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ORIGINAL ARTICLE

Historical biogeography of the lacertid lizard *Mesalina* in North Africa and the Middle East

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ABSTRACT

Aim We explored the phylogenetic relationships of species of *Mesalina*, using one nuclear and two mitochondrial loci. This genus of lacertid lizards is widely distributed in North Africa and the Middle East and our goal was to develop a scenario capable of explaining the current distribution and evolutionary patterns within the genus in the context of the wider historical biogeography of the region.

Location North Africa and the Middle East.

Methods The assembled dataset consisted of 193 *Mesalina* individuals, representing 12 species distributed across the geographical range of the genus. Bayesian and maximum likelihood methods were used to support phylogenetic inferences on two mitochondrial (cytochrome *b* and 16S ribosomal RNA) and one nuclear (beta-fibrinogen intron 7) markers. Palaeogeographical and palaeoclimatic data were used to support the inferred phylogeographical patterns.

Results *Mesalina* lizards exhibit high genetic diversity and complex phylogenetic patterns, leading to an unsatisfactory systematic hypothesis of one paraphyletic and three polyphyletic traditional species. The estimated divergence times place the origin of the genus in the early Miocene (*c.* 22 Ma) and the divergence of most currently recognized species in the middle to late Miocene. The inferred ancestral distribution suggests that the genus and most of its species originated somewhere in Arabia or the Middle East, with the exception of the *Mesalina olivieri* complex, which may be of African origin.

Main conclusions Phylogenetic reconstruction based on the three loci studied suggests a higher than expected cryptic diversity of *Mesalina* in North Africa and the Middle East. We suggest that the tectonic movements of the Arabian plate, coupled with the climatic changes occurring since the Miocene, may be responsible for the phylogeographical patterns of North African and Middle Eastern *Mesalina*.

Keywords

Cryptic diversity, historical biogeography, lacertid lizards, *Mesalina*, Middle East, Miocene, North Africa, palaeoclimatic oscillations, phylogenetic reconstruction, tectonics.

INTRODUCTION

The high level of diversity and endemism in the Mediterranean Basin, described as a 'global hotspot' (Brooks *et al.*, 2006), can be explained by its unique variety of habitats. These include mediterranean, mesic, alpine and xeric environments, and geomorphological configurations such as mountains, islands and deserts, resulting in a complex historical biogeography. Many studies have examined how this complex biogeography may have fuelled speciation processes and faunal assemblages in the European Mediterranean Basin. However, our knowledge of the evolutionary history of communities in the African and Asian Mediterranean Basin remains poor.

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Most of the phylogeographical effort in the non-European part of the Mediterranean Basin has focused on the Maghreb region of North Africa and Anatolia (Fonseca *et al.*, 2009; Kornilios *et al.*, 2011; Gonçalves *et al.*, 2012). Few studies have included representatives from the Middle East and the eastern part of North Africa (Gvozdik *et al.*, 2010; Migliore *et al.*, 2012) or, in particular, from the Arabian Peninsula, in spite of the latter's long and complex faunal interchange with the Middle Eastern and North African areas (Amer & Kumazawa, 2005; Carranza *et al.*, 2008; Pook *et al.*, 2009; Metallinou *et al.*, 2012).

The collision of the Arabian Peninsula and the Anatolian plate led to the closure of the Tethyan seaway (Rögl, 1999; Popov *et al.*, 2004) and the formation of the so-called *Gomphotherium* land bridge, which permitted terrestrial interchange between Eurasia and Africa during the early Miocene, *c.* 19 Ma (Rögl, 1999). The movement of the Arabian Peninsula also caused the emergence of several geographical barriers that played a significant role in the formation of the distribution patterns of North African and Middle Eastern species. Among the most important barrier-forming events were the opening of the Red Sea, the formation of the Sinai Peninsula, the two rifts on either side of Sinai (i.e. the Wādī 'Araba on the east and the Gulf of Suez on the west) and the formation of the Taurus and Zagros mountain ranges (Arnold, 1987; Disi *et al.*, 2001; Harzhauser *et al.*, 2007).

Climatic oscillations during the Cenozoic have also influenced the faunal composition of North Africa and the Middle East (Le Houérou, 1997; Anderson, 1999). During the Cenozoic, the climate has changed successively from warm and humid into the Quaternary glaciations (Zachos *et al.*, 2001). Tectonic changes, such as the uplift of the Tibetan Plateau and the East African Rift, have reinforced these climate changes and marked the onset of aridity in North Africa and Arabia (Micheels *et al.*, 2009). In particular, the first appearance of drier grasslands, followed by major faunal turnover, is documented during the mid-Miocene (*c.* 14 Ma) in Africa, following the global trend of increased aridity in the mid-latitudes (Flower & Kennett, 1994). The broader aridification events that eventually resulted in the formation of the Sahara are largely attributed to the Miocene–Pliocene (Le Houérou, 1997; Schuster *et al.*, 2006; Micheels *et al.*, 2009); desert conditions similar to the Sahara may have appeared by at least 7 Ma (Schuster *et al.*, 2006).

Molecular dating indicates that radiation and expansion of several desert taxa occurred before the Pliocene (Carranza *et al.*, 2008 and references therein; Metallinou *et al.*, 2012), favouring the view that desert conditions have existed since the Miocene, even if they were geographically restricted (Schuster *et al.*, 2006). These repetitive wet–dry cycles consequently led to habitat expansion or reduction [at least 8–10 cycles are believed to have occurred since the late Pliocene (Le Houérou, 1997)] and may have contributed to vicariance events (Douady *et al.*, 2003).

Lizards are considered to be ideal organisms for studying phylogeography, because of their low dispersal abilities and often specialized ecological niches, making them accurate markers of past climatic and ecological conditions (Camargo *et al.*, 2010). *Mesalina*, in particular, may serve as an excellent model group through which to explore the phylogeography of North Africa and the Middle East, given its wide distribution in both areas. Currently, the genus comprises 14 species that inhabit xeric habitats and which are found at a wide range of elevations (Schleich *et al.*, 1996; Sindaco & Jeremčenko, 2008). Some of them are widely distributed (such as *M. guttulata*, *M. olivieri*, *M. rubropunctata*, *M. brevirostris* and *M. pasteuri*), while others have a more restricted distribution (such as *M. martini*, *M. watsonana*, *M. balfouri*, *M. adramitana* and *M. simoni*) or are even micro-endemic (such as *M. bahaeldini* and *M. kuri*). There are two previous studies that discuss the phylogeography of the genus *Mesalina* (Kapli *et al.*, 2008; Šmíd & Frynta, 2012), but both were based on a limited number of species and samples. The present study provides a comprehensive phylogeny representing most of the currently recognized species, based on both mitochondrial (mt) and nuclear sequence data. In addition, the study provides a phylogeographical hypothesis that can explain the current distribution of *Mesalina* lizards using historical events in North Africa and the Middle East.

