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Genetic variability of *Mesalina watsonana* (Reptilia: Lacertidae) on the Iranian plateau and its phylogenetic and biogeographic affinities as inferred from mtDNA sequences

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Abstract. The lacertid lizard *Mesalina watsonana* is widely distributed on the Iranian plateau where it is one of the most common lizards. However, the intraspecific variability and the phylogenetic position of this species within the genus still remain unknown. We sequenced a 715bp long fragment of the mtDNA cytochrome *b* gene from lizards sampled in 10 localities covering the Iranian distribution range of the species. We identified four distinct and geographically isolated clades with an average genetic divergence between them ranging from 9.8 to 13.1% (*p*-distance) which is comparable to the values of genetic distance commonly reported between Lacertidae species. Analyses combining data from recently published phylogeny of the genus *Mesalina* with our dataset confirmed the monophyly of *M. watsonana*. The isolation of this species from the rest of the genus points out the important role of the Zagros Mountains uplift during the Miocene. It is possible that this geological event participated on the isolation of the ancestor of *M. watsonana* from the rest of the *Mesalina* lizards and together with the upheaval of the whole Iranian plateau provided suitable environmental conditions for rapid diversification of this species.

Keywords. *Mesalina watsonana*, Lacertidae, Iran, Zagros, mtDNA, phylogeny.

INTRODUCTION

The upland area of the Iranian plateau represents a unique biogeographical element in the Middle East, isolated from the neighboring territories (Anderson, 1968, 1999; Fisher, 1968; Coad, 1998). It encompasses most of the territory of Iran, reaching Afghanistan and Pakistan in the east. The geographical delimitation of the Iranian plateau is determined by high mountain ranges of the Zagros in the west, Elborz and Kopet Dagh in the north, lofty peaks of Hindu Kush in Afghanistan in the east and Makran and Sulaiman moun-

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tain ranges in the south and east of Pakistan (Fisher, 1968, Khan, 1980). This area has been extensively studied from the geological point of view due to persisting geological activity; thus, the orographical history and dating of major events are well-known (Tchalenko and Braud 1974; Dersourt et al., 1986; Mouthereau, 2011). The uplift of the Zagros Mountains was initiated by the collision of the Arabian lithospheric plate moving in the north-east direction and the Eurasian landmass, which took place from the Oligocene to the Miocene 35 – 20 million years ago (MYA) (Mouthereau, 2011). This event has led to a formation of unique environmental and climatic conditions on the uplifted plateau and thus separated Iranian highland from Mesopotamian lowland populations and resulted into (sub)specific differentiation of many species of amphibians and reptiles (Wischuf and Fritz, 1996; Feldman and Parham, 2004 - *Mauremys*; Hrbek et al., 2006 – *Aphanius*; Rastegar-Pouyani et al., 2006 - *Asaccus*).

For a long time, the lizards of the genus *Mesalina* Gray, 1838 were considered a part of the genus *Eremias* Fitzinger, 1834, until Szczerbak (1974) resurrected Gray´s generic name and separated these small lizards from the more robustly built genus *Eremias* distributed mostly in Central Asia. *Mesalina* lizards inhabit dry regions of Africa along the northern coast from Senegal to Somalia; some species penetrate through the Sahara desert into the Sahel. Their distribution spans eastwards throughout the Arabian Peninsula and Iranian plateau to Northwestern India. The genus *Mesalina* currently encompasses 14 described species. Whereas the majority of the species occur west of the Zagros Mountains, there are only two species inhabiting the eastern part of the genus range, with *M. watsonana* being the only one living right on the Iranian plateau (Anderson, 1999; Sindaco and Jeremčenko, 2008).

The first attempt to resolve phylogenetic relationships inside the genus *Mesalina* using molecular data was done by Joger and Mayer (2002). Their results brought justification for an elevation of the Socotran *M. kuri* to a species level, but did not provide any further phylogenetic information concerning the relationships among other studied taxa. Recently, Kapli et al. (2008) used partial cyt *b* and 16S rRNA sequences (mtDNA) to infer phylogeny and phylogeography of the North African members of the genus *Mesalina*. Their study revealed high genetic differences between eastern and western populations of the widespread *M. guttulata* and across the distribution of *M. brevirostris*. With the application of molecular clock calibration, they showed these intraspecific evolutionary splits took place between 7.1 to 9 MYA and may have been correlated with climate change in North Africa and southwest Asia during the Miocene (Kapli et al., 2008). However, neither Joger and Mayer (2002) nor Kapli et al. (2008) included *M. watsonana* in their analyses, so the phylogenetic position of this species still remains unclear. According to its morphology, it is considered a part of a monophyletic group with *M. guttulata* (Arnold, 1986).

