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Molecular phylogeny of three *Mesalina* (Reptilia: Lacertidae) species (*M. guttulata*, *M. brevirostris* and *M. bahaeldini*) from North Africa and the Middle East: Another case of paraphyly?

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ABSTRACT

Mesalina is a widespread lacertid genus occurring throughout the Saharo-Sindian region from North Africa to Pakistan. It has been through a series of taxonomic revisions, but the phylogenetic relationships among the species remain unclear. In this study we estimate the phylogeographic structure of *M. guttulata* across most of its distributional range and we evaluate the relationships between *M. guttulata* and the sympatric species *M. brevirostris* and *M. bahaeldini* using partial mitochondrial DNA (mtDNA) sequences (cyt *b* and 16S). *M. guttulata* and *M. brevirostris* represent species complexes, whereas *M. bahaeldini* considered before as *M. guttulata* is a recently described species with very restricted distribution. Here we present the first evidence that *M. guttulata* is a paraphyletic taxon with respect to *M. bahaeldini*, while *M. brevirostris* proves to be a polytypic species or even a species complex, confirming previous studies. Although mtDNA markers have several properties that make them suitable for phylogeographic studies, they are not free of difficulties. Phylogeographic inferences within and between closely related species can be mislead by introgression and retention of ancestral polymorphism (incomplete lineage sorting). However, the present distribution pattern, the estimated times of divergence and the significant variation in morphology within *M. guttulata* led us to accept that the paraphyletic pattern observed, is most likely due to inaccurate taxonomy. Our hypothesis is that what has hitherto been considered as intraspecific variation, actually reflects species-level variation. Furthermore, our biogeographic analyses and the estimated time of divergences suggest that the present distribution of *M. guttulata* was the result of several dispersal and vicariant events, which are associated with historical changes (climatic oscillations and paleogeographic barriers) of late Miocene and Pliocene period.

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1. Introduction

The reconstruction of phylogenies is of primary importance in the understanding of the dynamic patterns of evolution, that is, the biogeography of a group and the bases of its biological diversity at any level. Phylogeography is considered the bridge between population genetics and phylogenetic systematics (Avise, 2000), and has contributed considerably to our understanding of what factors have influenced population structure and species divergence (Avise et al., 1994).

Mesalina was one of the "sections" into which Boulenger (1921) divided the genus *Eremias*. The taxon gained full generic status later by Szczerbak (1989). It is a widespread genus, occurring throughout the Saharo-Sindian region from North Africa to Pakistan. Today, *Mesalina* is considered to comprise 14 species, but the taxonomy of the genus is still problematic and necessitates more detailed research. All species are relatively small and most of them attain sexual maturity within one year and therefore they probably have the quickest sequence of generations among North African lizards (Scleich et al., 1996).

Although the genus has been through a series of taxonomical revisions (e.g. Haas, 1951; Arnold, 1986a, b; Mayer, 1989; Joger and Mayer, 2002; Segoli et al., 2002); the relationships among the species remain unclear. The most interesting issue in *Mesalina* derives from the overlapping distributions of several species (Werner, 1988; Scleich et al., 1996), and the sympatry of up to three species in the same locality (Ross, 1988), raising questions on its biogeography and causing problems in its taxonomy. Three species (*M. rubropunctata*, *M. olivieri*, and *M. guttulata*) have wide distribu-

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tions, since they occur throughout North Africa, whereas the latter two extending also to the Middle East. According to Arnold (1986b) and Ross (1988) in the areas of sympatry the species present more intense morphological differences, while when allopatric they may be morphologically similar. Nevertheless, suspected hybrids between *M. guttulata* and *M. olivieri* occur rarely in Israel (Werner, 1988). This information has also been mentioned by Scleich et al. (1996) and In den Bosch (2001).

So far the phylogeny of the genus has been the subject of three studies. Arnold (1986a) proposed a division of the genus into two groups, according to hemipenal characters and suggested that the one subspecies of *M. guttulata* (*M. g. watsonana*) and three of *M. olivieri* (*M. o. simoni*, *M. o. balfouri* and *M. o. martini*) deserve full specific status. Moreover, in the same year (Arnold, 1986b) distinguished five holophyletic groups, based on copulatory organs. Apart from Arnold's (1986a,b) divisions the genus's phylogenetic relationships were explored only by Joger and Mayer (2002) comparing partial mtDNA (16S rRNA) sequences of seven species from eight localities, focusing on the raising of an insular population at the species level.