MATERIALS AND METHODS

Sampling

The assembled dataset consisted of 193 *Mesalina* individuals, representing 12 out of the 14 currently recognized species and various morphotypes that could not be assigned to any species based on their morphological characters. The two missing species are only known from their type localities, i.e. from Somalia (*M. ercolinii*) and Oman (*M. ayunensis*) (Sindaco & Jeremčenko, 2008). All the samples (Fig. 1) were sequenced for two mitochondrial DNA (mtDNA) loci, cytochrome *b* (cyt *b*) and 16S ribosomal RNA (*16S*). Seven samples of the genus *Gallotia* and two of the genus *Eremias* were used as outgroup taxa. A subset of 56 *Mesalina* samples and eight outgroup taxa (one *Eremias* and seven *Gallotia* species) were also sequenced for the nuclear marker beta-fibrinogen intron 7 (β-*fibint7*). For the estimation of divergence times, six additional taxa were used (*Psammodromus algirus*, *Podarcis cretensis*, *Podarcis peloponnesiacus*, *Podarcis bocagei, Podarcis carbonelli* and *Podarcis hispanicus*). For full details of all the specimens see Appendix S1 in the Supporting Information.

DNA extraction, amplifications and sequencing

Total genomic DNA was extracted as described in Aljanabi & Martinez (1997). Poorly preserved samples were treated as described in Austin & Melville (2006). For the polymerase chain reactions (PCR), we used six pairs of primers, three of which were designed in the current study (Table 1).

The PCR products were purified with a NucleoSpin PCR purification kit (Macherey–Nagel, supplied by Lab-Supplies

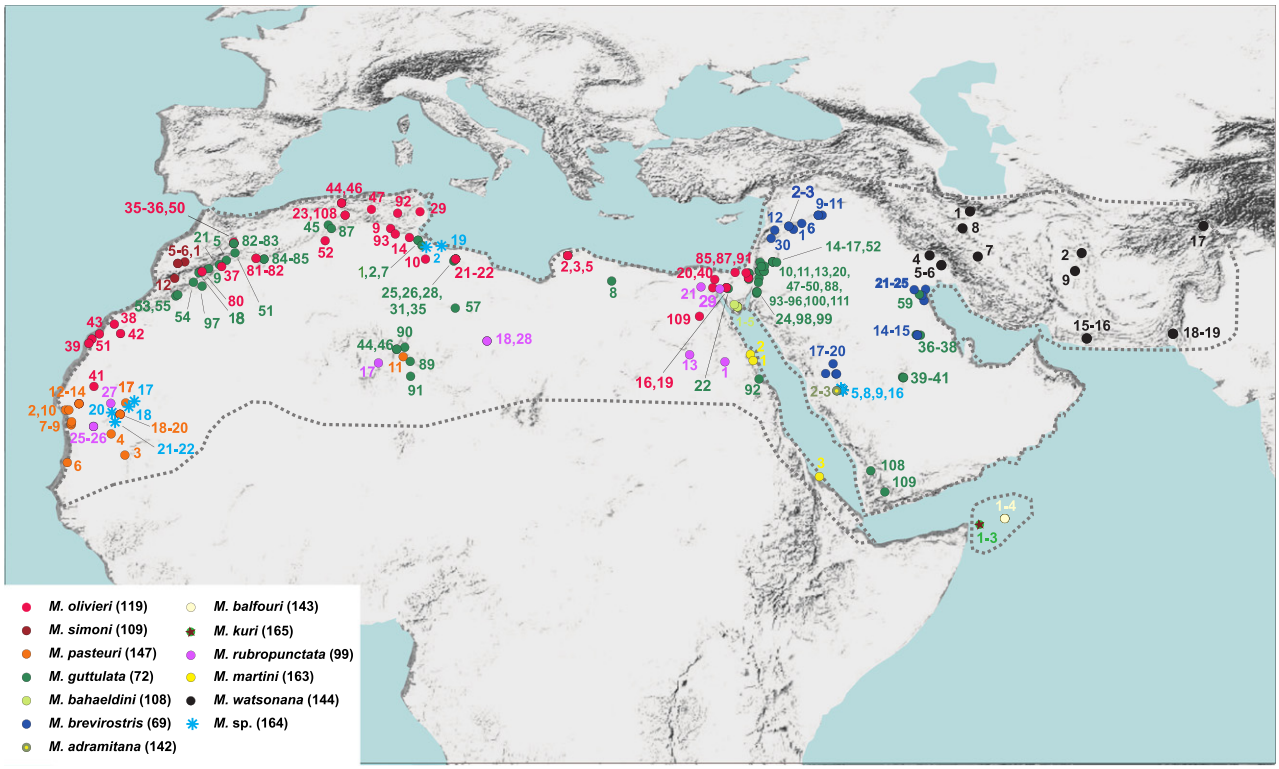

Figure 1 The locations of *Mesalina* samples used in the study of the historical biogeography of the lacertid lizard in North Africa and the Middle East. The code for each sample comprises the species code (given in parentheses in the figure key) and the specific sample code (depicted on the map). The dashed line represents *Mesalina*'s approximate distribution (Sindaco & Jeremčenko, 2008).

Table 1 The primers used for the amplification of three target loci in the study of the lacertid lizard *Mesalina* in North Africa and the Middle East.

Gene	Primer name	Sequence name	Length (bp)	Reference
cyt *b*	GLUDG-L	5′-TGACTTGAARAACCAYCGTTG-3′	*c.* 450	Palumbi *et al.* (1991)
	CB2-H	5′-CCCTCAGAATGATATTTGTCCTCA-3′		
	Mes_cytb_F	5′-CGWAAACAACACCCVATCCT-3′	*c.* 400	Current study
	Mes_cytb_R	5′-GATATTTGTCCTCADGGHA-3′		Current study
16S rRNA	16SAR-L	5′-CGCCTGTTTATCAAAAACAT-3′	*c.* 530	Palumbi (1996)
	16SBR-H	5′-CCGGTCTGAACTCAGATCACGT-3′		
	Mes_16S_F	5′-CCGCGGTATCCTAACCGTGCAA-3′	*c.* 500	Current study
	Mes_16S_R	5′-TTAATCGTTGAACAAACGAACC-3′		Current study
β-*fibint7*	BFXF	5′-CAGGGAGAGCTACTTTTGATTAGAC-3′	*c.* 600	Sequeira *et al.* (2008)
	BF8	5′-CACCACCGTCTTCTTTGGAACACTG-3′		Pinho *et al.* (2008)
	Mes_fib7_F	5′-AGAGACAATGATGGCTGGTATG-3′	*c.* 570	Current study
	Mes_fib7_R	5′-TGGAACACTGTTTCTTTGGGTC-3′		Current study

Scientific, Athens, Greece). Sequencing of the PCR product was performed either directly for the mitochondrial loci, using the corresponding PCR primers, or after cloning, choosing one clone per sample for the nuclear locus, into the pCR2.1/TOPO vector (Invitrogen, supplied by Antisel SA, Thessaloniki, Greece). Double-stranded sequencing of the DNA sequences was performed in both directions using a Big-Dye Terminator Cycle Sequencing (3.1) kit on an ABI-prism 377 automated sequencer, by the sequencing company Cellular and Molecular Immunological Applications (Cemia, Larissa, Greece). GenBank Accession numbers for the sequences produced by this study are given in Appendix S1.