In spite of the fact that *M. watsonana* is considered abundand and extremely common (Smith, 1935), we are not aware of any publication aimed directly on this species. In the present study, we focused on the genetic differentiation among the populations of *M. watsonana* from the Iranian plateau and the phylogenetic position of this species within the genus using mitochondrial cyt *b* gene sequences. This marker is traditionally used for assessing both intraspecific variability within lacertid lizard species and relationships among different genera (Poulakakis et al., 2003, 2005; Carranza et al., 2004; Podnar et al., 2004, 2005; Pavlicev and Mayer, 2009). An assessment of the Iranian populations may

help us not only uncover biogeographic history of the genus *Mesalina*, but also provide a reliable model revealing evolutionary history of this region.

MATERIALS AND METHODS

Sampling

A total of 16 individuals of *M. watsonana* from ten distinct geographical locations were used. The sampling sites were chosen to cover all the Iranian range of the species´ distribution (Fig. 1). Sequences of other lacertids, *M. guttulata* and *Ophisops elegans*, were used as outgroup species in the phylogenetic analyses. All tissue samples used in this study came from the material deposited in the collections of the Faculty of Science, Charles University in Prague and from the National Museum in Prague. To infer overall phylogenetic position of *M. watsonana* within the genus, sequences of other species already published elsewhere (Whiting et al., 2003; Kapli et al., 2008; Rastegar-Pouyani et al., 2010) were used. The complete list of the new material as well as GenBank accession numbers of elsewhere published sequences used in this study are given in Table 1.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from tissue pieces (tail tips, thigh muscles) using Qiagen DNAeasy® Tissue Kit (Qiagen) according to the manufacturer's protocol. We amplified a fragment

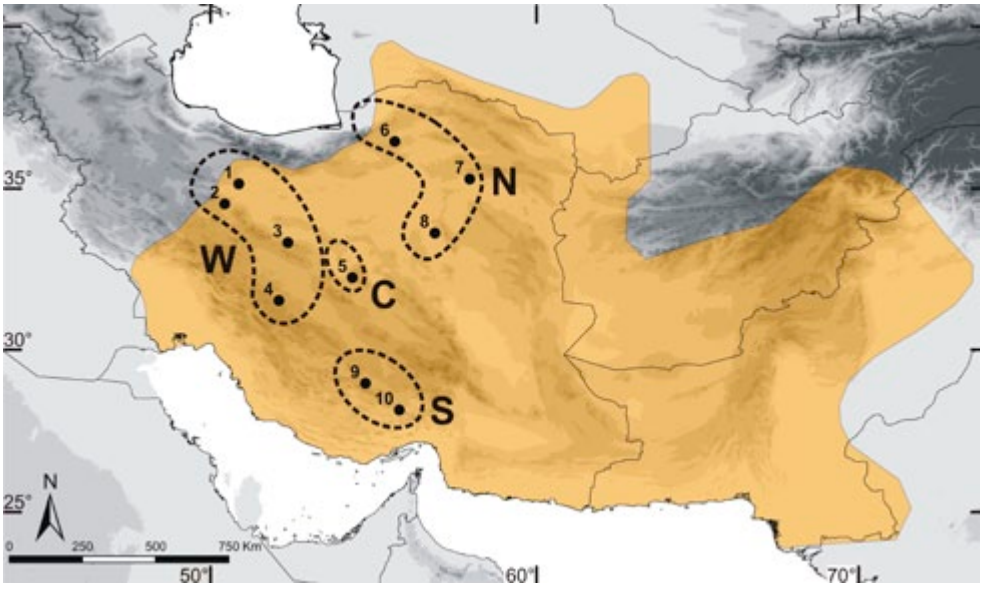

Fig. 1. Map of distribution of *M. watsonana* and the sample sites used for this study. Numbers correspond to the locality numbers in Tab. 1. Dashed lines delimit individual clades recovered from the genetic analyses.

Table 1. List of samples of *M. watsonana* including geographical origin and GenBank Accesion numbers, including sequences of *Mesalina* species taken from GenBank used for the taxon-wide phylogeny.