Analyses of intraspecific phylogeographic patterns have led to major advances in our understanding of historical biogeographical processes (Avise, 2000). In biogeographic theory, the natural processes of vicariance and dispersal are used to explain the biogeographical pattern of organisms. Although, vicariance is considered by many to have been the dominant force underlying biogeographical patterns of modern taxa, neither dispersal nor vicariance seems to be especially favored (Austin et al., 2003). Some patterns are explained by dispersal, some by vicariance. However, Fu (1998) stated that the distribution pattern of lacertids (Lacertidae) is probably explained best by dispersal. One of the most impressive dispersal events was that of the subfamily of Eremiadinae (in the Saharo-Sindian assemblage comprising the genus *Mesalina*) from Eurasia to Africa. The Lacertidae probably arose in the European area, with the Gallotiinae later reaching Northwest Africa and the Canary Islands, and the ancestor of the Eremiadini invading Africa in the mid-Miocene (Arnold et al., 2007). Mayer and Benyr (1995) proposed a colonization of Africa by lacertids 17–19 million years ago (mya), immediately after the first Neogenic contact between Eurasia and Africa (Steininger and Rögl, 1984). Arnold (Arnold, 2004) and Mayer and Pavlicev (2007), in agreement with the former scenario, suggested a secondary recolonization of southwest Asia from Africa by an ancestor of the 'Saharo-Sindian' group across a land connection that existed until the early Pliocene between the Horn of Africa and Arabia. This could probably have happened during the middle Miocene, since the separation of the "Saharo-Sindian" lineage within Eremidianae and its first radiation occurred at ~13 mya (Mayer and Pavlicev, 2007). The invasion of this ancestor gave rise to the xeric forms of *Eremias*, *Ophisops*, *Acanthodactylus* and *Mesalina*, the last three of which later colonized dry areas of North Africa (Arnold, 2004; Arnold et al., 2007; Mayer and Pavlicev, 2007). In other words, *Mesalina* evolved in Southwest Asia and dispersed into North Africa later. But when and how many times? Are there paleogeographic and paleoclimatic events that are associated with the divergence of *Mesalina*?

In this study we focus on three species of the genus (*M. guttulata*, *M. brevirostris,* and *M. bahaeldini*). The first one is the most widely distributed species of the genus, covering all North Africa and a great part of Middle East. It is currently regarded as monotypic, since the only subspecies *M. g. watsonana* gained full species status (Arnold, 1986a). According to Baha El Din (2006), it probably represents a species complex. The same hypothesis stands for the second species (*M. brevirostris*), considered in this study. This species is distributed in the Middle East and it comprises two subspecies *M. b. microlepis* and *M. b. fieldi*. The subspecies' validity is in question according to Disi et al. (2001), whereas Moravec (2004) and Arnold (1986a) stated that this polytypic species involves more than one taxon and requires further investigation. Finally, *M. bahaeldini* is located only in the high mountains of South Sinai and was considered until recently as *M. guttulata*. Segoli et al. (2002) based on morphological differences elevated it at species level.

Here we explore the phylogenetic relationships among Mediterranean populations of *M. guttulata*, *M. brevirostris*, and *M. bahaeldini* comparing partial mtDNA (cytochrome *b* and 16S rRNA genes) sequences, sampled from 20 (Fig. 1) populations from Morocco, Tunisia, Libya, Egypt, Jordan and Syria. The aim of this study is to (i) infer the intraspecific phylogenetic relationships of *M. guttulata* populations (ii) clarify the uncertainties concerning the validity of the its current taxonomy (already challenged by the splitting of marginal populations of *M. guttulata* as in the cases of *M. watsonana* and *M. bahaeldini*), and (iii) investigate this species' affinity with *M. brevirostris*, and *M. bahaeldini*. Furthermore, a possible biogeographic scenario to account for the current distribution of *M. guttulata* is proposed.

2. Materials and methods

2.1. DNA extraction, amplification and sequencing

The number, taxonomic status and geographic locations of the specimens used in this study are given in Appendix 1 and Fig. 1. Voucher specimens (31 of the three focal species and 2 for *M. olivieri*) are deposited in the Natural History Museum of Crete (NHMC), Greece. Total genomic DNA was extracted from small pieces of muscle or liver using standard methods (Sambrook et al., 1989). Two target genes were selected for molecular phylogenetic analysis. A partial sequence (~450 bp) of mitochondrial cyt *b* gene was amplified with the primers GLUDG-L and CB2-H (Palumbi, 1996). Then ~500 bp of the mitochondrial 16S rRNA gene was amplified by using the universal primers 16Sar and 16Sbr (Palumbi, 1996).

Amplification of all sequences involved an initial cycle of denaturation at 94 °C for 5 min, and 40 subsequent cycles of 94 °C for 1 min, 47 °C for 1 min and 72 °C for 1 min. PCR product of the mtDNA genes was purified with the NucleoSpin PCR purification kit (Macherey–Nagel). Double stranded sequencing of the PCR product was performed in both directions using the Big-Dye Terminator Cycle Sequencing (v. 3.1) kit on an ABI-prism 377 automated sequencer following the manufacturer's protocol. The primers used in cycle sequencing were the same to the ones used in the PCR amplifications.