Alignment

The alignment for the coding gene cyt *b* was performed with MAFFT 6 (Katoh & Toh, 2008) (http://align.bmr.kyushu-u.ac.jp/mafft/online/server) under the default settings. Subsequently, the cyt *b* nucleotide alignment was translated into amino acids and did not show any stop codons or indels. The nuclear and *16S* sequences were aligned using Fast Statistical Alignment (Bradley *et al.*, 2009), an approach that effectively reduces false-positive alignments, thus producing a 'safer' alignment for genes with high indel expectancy (such as non-coding genes or introns). Two datasets were formed: one concatenated for the two mtDNA loci for all the samples used in this study (193 ingroup and nine outgroup samples), and one for the nuclear locus (56 ingroup and one outgroup samples).

Model selection

The best-fit model of DNA substitution was chosen for each gene with jMODELTEST 2.1.1(Darriba *et al.*, 2012). The program was run under the following likelihood settings: three substitution schemes, base frequencies estimation, gamma shape and invariable sites estimation, which made a total of 24 models. The models including both a gamma distribution and invariable sites were ignored (Yang, 2006), thus the number of models available was reduced to 18. The models were evaluated using the Bayesian information criterion (BIC), which is considered to be the most accurate in jMODELTEST (Darriba *et al.*, 2012) under the NxL sample size mode.

Phylogenetic analysis

Bayesian inference (BI) and maximum likelihood (ML) analyses were performed. For both analyses, nucleotides were used as discrete, unordered characters. BI was carried out using the software MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001). The analysis was performed with four runs for 10^7 generations and eight chains, and the current tree was saved to file every 100 generations. This generated an output of 10^5 trees for every run. The performance of the runs was visualized using TRACER 1.5 (Rambaut & Drummond, 2009). The first 10^4 trees (10%) were discarded as 'burn-in' and a majority rule consensus tree was calculated from the remaining trees. The posterior probabilities were calculated as the percentage of samples recovering a clade ($\geq$ 95% indicated significant support) (Huelsenbeck & Ronquist, 2001). Two analyses were performed, one for the mtDNA (partitioned by gene) and one for the nuclear dataset.

Two ML analyses were carried out using RAxML 7.2.7 (Stamatakis, 2006) under the GTRGAMMA model. To ensure that the inferred ML tree was not a local optimum, 200 ML searches for each dataset were conducted. The Robinson–Foulds (RF) symmetric distance was employed to assess the topological similarity between these trees (Robinson & Foulds, 1981). The confidence of the branches of the best ML tree was assessed further based on 1000 rapid bootstrap replicates (under the GTRCAT model). The analysis was performed for both the mtDNA (partitioned by gene) and the nuclear dataset.

Divergence times

mtDNA cluster delimitation

The mtDNA dataset was used to estimate divergence times. In order to standardize molecular clock rates (Ho *et al.*, 2011), the sequence variation of the mtDNA dataset was divided into intra- and interspecies groups using the generalized mixed Yule–coalescent (GMYC) model (Pons *et al.*, 2006). The analysis was carried out using the R package SPLITS (SPecies LImits by Threshold Statistics; http://r-forge.r-project.org/projects/splits/) with both single and multiple-threshold methods. The ultrametric tree required for the analysis was built for the full mtDNA dataset after removing identical haplotypes. The tree search was performed with RAxML, as described above (i.e. the best out of 200 ML trees was chosen), and was converted to ultrametric using penalized likelihood with the TN algorithm with R8S 1.8 (Sanderson, 2003). The optimal smoothing parameter (evaluated to 1) was selected by cross-validation as described in the R8S manual.

Divergence times of *Mesalina* were estimated using BEAST 1.7.2 (Drummond & Rambaut, 2007) and one representative sample per 'species group' defined by GMYC. The dataset was realigned, and the best-fit substitution model was recalculated. The analysis was run for 5×10^7 generations with a sampling frequency of 1 per 1000 trees, from which 10% were discarded as burn-in. The models and prior specifications that were applied were as follows (otherwise by default): *16S*, GTR+G; cyt *b,* HKY+G; relaxed uncorrelated lognormal clock (estimate); Yule process of speciation; normal prior distribution for all calibration points.

With the lack of internal calibration points for *Mesalina*, we used 'external' ones to estimate the divergence times of the genus. The ages of the Canary Islands (reflecting the separation of the species of the genus *Gallotia*) were used as the primary calibration points, as in previous lacertid phylogenies (Arnold *et al.*, 2007; Cox *et al.*, 2010; Šmíd & Frynta, 2012). In order to cross-check the estimated times, we followed an independent strategy by using the separation of the island of Crete from the Peloponnese, reflecting the separation of *Podarcis cretensis* from *Podarcis peloponnesiacus* (Poulakakis *et al.*, 2003), along with the estimated times of the Iberian *Podarcis* (Kaliontzopoulou *et al.*, 2011). A third analysis was conducted with all available calibration points (Table 2). Finally, we performed a fourth analysis similar to the third, but adding the available nuclear sequences (under the HKY+G model) to the mitochondrial dataset (see Appendix S2).

Table 2 The calibration points (in bold) for each of three calibration strategies used in the study of the lacertid lizard *Mesalina* in North Africa and the Middle East: (1) the separation of representatives of the subfamily Gallotinae; (2) the separation of *Podarcis cretensis* from *Podarcis peloponnesiacus* along with the separation times of the Iberian *Podarcis* (*P. hispanicus* I/*P. bocagei, P. hispanicus* II/*P. carbonelli*); and (3) a combination of all calibration points. The median divergence times and the confidence intervals for the outgroup clades and the main nodes (A–G) of the tree (presented in Fig. 4) are also presented for each of the three calibration strategies.