Species	Sample	Locality; number in Fig 1	GPS	GenBank Acc no.	Source
M. watsonana	Gah 09	Gahkom, Iran; 10	28°10'54.01"N, 55°49'20.13"E	JN828632	This study
M. watsonana	Ard 14	Ardestan, Iran; 3	33°18'19.64"N, 52°24'2.39"E	JN828633	This study
M. watsonana	Bard 05	Bardeskan, Iran; 7	35°14'57.92"N, 57°58'47.05"E	JN828634	This study
M. watsonana	Izad 03	Izadkhast, Iran; 4	31°31'58.45"N, 52°7'32.09"E	JN828635	This study
M. watsonana	Izad 04	Izadkhast, Iran; 4	31°31'58.45"N, 52°7'32.09"E	JN828636	This study
M. watsonana	Izad 08	Izadkhast, Iran; 4	31°31'58.45"N, 52°7'32.09"E	JN828637	This study
M. watsonana	May 06	Mayamey, Iran; 6	36°24'10.17"N, 55°41'13.30"E	JN828638	This study
M. watsonana	Salaf 07	Salafchegan, Iran; 2	34°29'20.17"N, 50°28'12.90"E	JN828639	This study
M. watsonana	Tab 11	Tabas, Iran; 8	33°35'57.37"N, 56°54'44.12"E	JN828640	This study
M. watsonana	Vaz 01	Vazireh, Iran; 9	28°59'50.53"N, 54°46'52.49"E	JN828641	This study
M. watsonana	Vaz 10	Vazireh, Iran; 9	28°59'50.53"N, 54°46'52.49"E	JN828642	This study
M. watsonana	Kush 001	Kushk-e Nosrat, Iran; 1	35°6'39.11"N, 50°53'51.71"E	JN828643	This study
M. watsonana	Kush 02	Kushk-e Nosrat, Iran; 1	35°6'39.11"N, 50°53'51.71"E	JN828644	This study
M. watsonana	Kush 12	Kushk-e Nosrat, Iran; 1	35°6'39.11"N, 50°53'51.71"E	JN828645	This study
M. watsonana	Kush 13	Kushk-e Nosrat, Iran; 1	35°6'39.11"N, 50°53'51.71"E	JN828646	This study
M. watsonana	Anj	Anjireh, Yazd, Iran; 5	32°13'60.00"N, 54°22'60.00"E	JN828647	This study
M. guttulata	Jem 109	Ghayl Ba Wazir, Yemen	14°54´36"N, 49°02´14"E	JN828648	This study
M. guttulata		Jordan	31°15'11.16"N, 35°36'48.6"E	EF555250	Kapli et al. 2008
M. guttulata		Libya	30°27'57.24"N, 24°32'11.76"E	EF555254	Kapli et al. 2008
M. guttulata		Morocco	32°02'49.92"N, 4°24'31.68"W	EF555255	Kapli et al. 2008
M. guttulata		Morocco	31°24'06.48"N, 5°43'39.36"W	EF555256	Kapli et al. 2008
M. guttulata		Tunisia	33°31'21"N, 9°59'33"E	EF555268	Kapli et al. 2008
M. guttulata		Tunisia	33°09'00.72"N, 10°17'23.64"E	EF555270	Kapli et al. 2008
M. guttulata		Jordan	29°34'13.44"N, 35°24'45.68"E	EF555279	Kapli et al. 2008
M. guttulata		Egypt	28°25'58.79"N, 29° 4'58.21"E	AY217815	Whiting et al. 2003
M. brevirostris		Iran		FJ416173	Rastegar-Pouyani et al. 2010
M. brevirostris		Syria	35°25'02.64"N, 40°19'11.28"E	EF555264	Kapli et al. 2008
M. brevirostris		Syria	35°25'36.48"N, 40°01'40.08"E	EF555266	Kapli et al. 2008
M. brevirostris		Syria	34°17'35.16"N, 36°45'55.8"E	EF555267	Kapli et al. 2008
M. rubropunctata		Egypt	24°24'N, 33°01'01.2"E	EF555274	Kapli et al. 2008
M. bahaeldini		Egypt	28°32'26.88"N, 33°58'51.6"E	EF555243	Kapli et al. 2008
M. olivieri				EF555247	Kapli et al. 2008
M. olivieri				EF555249	Kapli et al. 2008
M. olivieri		Tunisia	32°07'43.32"N, 10°33'49.68"E	EF555272	Kapli et al. 2008
M. olivieri				EF555273	Kapli et al. 2008
M. simoni		Morocco	31°54'43.2"N, 7°30'18"W	EF555259	Kapli et al. 2008
Ophisops elegans	Fam35	Famenin, Iran	35°08'45.68"N, 48°52'33.40"E	JN828649	This study

of about 700bp of the mtDNA cytochrome *b* gene with primers L14841 (Kocher et al., 1989) and CB3H (Palumbi, 1996). Amplification involved an initial cycle of denaturation at 93°C for 2 min, 41 subsequent cycles of 93°C for 1 min, 46°C for 1 min and 72°C for 1 min, and final step of extension at 72°C for 10 min. PCR products were subsequently purified using QIAquick® PCR Purification kit (Qiagen) following manual therein. Sequencing was conducted on ABI PRISM 3100 Avant Genetic Analyzer at the Laboratory of DNA sequencing, Faculty of Science, Charles University in Prague.

