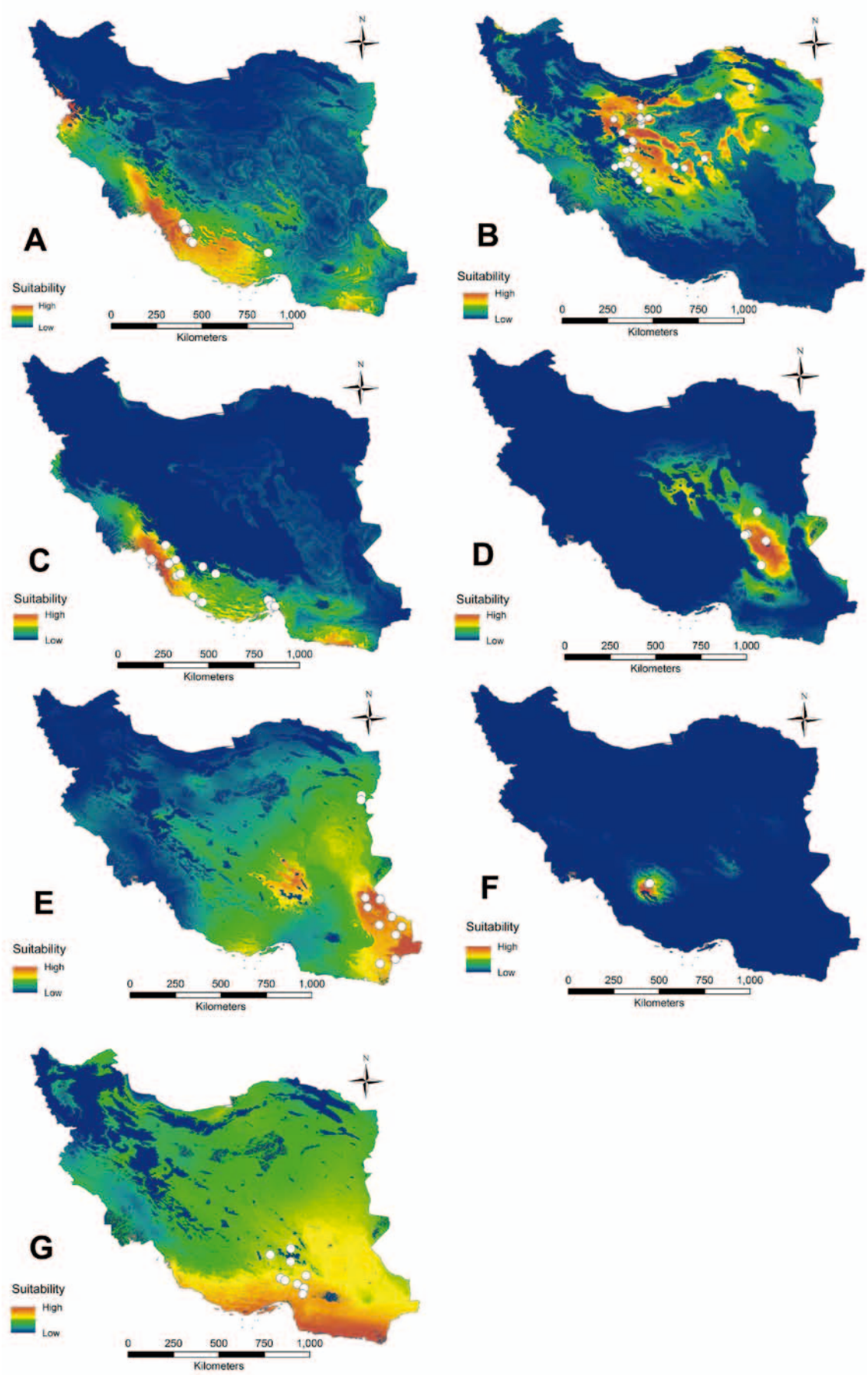

Figure 4. Potential species distribution models of spider geckos on the Iranian Plateau. A) *C. agamuroides*; B) *A. persica*; C) *C. gastrophole*; D) *R. misonnei*; E) *A. cruralis*; F) *C. persepolense*; G) *A. kermanensis*. The colours refer to the level of suitability as presented in the figure legends.