Taxon/clade	First calibration	Second calibration	Third calibration
Lacertidae	37 (16.7796, 47.4143)	39.2 (14.2916, 49.3443)	39 (17.7353, 47.918)
Gallotinae	**(17.5, 19.66)***	16.3 (6.4903, 23.9067)	**(17.5, 19.66)***
Eremiadini	32.1 (9.7422, 42.9423)	34 (12.208, 45.4261)	33.9 (11.2614, 43.299)
Gallotia	**(10.72, 12.61)***	12.6454 (5.1467, 23.9067)	**(10.72, 12.61)***
G. galloti/*G. caesaris*	**(3.14, 3.74)***	3.7 (1.3667, 5.9333)	**(3.14, 3.74)***
P. cretensis/*P. peloponnesiacus*	4.1 (1.2077, 6.2228)	**(5, 5.5)†**	**(5, 5.5)†**
P. hispanicus I/*P. bocagei*	4.3 (0.9577, 6.236)	**(5.17, 6.05)‡**	**(5.17, 6.05)‡**
P. hispanicus II/*P. carbonelli*	6 (1.7439, 8.7189)	**(3.81, 6.19)‡**	**(3.81, 6.19)‡**
A	21.64 (6.3362, 29.3819)	23.22 (8.1284, 31.4332)	22.97 (7.173, 29.7283)
B	16.14 (6.2939, 21.416)	17.37 (8.3679, 23.3509)	17.13 (7.5195, 21.6096)
C	13.28 (5.1524, 17.6051)	14.35 (7.8642, 19.1917)	14.13 (6.6048, 17.9911)
D	12.4 (5.4225, 16.4303)	13.41 (8.3262, 17.9137)	13.2 (8.0621, 16.9818)
E	10.4 (3.8265, 14.0336)	11.3 (5.3787, 15.0396)	11.1 (4.8829, 14.2132)
F	10.66 (4.8061, 14.5633)	11.51 (7.2775, 15.726)	11.3 (7.0987, 15.0536)
G	8.3 (3.0386, 11.1744)	8.94 (4.5072, 12.2067)	8.78 (4.2696, 11.7635)

*Cox *et al.* (2010); †Poulakakis *et al.*(2003); ‡Kaliontzopoulou *et al.* (2011).

The results of all the analyses performed with BEAST were analysed in TRACER. The annotations of the maximum clade credibility tree were computed in TREEANNOTATOR 1.7.2 (Drummond & Rambaut, 2007).

Ancestral area reconstruction

Bayesian binary Markov chain Monte Carlo (MCMC) (BBM) implemented in RASP (Yu *et al.*, 2010) was employed to reconstruct the possible ancestral distribution areas of *Mesalina*. We used the mitochondrial ultrametric tree inferred by BEAST as input to the program, after trimming all outgroup taxa except *Eremias.* The distribution range of *Mesalina* was divided into two main areas, Africa and Arabia/Middle East (the root distribution was set to outgroup distribution). Ten MCMC chains were run in two independent analyses for 5×10^6 generations under the JC+G (Jukes–Cantor + gamma) model. The state was sampled every 100 generations.

RESULTS

Alignment

A total of 1051 base pair (bp) alignments for the two mtDNA loci (*16S*, 590 bp; cyt *b*, 461 bp) was obtained for the total mtDNA dataset. The ingroup alignment contained 386 variable sites, increasing to 441 when the outgroup taxa were included. The length of the nuclear alignment was 957 bp for 64 sequences. It contained 186 and 227 variable sites for the ingroup and the whole alignment, respectively. β-*fibint7* was indel rich and the size of indels varied from 1 to 93 bp (between *M. watsonana* and all other sequences).

Phylogenetic analysis

Mitochondrial dataset

BI was run under the GTR+G model for both partitions. Three of the parameters, shape parameter (*alpha*), relative partition rates (*m*) and tree length (TL), resulted in a double-peak posterior distribution for three out of the four runs of the analysis. The TL (in all four runs) was one order of magnitude larger than that inferred by ML. The phenomenon of long tree solutions and unexpected values for *m* and *alpha* has been reported previously in BI with no necessary effect on the optimization of the tree topology (Marshall, 2010). In the present case the BI topology was congruent with the best ML tree (log-likelihood, ln*L*, = −13217.86) drawn from the 200 ML inferences (Fig. 2). The topological variance among the 200 ML trees based on the RF distances was low (0.09).

Our results suggest that *Mesalina* is monophyletic under both Bayesian and ML conditions of analysis. *Mesalina guttulata* is paraphyletic with respect to *M. bahaeldini*, whereas *M. olivieri*, *M. pasteuri* and *M. simoni* are polyphyletic. The phylogenetic tree was divided into four well-supported clades. The *M. watsonana* lineage had high statistical support, 1.00 and 100 posterior probability (PP) and bootstrap support (BS), respectively, and occurred in the easternmost part of the *Mesalina* distribution (Fig. 2). The relationship of the remaining three clades, (1) *M. martini*, (2) the *M. guttulata* complex, which included *M. guttulata* and *M. bahaeldini*,