Phylogenetic analyses

Alignment of concatenated sequences was performed with Clustal W (Thompson et al., 1994) as implemented in BioEdit 7.0 (Hall, 1999) and checked manually. Prior to analyses, all sequences were translated into amino acids using vertebrate mitochondrial translation code. This did not reveal any stop codons or gaps suggesting that all protein coding sequences were functional and no pseudogenes were amplified. The alignment for the dataset including *M. watsonana* and selected outgroups (referred to as *watsonana* dataset hereupon) was 715 bp long. The second dataset including *M. watsonana* sequences and additional sequences of *Mesalina* species retrieved from GenBank (referred to as taxon-wide dataset hereupon) resulted in an alignment with a limited overlap of 321 bp of our own, and the GenBank data. Average genetic uncorrected distances (*p*-distance) between individuals and mitochondrial clades were calculated in MEGA 2.1 (Kumar et al., 2001).Three different phylogenetic analyses were performed: Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). MP analysis was performed in PAUP* 4.0b10 (Swofford, 2003) under heuristic search criterion with 100 random stepwise addition and tree-bisection-reconnection (TBR) branch swapping. ML analyses were conducted using PhyML 3.0 (Guindon and Gascuel, 2003) with the nearest neighbor interchange (NNI) tree improvement, and adopting the HKY+I+G model as the best fitting model of substitution for both datasets. The most appropriate model of sequence evolution under both Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) was selected by jModelTest 0.1.1 (Posada, 2008). Nodal support for MP and ML trees was assessed by 1000 bootstrap pseudoreplications (Felsenstein, 1985). Bayesian analyses were performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) implementing the HKY+I+G model, with two runs and four chains for each run for $5x10^6$ generations with the sampling frequency of every 100^{th} generation that produced 50 000 sampled trees. After each analysis and after the assurance that the log-likelihood scores achieved stationarity (as plotted against generation time), the first 10% of the trees (5000) was discarded as a burn-in. A 50% majority rule consensus tree was then produced from the posterior distribution of the trees, and posterior probabilities calculated as the percentage of a sampled tree recovering any particular clade (Huelsenbeck and Ronquist, 2001).

Molecular clock calibration

To estimate divergence dates among individual lineages, we used a Bayesian coalescent approach as implemented in the software BEAST v1.6.1 (Drummond and Rambaut, 2007). Because our major interest was to estimate the outbranching date of *M. watsonana* from the rest of the species, we performed the calibration on the dataset consisting of all available *Mesalina* species from GenBank. We used only one representative animal for each haplotype for this analysis. Kapli et al. (2008) used the dating of the split between *Gallotia stehlini* and *G. simonyi* calculated by Maca-Meyer et al. (2003) as a calibration point. However, the dating of diversification of *Gallotia* lizards was recently reassessed by Cox et al. (2010) and all the major diversification events were moved farther to the geological history. Therefore, we based our calibration on the data of Cox et al. (2003) and employed the splits between *Psammodromus* lizards and *Gallotia stehlini* and between *G. stehlini* and

G. simonyi as calibration points (17 – 20 MYA, 11 – 13 MYA respectively). As a next calibration point we used the diversification of *Timon lepidus* from *Dalmatolacerta oxycephala* 5.3 MYA as used by Hispley et al. (2009), which was based on Estes´s (1983) paleontological findings. The following sequences used for molecular clock calibration were also used as outgroups in the taxon-wide dataset in other phylogenetic computations: *P. algirus* (EU116517), *G. simonyi* (AF101219), *G. stehlini* (AY154899), *T. lepidus* (GQ142119), *D. oxycephala* (GQ142129). We employed the Yule tree prior as suggested for the species-level phylogenies, with randomly generated starting tree, an uncorrelated lognormal relaxed molecular clock model and under the HKY+I+G model. The priors for the most recent common ancestors (tmrca) for the calibration points were specified as having normal distribution. The analysis was run twice with $5x10^7$ generations; achieved stationarity and convergence of both runs were checked in Tracer 1.5 *(*Rambaut and Drummond 2007) and a burn-in of 20% performed in TreeAnnotator 1.6.1 (Drumond and Rambaut 2007).

RESULTS

The *watsonana* dataset alignment consisted of 715bp out of which 263 positions were variable and 170 parimony informative. All phylogenetic methods resulted in nearly identical tree. MP resulted in 10 equally parsimonious trees, ML analysis resulted in a topology with lnL= -2975.12 which was comparable to the one recovered by MrBayes (mean lnL = -2992.16). The final parameter estimates (obtained from PhyML) for this dataset were: gamma shape parameter = 0.188, Ti/Tv ratio = 10.544, base frequencies f(A)= 0.26, f(C)= 0.30, f(G)= 0.14, f(T)= 0.30. Sixteen analyzed samples were represented by 12 different haplotypes. All *M. watsonana* samples formed a clade with high bootstrap and posterior probabilities support (MP/ML/BI: 100/93/1). Four main and well-defined clades of this species were recovered from all analyses: 1) the southern lineage including three animals from localities Gahkom and Vazireh (100/89/-; average *p*-distance within this group equal to 0.7%); 2) the central lineage consisting of only one sample from Anjireh, 3) the northern lineage including animals from Bardeskan, Tabas and Mayamey (100/92/1; average within-group *p*-distance equal to 1.8%), with Tabas and Bardeskan samples clustering together with high support (93/98/1), and 4) the western clade, including samples from Izadkhast, Ardestan, Salafchegan and Kushk-e Nosrat (90/93/0.99; average within-group *p*-distance equal to 4.5%). Inside the last group, two sister lineages were recognized, one formed by three animals from Izadkhast (100/99/1, two of them sharing the same haplotype), and another one from the six remaining samples (99/96/1, two haplotypes present twice). Although the four major evolutionary branches were very well supported, mutual relationships among them remain unresolved (Fig. 2). Genetic distances between them were surprisingly high (uncorrected *p*-distance ranging from 9.8 to 13.1%). Average between-group uncorrected genetic distances are summarized in Tab. 2.