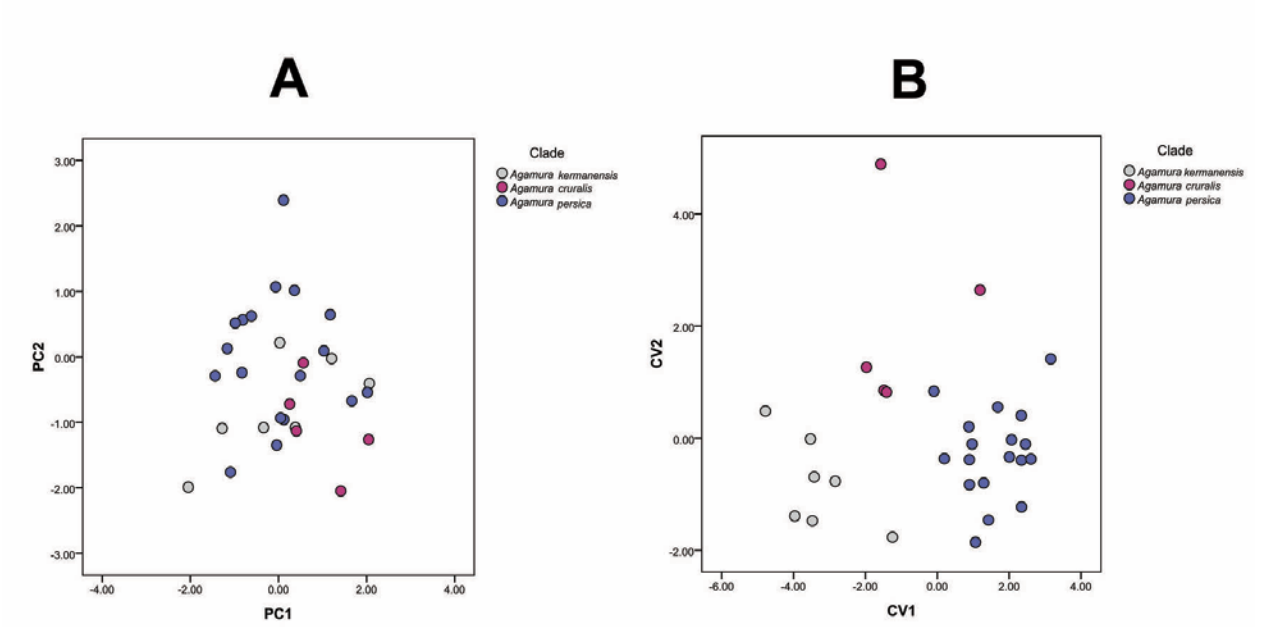

Figure 5. Ordination of principal component 1 (PC1) against PC2 (A) and canonical variate 1 (CV1) against CV2 (B) for all significant characters among *Agamura* clades in the Iranian Plateau

from the genus *Agamura*. The Genus *Rhinogekko* is considered as sister genus to *Bunopus* and confirm the previous assumptions (Szczerbak & Golubev, 1996) (Fig. S3) and separated from the *Agamura* clade with high posterior probability and bootstrap support values.

The haplotype network constructed for nuclear marker MC1R showed the similar pattern of a concatenated phylogenetic tree. According to the network (Fig. 3), separation of *Rhinogekko* and *Cyrtopodion* from the genus *Agamura* is confirmed, but the complexity in the genus *Agamura* remains. *Agamura kermanensis* is clearly differentiated from other clades with more mutations, but other two species have more similarity to each other.

Divergence time estimates

The analyses were run based on two mitochondrial genes (12S and Cyt*b*) and finally, both trees linked together (Figs. 2, S2) and the results indicated that *Rhinogekko* split from angular-toed geckos around 17 million years ago (Mya; 95% HPD: 9.5-31.0 Mya). Divergence between *Cyrtopodion* and *Agamura* started through early-mid-Miocene ca. 15 Mya (95% HPD: 7.8-25.8 Mya). Speciation within the *Cyrtopodion* clade appears to have occurred 11 Mya (95% HPD: 5.2-18.5 Mya). The split between *C. gastrophole* and *C. agamuroides* group took place around 7 Mya (95% HPD: 3.5-12.7 Mya) and the separation between *C. agamuroides* and *C. persepolense* occurred 2.8 Mya (95% HPD: 1.1-5.0 Mya). The cladogenesis of spider geckos started approximately in early Pliocene ca. 4.3 Mya (95% HPD: 2.2-7.8 Mya) and the divergence of *A. persica* and *A. cruralis* have occurred 3.2 Mya (95% HPD: 1.6-5.6 Mya).

Spider geckos in the Iranian Plateau are distributed in all areas within the plateau across the mountains, plains and deserts. The ancestor of spider geckos was retrieved from the central part of the Iranian Plateau as the distribution of most of them covered the central part (Supplementary Materials, Fig. S4). Among spider geckos, *Rhinogekko misonnei*, *Agamura persica*, *A. cruralis* and *A. kermanensis* are distributed in the Central Plateau. *Cyrtopodion* species that were used in this study are from the Zagros area. It seems the spider gecko clade first appeared from the central part of the Plateau, because few of them are distributed in other parts.