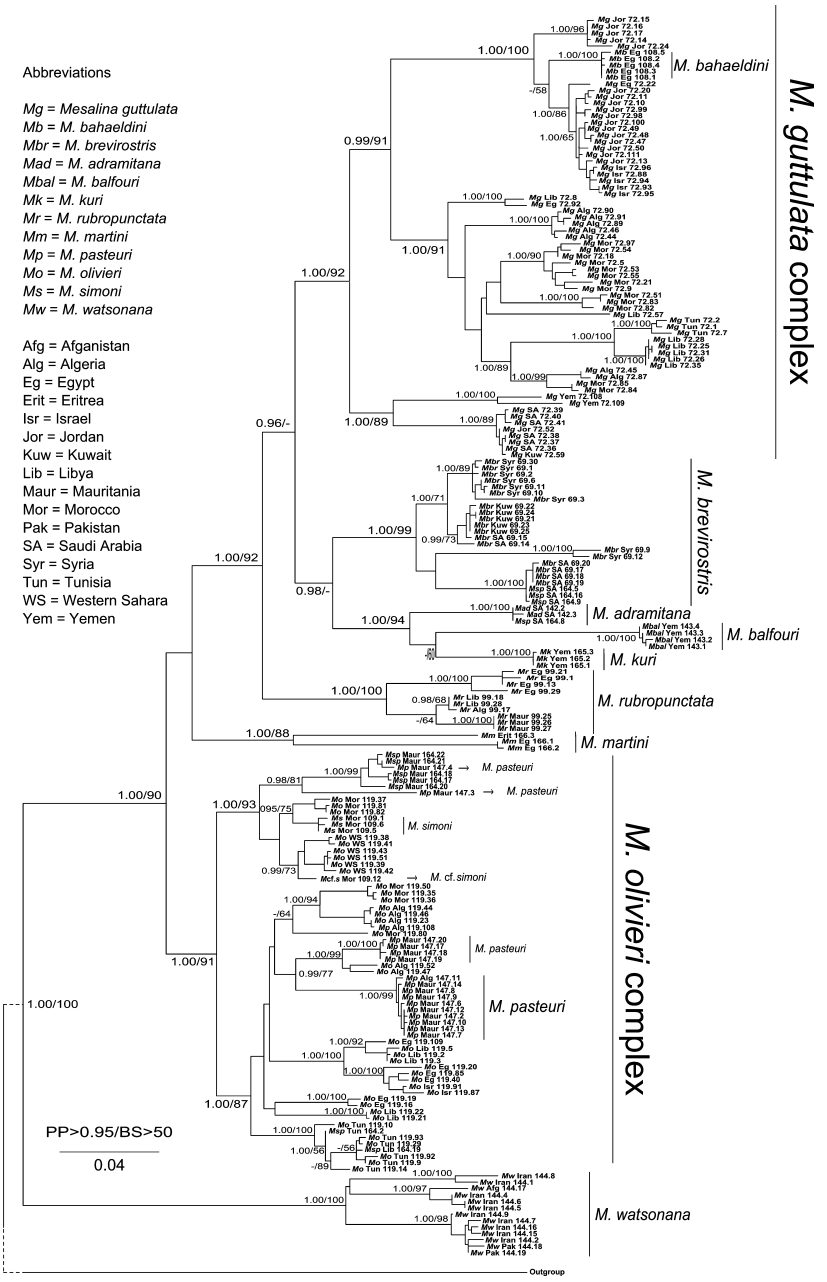

Figure 2 The phylogenetic relationships of 193 *Mesalina* samples from North Africa and the Middle East, according to the maximum likelihood (ML) method. The numbers on the branches indicate the posterior probabilities (PP) of the Bayesian inference (only values above 0.95 are shown) followed by the bootstrap supports (BS) of the ML method (only values above 50 are shown). Seven samples of the genus *Gallotia* and two of the genus *Eremias* were used as outgroup taxa.

with allies (i.e. *M. brevirostris*, *M. rubropunctata*, *M. adramitana*, *M. kuri* and *M. balfouri*), and (3) the *M. olivieri* complex, which included *M. olivieri*, *M. pasteuri* and *M. simoni*, was not resolved by any of the analyses.

Nuclear dataset

BI (under the HKY+G model) and ML analyses resulted in similar topologies (ln*L* = −4305.41 and −4216.81, respectively) that were partially congruent to the mtDNA tree. The topology of β-*fibint7* was divided into three clades that supported the three main groups within the genus: (1) *M. watsonana* (100 BS, 1.00 PP); (2) *M. guttulata* and allies (80 BS, 0.98 PP); and (3) the *M. olivieri* complex (61 BS, – PP). *Mesalina martini* appeared to be nested within the clade of the *M. olivieri* complex, but with low statistical support (Fig. 3).

Divergence times

GMYC analysis identified 73 and 74 independently evolving mtDNA lineages under the single ($\log L_{null}$ = 84.43596, $\log L_{GMYC}$ = 93.42465, P = 0.0004) and multiple ($\log L_{GMYC}$ = 97.93994, P = 0.0001) threshold options. Given that none of the results was significantly better and the single-threshold version of the method outperformed the multiple-threshold version (Fujisawa & Barraclough, 2013), we employed the results of the single-threshold version (see Appendix S3).

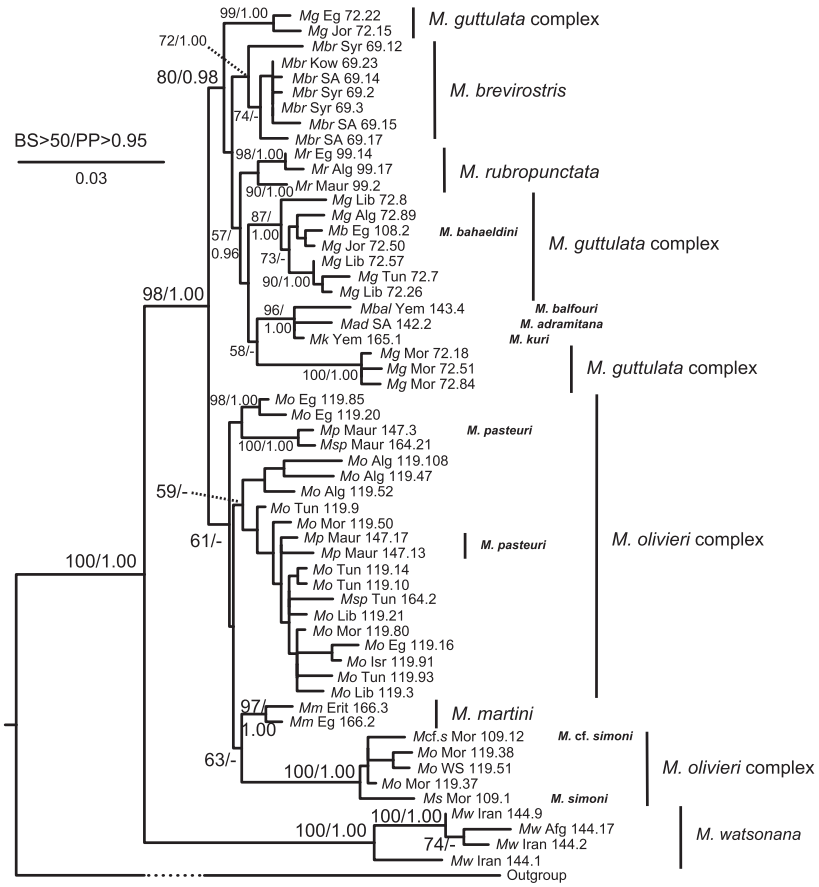

Figure 3 Phylogenetic relationships derived from the maximum likelihood (ML) analysis of the beta-fibrinogen intron 7 sequences of the subset of 56 *Mesalina* samples from North Africa and the Middle East. The numbers on the branches indicate the posterior probabilities (PP) of the Bayesian inference (only values above 0.95 are shown) followed by the bootstrap supports (BS) of the ML method (only values above 50 are shown). Seven samples of the genus *Gallotia* and two of the genus *Eremias* were used as outgroup taxa. Abbreviations as in Fig. 2.

All four independent BEAST runs (three based on mtDNA: Fig. 4; one based on mtDNA with nuclear sequences: Appendix S2) produced a congruent topology with the tree inferred for the complete mtDNA dataset (Fig. 2). Trace plots indicated that all analyses had reached convergence for all parameters with good mixing (effective sample size >200). All calibration strategies resulted in similar estimations for the divergence events of the genus (Fig. 4, Appendix S1). The data suggested that the origin of *Mesalina* dates back as early as the Miocene (*c.* 22 Ma), with *M. watsonana* diverging from the other *Mesalina* clades at that time. Subsequent cladistic events (Fig. 4) leading to the currently recognized species or species complexes occurred in the middle to late Miocene.