MP analysis of the taxon-wide dataset produced 16 most parsimonious trees. The 50% majority-rule consensus tree was nearly identical to the results of other computational approaches except of low bootstrap values for higher clades. ML analysis resulted in a topology with lnL= -5494.08009, Gamma shape parameter with 4 discrete categories = 0.219, Ti/Tv ratio = 8.798, nucleotide frequencies: f(A)= 0.27, f(C)= 0.29, f(G)= 0.14, f(T)= 0.30. Mean log-likelihood values obtained from bayesian analysis was equalt to -5461.980505. All computational methods provided the same topology for the subtree of

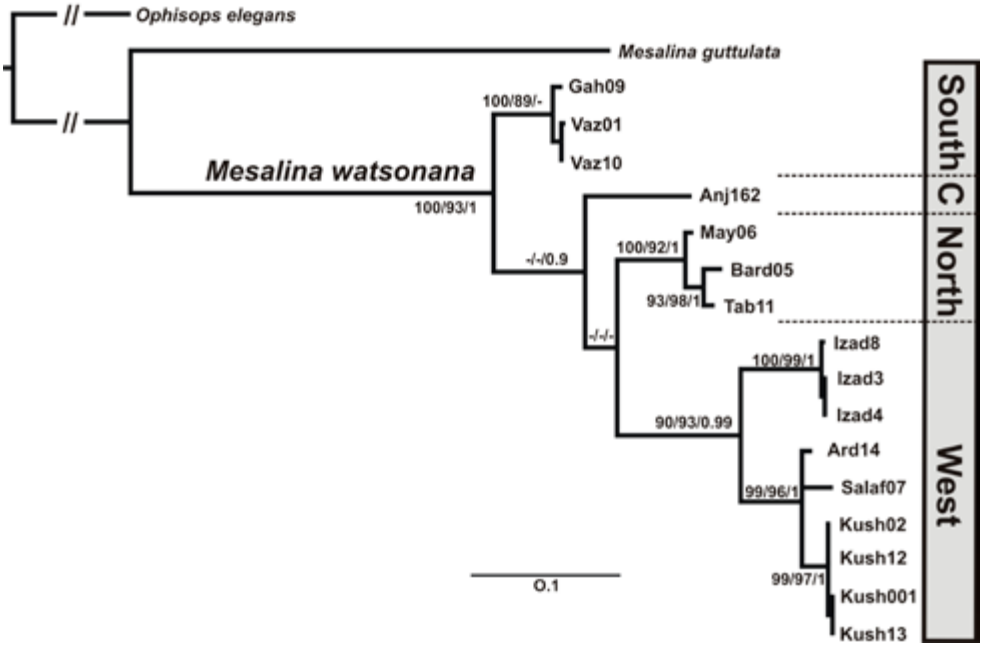

Fig. 2. Bayesian inference phylogram of *M. watsonana* based on 715bp fragment of cyt *b*. MP and ML bootstrap support values above 70% and BI posterior probabilities above 0.9 are indicated above branches.

Table 2. Average uncorrected genetic distances (*p*-distance) between individual clades of *M. watsonana* from the Iranian plateau based on 715 bp fragment of cyt *b*. Within-group distances are reported in corresponding fields on the diagonal.