Species distribution modelling

The results of Maxent modelling shows good AUCs for all models: *A. cruralis* AUC = 0.896±0.117; *A. persica* AUC = 0.879±0.028; *A. kermanensis*. AUC = 0.865±0.071; *Cyrtopodion persepolense* AUC = 0.997±0.001; *C. agamuroides* AUC = 0.864±0.188; *C. gastrophole* AUC = 0.962±0.013; *Rhinogekko misonnei* AUC = 0.969±0.029. The predicted map indicated that suitable areas confirmed the current distribution of the species (Figs. 1, 4). The most important climate variables are presented in Table 2.

Morphological examination

Twenty-seven morphological characters were examined among three distinct clades of the genus *Agamura*. Based on the analysis of variance (ANOVA) for metric characters, five characters were distinguished as significant characters ($P < 0.05$) (Head Height (HH), Snout Length (SL), Interorbital distance (IO), Hind Limb Length (HLL) and Fore Limb Length/Snout-Vent length (FLL/SVL)). The meristic characters were analysed by Kruskal-Wallis H test and two characters were distinguished as significant (Number of scales across widest part of

abdomen (NSA), Number of scales between postmental scales and vent (NPV) (Table 3). PCA and CVA were calculated using significant characters obtained in the previous stage. In the PCA, the first three components explained 87.38% of total variance and in the CVA, the first two components explained 100% of total variance of characters (Fig. 5; Table 4, 5).

DISCUSSION

Phylogeny, diversity and endemism

The molecular results confirmed the previously known and described species belong to the genus *Cyrtopodion* (Nazarov et al., 2009; Nazarov et al., 2012) and revealed three distinct lineages of the genus *Agamura* and confirmed their species status (Fig. 2). *Agamura* was defined as a monophyletic genus with four distinct species (Szczerbak & Golubev, 1996), but was recently revised morphologically and three of them were excluded from the genus (Anderson, 1999). Two subspecies were considered for *Agamura persica*: *A. p. cruralis* for the eastern population and *A. p. persica* for the western population, with some differences in morphological characters including the number of dorsal scales and shape of the tubercles (Anderson, 1999). *Rhinogekko misonnei* is a representative of the genus *Rhinogekko* and was distinctly separated from *Agamura* (high bootstrap support and posterior probability values). It has previously been considered in the genus *Agamura* by Szczerbak & Golubev (1996) and its distinction presented recently (Anderson, 1999; Krysko et al., 2007; Sindaco & Jeremčenko, 2008). A high level of genetic differentiation between *Rhinogekko* and *Agamura* populations in our study strongly supports the exclusion of *R. misonnei* within the genus *Agamura*. *Agamura gastropholis* was one of the members of *Agamura* according to Szczerbak & Golubev (1996), but in the present study, this species was clustered within the genus *Cyrtopodion*. These geckos are common in the Iranian Plateau and have been recorded several times from arid regions of central and southern parts of Iran (Anderson, 1999; Nazarov et al., 2009; Moradi et al., 2011), but there were, until this study, no documented morphological or molecular evidence, and indeed no taxonomic study, on these geckos in the area.

The Zagros Mountains are presented as an important region of endemism in west and southern Iran (Gholamifard, 2011; Hosseinzadeh et al., 2014). Recently, several species of lizards and snakes have been described, uncovering a deep history of radiations in the region (Nazarov et al., 2009; Ahmadzadeh et al., 2012; Rajabizadeh et al., 2012). All of the described species are endemic to the Iranian Plateau and their cladogenesis began in the Miocene. Interspecific variation of 12S and Cyt*b* genes among the studied species is about 14-15% and 19-20% respectively, which is very high (Table 1). Species distribution modelling revealed that potential areas of distribution of these species did not have any overlap (Fig. 4), but different climate variables affect a species presence in a precise region (Hosseinian Yousefkhani et al., 2016). BIO6 (minimum temperature in coldest month) is the important variable for the presence of three species (*Cyrtopodion agamuroides*, *C. gastrophole* and *R. misonnei*) (Table 2). Divergence between *Cyrtopodion* and *Agamura* took place 15 Mya by uplifting of the Zagros Mountains in the mid-Miocene and then cladogenesis within these clades occurred during the Pliocene (Fig. 2) with different climate conditions.

Morphological variations between Agamura clades include the size of the body and especially in the length of limbs, which is longer in the eastern population than the western one. Ground structures in different parts of the range were observed directly during fieldwork and there are large rocks in the eastern and southern part of Iran, whereas the size of rocks decreases in central Iran. Habitat features like larger rocks are related to the structure of adjacent mountains produced by geological events and pressures over millions of years (Anders et al., 2010).