Ancestral area reconstruction

The two runs of the BBM analysis for the major nodes of the tree produced identical results, with a 0.0003 distance between them (Fig. 4). These data suggested that *Mesalina* species originated somewhere in Arabia/the Middle East, with the exception of the *M. olivieri* species complex, which may be of African origin.

DISCUSSION

Mesalina shows high genetic diversity and marked incongruence of phylogeny with currently accepted systematics (para/polyphylies at species level), for both mtDNA and nuclear datasets. The unresolved phylogeny inferred by the nuclear gene compared with the mtDNA analysis may be explained by the slower rate of evolution and incomplete lineage sorting of ancestral polymorphism for the nuclear genes (Moore, 1995). This phenomenon has been reported elsewhere for other lacertid lizard species (Pinho *et al.*, 2008; Fonseca *et al.*, 2009).

Systematic implications

Even though the systematic implications for the status of the genus are beyond the scope of the present study, it is worth noting that the loci used are highly diversified among *M. guttulata*, *M. brevirostris*, *M. olivieri* and *M. watsonana*, indicating the presence of species complexes. This diversity agrees with previous phylogenetic studies of the genus (Mayer *et al.*, 2006; Kapli *et al.*, 2008; Šmíd & Frynta, 2012). The morphological and ecological variation within these four taxa (Moravec, 2004; Werner & Ashkenazi, 2010) or the whole genus (Arnold, 1986) corroborates that hypothesis. Several studies on reptiles have reported complex phylogenetic patterns and extensive incongruence with the current systematic hypothesis in North Africa and the Middle East (Fonseca *et al.*, 2009; Pouyani *et al.*, 2010; Gonçalves *et al.*, 2012), suggesting that the biodiversity of the area has been underestimated.

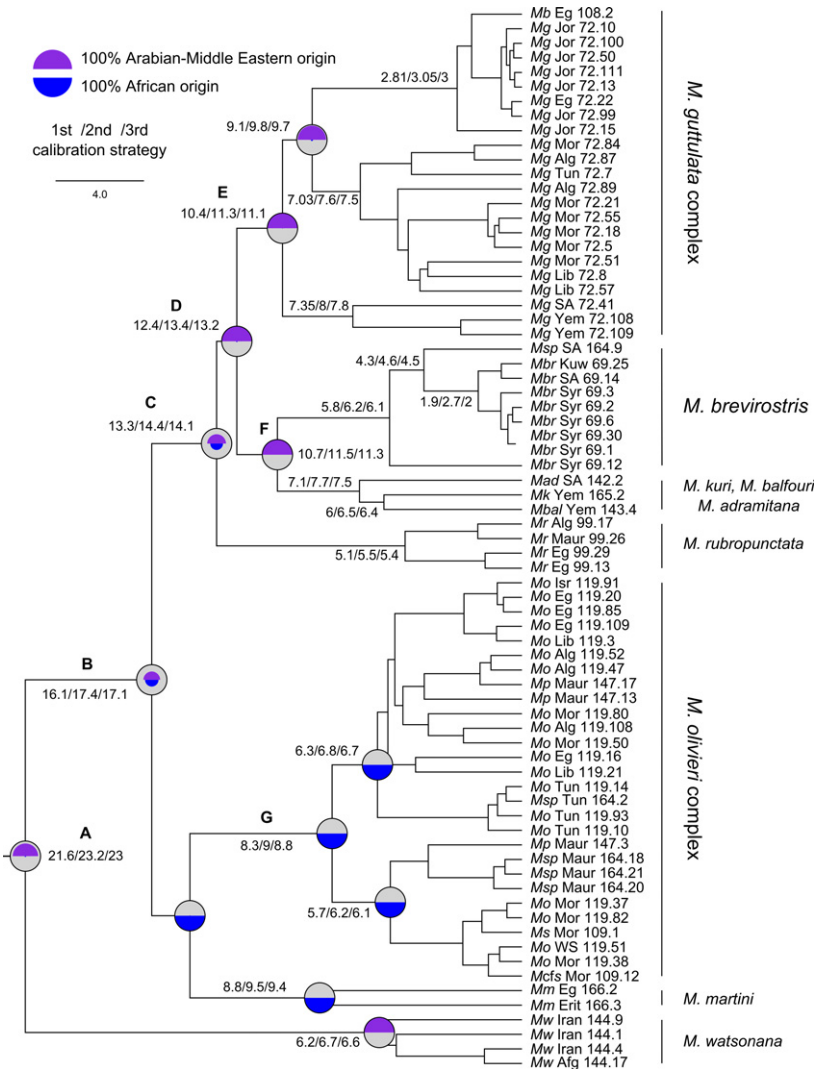

Figure 4 Calibrated tree as inferred by BEAST, based on the mitochondrial data of the 73 representatives of *Mesalina*'s 'species groups' defined by the generalized mixed Yule–coalescent (GMYC) model. The numbers on the branches indicate the median divergence times estimated according to the first (using separation times among species of the subfamily Gallotinae), second (using separation times among species of the genus *Podarcis*) and third (a combination of all calibration points) calibration strategies. The results of the ancestral area reconstruction are given for the main nodes of the tree; the upper semicircle is proportional to the possibility that the corresponding clade originated in Arabia/Middle East and the lower is proportional to the possibility that it originated in Africa. Abbreviations as in Fig. 2.

Phylogeography

The estimated divergence times support the initial differentiation of *Mesalina* in the early Miocene (*c.* 22 Ma), which is almost 6 Myr earlier than previous estimations (Šmíd & Frynta, 2012) and 2 Myr prior to the formation of the *Gomphotherium* land bridge (Rögl, 1999). Our data suggest an earlier divergence of the family (Table 2) prior to any connection between Arabia and Eurasia. The latter event is associated with the origin of Eremiadini, a tribe of Lacertinae that includes *Mesalina*, which is thought to have first dispersed into Arabia at this time (Arnold *et al.*, 2007; Pavlicev & Mayer, 2009).