	S	C	N	W
South	0.007			
Central	0.100	-		
North	0.099	0.098	0.018	
West	0.131	0.120	0.108	0.045

M. watsonana as described above. In this taxon-wide phylogeny, all samples of *Mesalina* form a monophyletic clade (82/79/0.92) consisting of two main branches – one represented by all of the samples of *M. watsonana* clustering together with one GenBank sample of *M. brevirostris* (FJ416173) (99/96/1) and the other lineage formed by all the remaining species, although this clade has only moderate support (-/79/0.94). Mutual relationships among individual *watsonana* clades remain as described above. For comments on the GenBank sample nested within *watsonana,* see discussion. Within the second group, *M. olivieri* and *M. simoni* formed a separate clade (-/83/0.96) sister to a cluster of *brevirostris*, *guttulata*, *bahaeldini* and *rubropunctata* (-/74/0.94). *M. brevirostris* stands apart

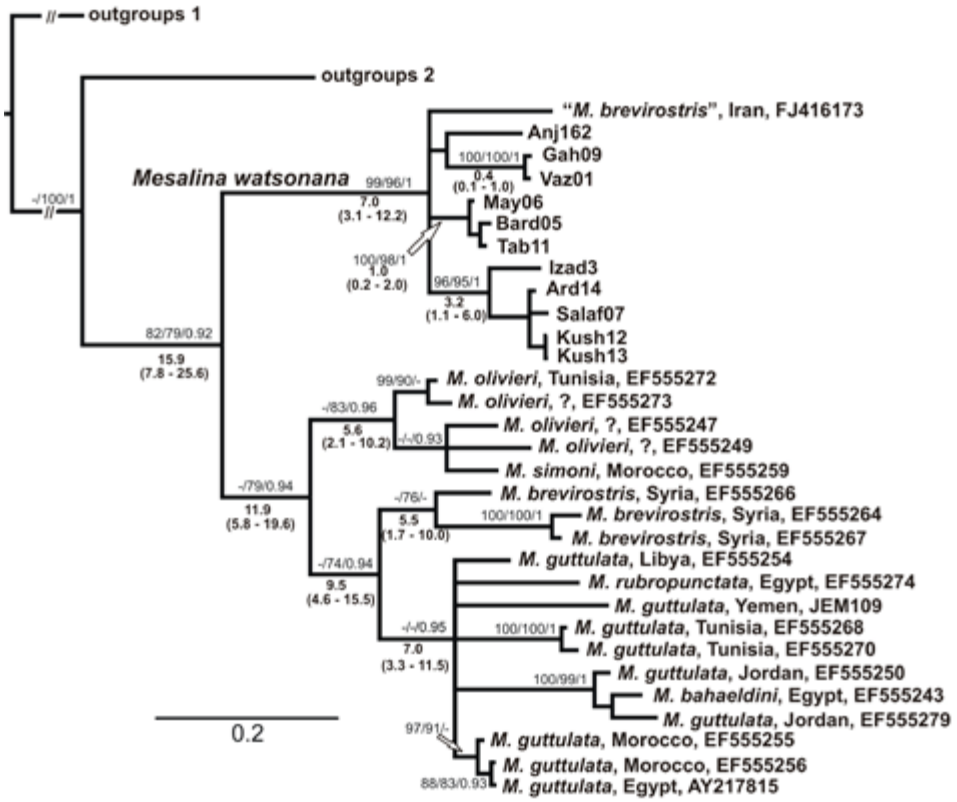

Fig. 3. Taxon-wide phylogeny of the genus *Mesalina*. The tree is a 50% majority rule consensus tree of the Bayesian analysis. MP and ML bootstrap support values above 70% and BI posterior probabilities above 0.9 are indicated above branches. Age estimates are indicated below nodes in bold and include the mean and the HPD 95% confidence interval.

from the other three species, however, with a very week support (-/76/-). The cluster of *guttulata*, *bahaeldini* and *rubropunctata* (-/-/0.95) forms an unresolved polytomy rendering *guttulata* paraphyletic. Topology of this group is in a general agreement with the tree presented by Kapli et al. (2008), except of paraphyly of *M. guttulata* with respect to *rubropunctata* and *bahaeldnini* and lower resolution of mutual relationships. We ascribe all this to shorter sequences of mtDNA used in the taxon-wide analysis. The new sample of *guttulata* from Yemen (JEM109) clusters with its conspecific sequences. The BI tree of the taxon-wide dataset is depicted in Fig. 3. Average between-species uncorrected *p*-distances are reported in Tab. 3.

The molecular clock analysis brought the following results: separation of Iranian *M. watsonana* from the other species occurred 15.9 MYA, the 95% Highest Posterior Density (HPD) interval ranged between 7.8 – 25.6 MYA. Within *M. watsonana*, the basal radiation resulting in the four independent clades took place 7.0 MYA (HPD: 3.1 – 12.2). Within the Western clade, the specimens from the locality Izadkhast branched out from

Table 3. Average uncorrected genetic distances (*p*-distances) between species or species groups of *Mesalina* used in this study, based on 321 bp of cyt b. Species with unresolved phylogeny are merged into one group. Mo – *Mesalina olivieri*, Ms – *M. simoni*, Mbr – *M. brevirostris*, Mba – *M. bahaeldini*, Mg – *M. guttulata*, Mr – *M. rubropunctata*, Mw – *M. watsonana*, Mbr* - probably misidentified *M. watsonana* determined as *M. brevirostris* by Rastegar-Pouyani et al. (2010).