Biogeography of spider geckos

South-eastern and southern Iran are the probable regions for the spider gecko's diversification. The collision of the Arabian and Eurasian Plates about 35 - 20 Mya (Dercourt et al., 1986; Mouthereau, 2011; McQuarrie & Van Hinsbergen, 2013) and the Zagros Mountain orogeny and consequent climate change are likely to have played an important role in diversification within spider geckos. According to our results, long branches with high bootstrap supports indicated a deep divergence of Rhinogekko from other spider geckos (17 Mya) and the Cyrtopodion from *Agamura* (15 Mya). But divergence time less than 4 Mya among *A. persica* and *A. cruralis* clades indicated to the historical taxon that originated in south-east Iran. *Rhinogekko* is restricted to the Lut Desert boundary and limited dispersal apparently caused by climate factors such as hot and dry conditions in the Lut area (Pourkhorsandi & Mirnejad, 2000). Closure of the new Tethys by collision with Arabia created the Zagros Basin (Khadivi, 2010), but contact with the Lut Block (Lut Block is a rigid plate on the Iranian Plateau) created a hot and dry region. The *Cyrtopodion* clade diversified in the late Miocene as a result of uplifting of the Zagros Mountains and aridification, in addition to trapping and isolating populations of this clade in the south Zagros valleys, which directly affected the variation among them. All species examined in this study are sensitive to temperature (Table 2) in different months of year except *C. persepolense*, which is dependent on annual precipitation. This isolation and diversification is directly reflected by the Zagros uplifting which affects precipitation in south and central Iran (Ramstein et al. 1997).

The main splits among spider geckos took place by vicariance in the mid-Miocene and other divergences within clades mainly represent different dispersal waves, aridification, climate change and restriction among valleys. Climate change and aridification directly affect *R. misonnei*; restriction among valleys is a common method for cladogenesis in the *Cyrtopodion* clade, as the newly described species belonging to this genus

are isolated among valleys and restricted to their small range. Cladogenesis in the genus *Agamura* may occurred by both vicariance events and dispersal waves on the Iranian Plateau from 15 Mya (Fig. 2). The southern clade (taxa. kermanensis) was isolated by the uplifting of the Lalezar Mountain in southern Kerman and the two others (*A. persica* and *A. cruralis*) diverged from each other about 3 Mya by occupying different niches.

Taxonomic implications

The monophyly of *Cyrtopodion* and *Agamura* is confirmed and *Rhinogekko* is placed at the root of the tree as the first diverged spider geckos in south-east Iran. However, the Agamura divided into three clades that are phylogenetically distinct from others, and each clade might represent a distinct species (Fig. 2). This is confirmed by our analyses of the three major clades of *Agamura* represented on the Iranian Plateau; two of them refer to the species previously known as *A. cruralis* (eastern clade, including the type locality of *A. cruralis* from southern Baluchestan, Bahukalat) and *A. persica* (western clade) (Hora, 1926; Szczerbak & Golubev, 1996). The third clade in *Agamura* (*A. kermanensis*) diverged widely from the two others (13.2% and 11.7%; genetic differentiation from *A. cruralis* and *A. persica* in Cyt*b*, respectively). *Agamura kermanensis* can be considered as a new taxon based on morphological and molecular evidence and will be described soon by authors. The morphological variation between the newly explored clade and two other *Agamura* clades is clearly explained in Figure 5. Species niche modelling confirmed this separation by estimating separate suitable areas (Fig. 4). The haplotype network based on the nuclear marker clearly showed separation of *Agamura kermanensis* (Fig. 3).

CONCLUSIONS

The present study provides a biogeographical view of the spider geckos in the Iranian Plateau. The phylogenetic history and divergence times of spider geckos support the geological events during the Miocene period. The monophyly of *Agamura* and *Cyrtopodion* indicate an important role of Zagros orogeny in the isolation of these lineages during the mid-Miocene. Following dispersal, *Agamura* rapidly diversified in the Iranian Plateau. The southern population in Kerman province showed a distinct clade from other *Agamura* populations. The southern Zagros slopes were involved in different collision events with Arabia and then new valleys appeared. Radiation within *Cyrtopodion* clade refers to these valley creations taking place during the late Miocene. *Rhinogekko* is a distinct clade that first diverged from all other spider geckos by closing the new Tethys about 17 Mya in the Lut region. Finally, our phylogenetic analyses suggest that morphological diversity among these geckos arose by historical process such as Zagros orogeny, which can explain the basal divergence within the group. The southern clade will be described as a new taxon in a separate article by authors in future.

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