The estimated divergence times and thus the historical scenario proposed in the present study differ from previous studies (Kapli *et al.*, 2008; Šmíd & Frynta, 2012), potentially because we included representatives for a substantial number of new lineages and distribution areas. A simplified illustration of the proposed biogeographical scenario is presented in Fig. 5. A short-term connection between Arabia and Eurasia prior to the *Gomphotherium* land bridge allowed mammalian taxa to disperse from Africa into Eurasia (Harzhauser *et al.*, 2007). Our estimation for the initial split of the genus (*c.* 21.6–22.2 Ma) coincides with this phase. One lineage of the genus (currently recognized as *M. watsonana*) remained in Eurasia, while another lineage dispersed into Arabia giving rise to the remaining currently recognized *Mesalina* species. Subsequent tectonic events in the collision zone (Popov *et al.*, 2004) and the uplift of the Zagros Mountains (Agard *et al.*, 2011) prevented the two lineages coming into contact again. A similar scenario has been proposed for the viper *Echis carinatus*, which was isolated in Asia *c.* 20 Ma (Pook *et al.*, 2009).

The second major splitting of the *Mesalina* genus occurred at *c.* 16–17 Ma, which coincides with both tectonic and climatic changes in the area. During this period, the Arabian plate experienced a major transgression (Popov *et al.*, 2004; Autin *et al.*, 2009), which was coincident with a drastic decrease in temperature following the mid-Miocene climatic optimum (*c.* 15–17 Ma) (Zachos *et al.*, 2001). One lineage

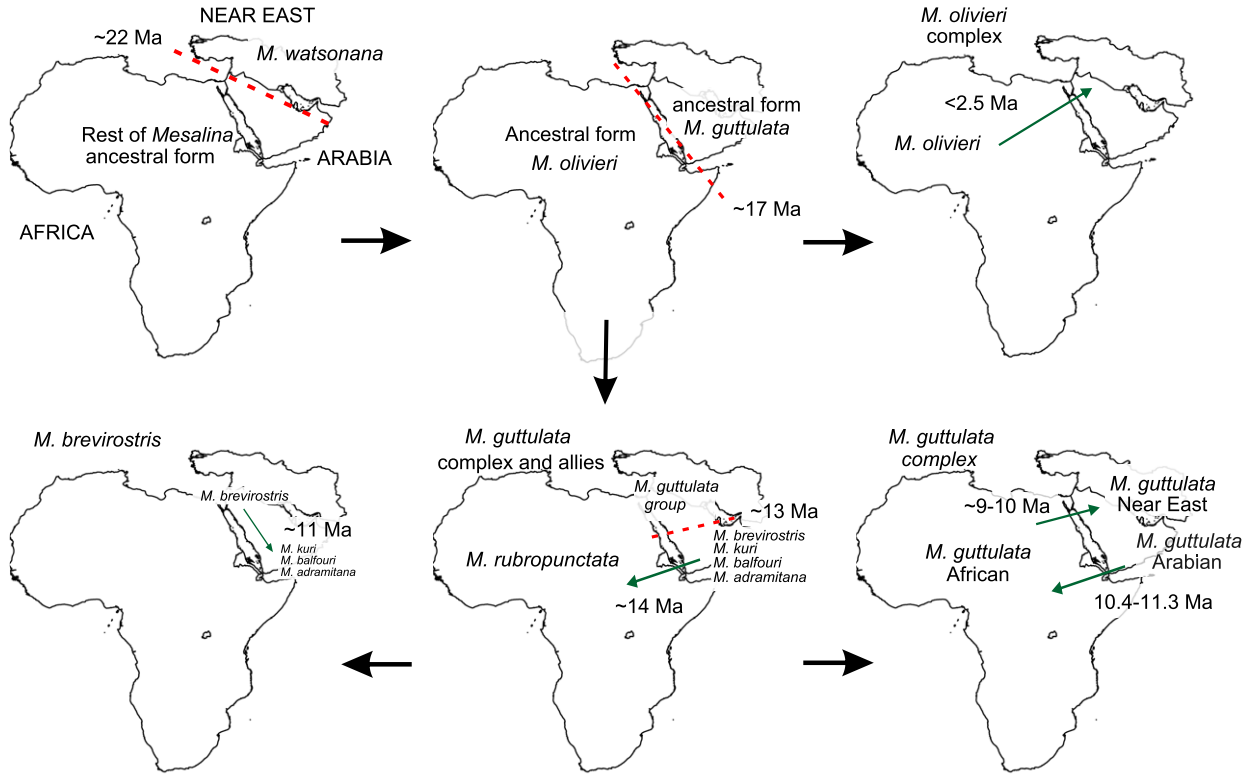

Figure 5 Simplified illustration of the proposed biogeographical scenario for the historical distribution of *Mesalina* in North Africa and the Middle East.

potentially dispersed into Africa, leading to the *M. olivieri* complex, while its sister clade remained in Arabia. Future studies using additional samples and loci may help resolve the position of *M. martini*.

The complex history of the Arabian Plate has been considered the driving force for the divergence of other reptile taxa (Amer & Kumazawa, 2005; Pook *et al.*, 2009; Metallinou *et al.*, 2012) in the early and middle Miocene. In studies of *Echis* (Pook *et al.*, 2009) and *Stenodactylus* (Metallinou *et al.*, 2012), the estimation of the divergence events were older (19.4 and 21.8 Ma for the two genera, respectively) than *Mesalina* (*c.* 16–17 Ma), coinciding with the intense volcanism occurring throughout the Red Sea (*c.* 24 Ma) and the subsequent rifting (*c.* 20 Ma). The divergence of the African clades of *Uromastyx* from the Arabian clades is estimated to be more recent (11–15 Ma) concurrent with climatic changes (Amer & Kumazawa, 2005). Overall, the opening of the Red Sea has been a multiphase process (Popov *et al.*, 2004), with volcanic events occurring since the late Oligocene until the initiation of the spreading in the early Miocene (*c.* 19–18 Ma). Consequently, variable divergence times that correspond to the volatile nature of the geology of the region at that period can be expected.

Mesalina guttulata and allies: the Arabian clade

Our results suggest that the origin of this clade is somewhere in Arabia/the Middle East in the mid-Miocene (*c.* 13–14 Ma). The early to middle Serravallian (*c.* 12–13 Ma) corridor (Jones, 1999 and references therein) may have allowed the dispersal of ancestral *M. rubropunctata* into Africa. The major cooling step after the mid-Miocene climatic optimum (14.1–14.8 Ma) and the resultant increased aridification of the mid-latitudes (Flower & Kennett, 1994) may have further induced the separation of *M. rubropunctata* from *M. guttulata*.

During the late Serravallian–Tortonian (*c.* 11–9 Ma), two land bridges are assumed to have connected Arabia and Africa: one in the north, in the area of Sinai, and one in the south, in the Gulf of Aden (Jones, 1999). Two dispersal events using these two routes could explain the current distribution of *M. guttulata*. In accordance with this hypothesis, the subsequent opening of the Gulf of Aden in the south and the formation of the Nile River later in the north (Goudie, 2005) may have prevented the populations reuniting. This period is known for major mammalian dispersal events, induced by both climatic changes and intercontinental relationships (Elewa, 2005). Climatic change in the circum-Mediterranean (Elewa, 2005 and references therein) could have been the driving force for both the dispersal of the ancestral *M. guttulata* populations as well as the parallel splitting of *M. brevirostris* from the rest of the Arabian lineages (i.e. *M. kuri*, *M. balfouri* and *M. adramitana*).