	Mo + Ms	Mbr	Mba + Mg + Mr	***Mbr**** **FJ416173**
Mo + Ms				
Mbr	0.150			
Mba + Mg + Mr	0.148	0.151		
Mbr* **FJ416173**	0.194	0.181	0.186	
Mw	0.170	0.179	0.188	0.121

the rest 3.2 MYA (HPD: 1.1 – 6.0). In the Northern clade, the sample from Mayamey (May06) separated from the two others (Bard05, Tab11) 1.0 MYA (HPD 0.2 – 2.0). Diversification dates of the remaining species predate the results of Kapli et al. (2008) because of different calibration settings. In our analyses, the basal split between *olivieri* + *simoni* clade and the cluster of *brevirostris*, *rubropunctata*, *guttulata* and *bahaeldini* took place 11.9 MYA (HPD: 5.8 – 19.6), *brevirostris* branched off 9.5 MYA (HPD: 4.6 – 15.5) and the radiation of *gutulata*, *rubropunctata* and *bahaeldini* occured 7.0 MYA (HPD: 3.3 – 11.5).

DISCUSSION

Intraspecific variability of M. watsonana

In our work, we used samples of *M. watsonana* from ten localities throughout the Iranian species´ distribution range. Phylogenetic analyses based on the 715bp fragment of cyt *b* indicate existence of four highly supported main clades of *M. watsonana* occurring in separate geographic regions across the Iranian plateau: the Southern clade from the southernmost tip of the Zagros mountains, the Central clade from the plains in the middle of Iran, the Western clade from the foothills of the Zagros, and the Northern clade. The western clade can be further divided into two separate lineages, one from the locality Izadkhast, another from the localities Salafchegan, Ardestan and Kushk-e Nosrat. Obvious geographic isolation of the *M. watsonana* clades is, however, remarkable. Whereas the separation of the Northern clade can be explained by the presence of vast desert areas of Dasht-e Lut and Dasht-e Kavir in central Iran that were repeatedly flooded with saltwater (Anderson, 1999), the divergence between Western, Central and Southern clades can not be clarified by presence of any apparent natural barrier. Moreover, high activity and low biotope preferences of *Mesalina* lizards (Smith, 1935; Anderson, 1968; Baker et al., 2004) suggesting high dispersal ability are in contrast with the presence of such diversified clades. On the other hand, high diversity of reptilian species or species groups was already reported from this particular territory for *Bunopus tuberculatus* (Červenka et al., 2008) and *Eremias persica* (Rastegar-Pouyani et al., 2010).

All four *watsonana* clades are mutually separated by high genetic distances ranging from 9.8 – 13.1% (see Tab.2). These distances are in concordance with those of other *Mesalina* species found by Kapli et al. (2008). According to their findings, genetically remotest populations of *M. brevirostris* are separated by 11.5%, intraspecific variability of *M. gututlata* reaching up to 15.6%. Taking into account Harris´s (2002) average genetic distance between congeneric species of reptiles being 13.6%, the real diversity of the genus *Mesalina* is still underestimated and should be revised. Our inability to resolve phylogenetic relationships among *watsonana* clades can be either caused by fast evolution and thus high saturation rate of the cyt *b* or by rapid radiation of *Mesalina* in Iran. Fast radiation of the whole Lacertidae was already suggested by Mayer and Pavlicev (2007) and Pavlicev and Mayer (2009) and could have taken place in *Mesalina* as well. We did not, however, cover the entire distribution of *M. watsonana*. Samples from eastern populations from Afghanistan and Pakistan might affect the tree topology and might disclose closer relationships among the clades. Moreover, additional genetic data (including nuclear) and detailed morphological study encompassing material from the type locality (between Karachi and Sukkur, Sind, Pakistan) are essential for any taxonomic revisions. Thus, we suggest to keep considering *M. watsonana* as a single species, although it has high intraspecific genetic variability.

Comments on the phylogeny of Mesalina

By combination of our data with the GenBank sequences, we provide a taxon-wide phylogeny of the genus *Mesalina*. According to the analyses of all *Mesalina* sequences available, *M. watsonana* always forms a separate clade clearly distinct from all the other species. The lineage of GenBank species has basically the same topology as published by Kapli et al. (2008) with *olivieri* and *simoni* forming a clade sister to a lineage of *brevirostris* + *guttulata* + *bahaeldini* + *rubropunctata*. *M. brevirostris* forms a sister lineage to the three remaining species, albeit with low to moderate support. The clade consisting of *guttulata* + *bahaeldini* + *rubropunctata* remains polytomic. The position of *rubropunctata* differs from the results of Kapli et al. (2008) based on 16S rRNA, where it formed a clade close to *brevirostris*, although without a reliable bootstrap support. Its placement within the *guttulata* clade in our analyses could result from the usage of a short fragment of mtDNA for the analyzes. The nesting of *bahaeldini* within *guttulata* remains consistent with Kapli et al. (2008).