Further splitting of the North Arabian *M. guttulata* populations (*M. bahaeldini* and the two *M. guttulata* lineages in Jordan) was probably the result of the Suez rift, the opening

of the Dead Sea, and the Wādī 'Araba line. The latter is located along the fault separating Africa and Arabia, and it plays a filtering role in faunal movement from and to Africa because of its extremely arid conditions (Arnold, 1987; Disi *et al.*, 2001). The mid-Pliocene aridification shift (*c.* 3.2–2.6 Ma; DeMenocal, 2004) probably worsened the conditions of Wādī 'Araba making it impossible to inhabit. Assuming that *M. guttulata* populations were present in the area prior to the aridification shift, the subsequent extreme arid conditions of the Wādī 'Araba could have split them into eastern and western populations. *Mesalina brevirostris* and *M. rubropunctata* failed to cross the fault westwards and eastwards, respectively (Arnold, 1987), indicating that they reached the area later than its aridification. The restricted distribution of *M. brevirostris* in South Sinai (Baha El Din, 2006) is more likely to be the result of stepping-stone dispersal from the opposite Arabian shore (evidenced by the presence of *M. brevirostris* on the island of Tiran), rather than a crossing of the Wādī 'Araba. *Mesalina olivieri*, on the other hand, is clearly present on both sides of the fault (Disi *et al.*, 2001). It remains unclear whether the populations of the latter are isolated for reasons similar to the case of *M. guttulata*.

Mesalina olivieri species complex: the African clade

This complex forms two well-supported lineages, one occupying the western part of the Maghreb region (Morocco, western Sahara and Mauritania) and the other extending from the Maghreb up to the Near East. Both lineages co-occur in Mauritania and Morocco. Similar dispersal patterns have been reported for the three main lineages of *Agama* lizards that co-occur in Mauritania (Gonçalves *et al.*, 2012). Both *Agama* and *M. olivieri* distribution patterns could be explained by the fact that mountainous areas such as Morocco and Mauritania may have acted as 'climatic islands', serving as refugia during drastic climatic changes (Schleich *et al.*, 1996; Pepper *et al.*, 2011; Brito *et al.*, 2013).

The major split of the *M. olivieri* complex is estimated to have occurred *c.* 8 Ma. A similar divergence event has been reported for *Agama,* with *A. tassiliensis* diverging from the *A. boueti* and *A. impalearis* sister clade at 8.15 Ma (Gonçalves *et al.*, 2012). Both events coincide roughly with the global transition from humid- (C_3) to arid-adapted (C_4) vegetation, which was initiated at about 7.8 Ma (Cerling *et al.*, 1997; Zachos *et al.*, 2001). The diversification of other dry-adapted North African reptiles (e.g. *Acanthodactylus*, *Stenodactylus* and *Agama*) has also been estimated to have occurred in the late Miocene (Fonseca *et al.*, 2009; Gonçalves *et al.*, 2012; Metallinou *et al.*, 2012). Thus our data support reports by previous authors (Carranza *et al.*, 2008; Metallinou *et al.*, 2012) that desert conditions prevailed in the Miocene and allowed xerophilic fauna to radiate.

Tunisian populations have been found to form a distinct lineage in many North African reptile and amphibian species (Recuero *et al.*, 2007; Carranza *et al.*, 2008; Kaliontzopoulou *et al.*, 2011). In the case of *M. olivieri*, the isolation of the Tunisian population was estimated to have occurred in the Messinian period. In the late Miocene, major orogenetic events occurred in the area (Bouaziz *et al.*, 2002), potentially causing a vicariance event between Tunisian and other North African populations. This area seems to support a distinct biogeographical unit within North Africa, regardless of the events that pre-date reptile lineages in Tunisia.

Finally, the colonization of the Middle East by populations of *M. olivieri* was potentially facilitated during the desiccation of the Nile, which occurred in the Pleistocene and lasted about a million years (Baha El Din, 2006).

CONCLUSIONS

The genus *Mesalina* may be divided into three main geographical groups according to the origin and distribution of the species or species complexes: (1) Iran–Afghanistan–Pakistan (*M. watsonana*); (2) Arabia and the Near East (*M. guttulata* species complex, *M. brevirostris*, *M. balfouri*, *M. adramitana* and *M. kuri*) with related taxa of African radiation (*M. rubropunctata* and a substantial part of the *M. guttulata* species complex); and (3) North Africa (*M. olivieri* species complex, with possible inclusion of *M. martini*). Similar clustering has been identified in other reptile taxa with similar distributions (e.g. *Echis*, *Uromastyx* and *Stenodactylus*). The movement of the Arabian Peninsula appears to have initiated the main diversification event reported for all the corresponding studies. In this particular case, the changing geography of the Arabian Peninsula appears to have allowed the dispersal of *Mesalina* back and forth from Africa to Arabia, multiple times.

Both Africa and Arabia are characterized by vast lowlands that, in periods of extreme climate conditions (i.e. aridity and low temperatures), do not form suitable habitats even for desert-adapted taxa. Potentially this is the reason why most of the diversity is concentrated in the mountainous areas (e.g. Morocco, Mauritania, Yemen and the western coast of Saudi Arabia), which served as refugia (Schleich *et al.*, 1996; Brito *et al.*, 2013) during hyper-arid periods, as has been documented for the Australian mountains of the arid zone (Pepper *et al.*, 2011). Both globally and in the study area (Zachos *et al.*, 2001; Le Houérou, 2003; Schuster *et al.*, 2006), climatic changes, towards colder/more arid conditions, have occurred multiple times after the mid-Miocene climatic optimum, playing a key role in the speciation patterns of the reptile fauna inhabiting both North Africa (Brito *et al.*, 2013) and Arabia.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Geographical origin of samples and GenBank accession numbers.

Appendix S2 Divergence times based on both mitochondrial and nuclear data.

Appendix S3 The mitochondrial DNA clusters recognized by the GMYC model under the single threshold option.

BIOSKETCH

Paschalia Kapli's interests are molecular phylogenies that address questions of species evolution, speciation processes and historical biogeography.

Author contributions: P.K. obtained the sequences, analysed the data and wrote the manuscript; N.P. and P.L. designed and supervised the research and refined the manuscript; P.A.C. contributed substantially in conducting the research and improving the manuscript; all authors collected or provided samples, read and improved the final manuscript.

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