In accordance with its geographic isolation, *M. watsonana* forms a monophyletic clade deeply separated from the rest of the genus. This species was long considered a subspecies of *M. guttulata* until Arnold (1986) elevated this form to a species rank on the basis of detailed hemipenial morphology. Taking this into account, one would suppose closer relationship of these two forms. Not only they are separated by a high genetic distance, moreover there are also several other evolutionary lineages (=species) closer to *M. guttulata*. Similar morphology leading former authors to consider *M. watsonana* a subspecies of *M. guttulata* is in this case nothing but phenotypic conservativeness of *Mesalina* lizards. This may be caused by the adaptation to xeric environmental conditions and similar habitat types spanning across *Mesalina* distribution range without any obvious selective pressure affecting their morphology.

The new *guttulata* sample from Yemen clusters well to the rest of *M. guttulata* specimens. Genetic distance separating Yemeni and Egyptian *M. guttulata* (14.1%, data not shown) suggests a long-time independent evolution.

Yet another noteworthy sequence was a sample of *M. brevirostris* (FJ416173) nested within the *M. watsonana* clade. This was most probably caused by misidentification of the animal belonging to the latter species. This animal was collected in a region where the distribution of both species overlap (Anderson, 1999). However, if this specimen really fits the morphological characteristics of *M. brevirostris*, then we might be dealing with hybridizaiton and gene introgression from one species to another. In any case, detailed study of the zone of sympatry should be carried out in order to clarify the phylogenetic position of Iranian populations of *M. brevirostris* with respect to the Levantine populations of this species and of the sympatric *M. watsonana*.

Biogeography

M. watsonana is the only member of the genus inhabiting the Iranian plateau. Although its distribution partly overlaps with *M. brevirostris* in the Mesopotamian plain and in the southeastern Pakistan, *M. watsonana* is the only species exceeding beyond the Zagros and the Sulaiman ranges (Khan, 1980; Anderson, 1999). This striking pattern can be, however, explained by the geological history of this region. The collision of the Arabian tectonic plate to the Eurasian landmass took place 35 - 20 MYA (Dercourt et al., 1986 Mouthereau, 2011), its drifting movement in the northeast direction resulted in the continuous uplift of the Zagros Mountains which culminated 12.4 – 10 MYA (Sborshchikov et al., 1981; Mouthereau, 2011). Thus, the outbranching time of *M. watsonana* which is dated back to the middle Miocene (15.9 MYA) can be correlated with this geological event. After its formation, Zagros established as the major biogeographic barrier in the Middle East and hampered *Mesalina* the east-west dispersion.

Our findings regarding the evolutionary history of *Mesalina* lizards predate the results of Mayer and Pavlicev (2007) and of Kapli et al. (2008) and they are in concordance with those of Hipsley et al. (2009), who suggest earlier diversification of lacertids with the split of Ethiopian and Saharo-Eurasian clades (sensu Mayer and Pavlicev, 2007) dated back to the middle Eocene (43.2 ± 5.6 Myr) and the radiation of Saharo-Eurasian xerophilous forms (*Acanthodactylus*, *Omanosaura*, *Ophisops*, *Eremias* and *Mesalina*) following soon after (40.2 ± 5.1 Myr). Hipsley et al. (2009) date the divergence between *M. guttulata* and *M. brevirostris* to the late Miocene (10.3 ± 2.9 Myr). According to available data, the ancestors of the genus *Mesalina* penetrated from the east Africa into Asia, spread over the southwest Asia and re-entered North Africa again (Kapli et al., 2008). *M. watsonana* might have separated even before the uplift of the Zagros Mountains took place, but the ongoing raise of the massif isolated this species from the rest of the genus and thus prevented gene flow. Subsequently, the remaining *Mesalina* species then diversified in North Africa in several steps ~ 11.9 MYA (separation of *olivieri* + *simoni*), ~ 9.5 MYA (separation of *brevirostris*) and ~ 7.0 MYA (*guttulata* clade radiation). Thus, as we can see, both dispersal and vicariance processes may have played an important role in the evolution of the *Mesalina* group. Within *M. watsonana*, the split into the four discovered lineages took place in the late Miocene (5.6 MYA). Since there are no apparent geographical barriers

limiting the dispersal of these lizards on the Iranian plateau, it remains disputable whether the four lineages became isolated via vicariant in situ diversification or by dispersion of already separated populations.

In our study, we brought a new view on the phylogeny of the genus *Mesalina*. The results presented confirm the monophyly of *M. watsonana* and its isolated position within the genus, which is supported by historical biogeographic events. Nevertheless, broader sampling accross the entire distribution and more genes (including nuclear) involved might help bring closer insight into the phylogeny and phylogeography of these lizards.

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