For the phylogenetic analysis, seven additional sequences of the genus *Mesalina* (three *M. guttulata*, and two *M. brevirostris*) were retrieved from Genbank, whereas the corresponding sequences of the taxa *Ophisops elegans*, *Acanthodactylus erythrurus*, and *A. cantoris* were used as outgroups (references and accession numbers of the sequences retrieved from Genbank are given in Appendix 1).

2.2. Alignment, genetic divergence, and model selection

The alignment of the concatenated cyt *b* and 16S sequences was performed with Clustal X (Thompson et al., 1997), and corrected by eye. Cytochrome *b* sequences were translated into amino acids prior to analysis and did not show any stop codons or indels, suggesting that all were functional. The alignment used is available upon request from the authors. GenBank Accession Nos. for the sequences produced for this study are EF555241-46, EF555248, EF555250-58, EF555260-70, EF555272, EF555275-79 and EF555283-88, EF555290, EF555292-300, EF555302-12, EF555314, EF555317-21 for cyt *b* and 16S rRNA, respectively.

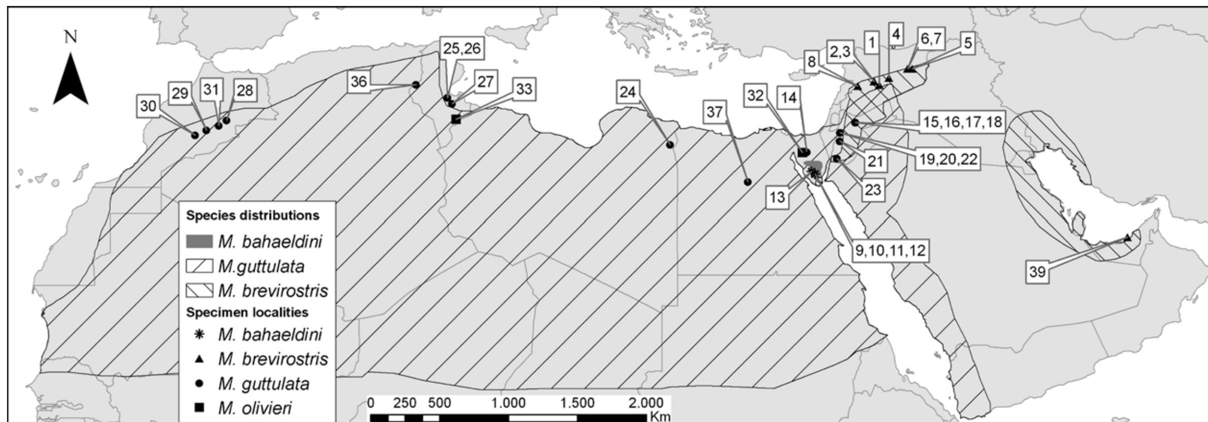

Fig. 1. Species and localities of specimens examined. Numbers correspond to codes in Appendix 1a. Distributions are indicative. Further eastern range of *M. brevirostris* and *M. guttulata* need revision and thus not presented.

The Akaike Information Criterion (AIC), (Akaike, 1974) as implemented in Modeltest (v.3.07) (Posada and Crandall, 1998), was used to choose the best-fit model of DNA substitution. AIC indicated that for the cyt *b*, 16S rRNA and for the whole dataset (the two genes combined) the General Time Reversible (GTR) (Rodriguez et al., 1990) model + I + G showed a significantly better fit than the other less complicated models.

Sequence divergences were estimated in MEGA (v 4) (Tamura et al., 2007) using the Tamura and Nei (1993) model of evolution.

2.3. Phylogenetic analyses

Phylogenetic inference analyses were conducted using Bayesian inference (BI), maximum parsimony (MP), neighbor-joining (NJ) and maximum likelihood (ML) methods. Nucleotides were used as discrete, unordered characters.

2.4. Neighbor-joining and maximum parsimony

Maximum-parsimony (MP) and neighbor-joining (NJ) analyses were performed with PAUP∗ (v.4.0b10) (Swofford, 2002). NJ was run under the GTR + I + G model, while MP was carried out (heuristic searches) using stepwise addition and performing tree-bisection–reconnection (TBR) branch swapping (Swofford et al., 1996). Confidence in the nodes of NJ and MP trees was assessed by 1000 bootstrap replicates (Felsenstein, 1985).

2.5. Bayesian inference and maximum likelihood

Bayesian inference (BI) analysis was performed with the software MrBayes (v3.1) (Ronquist and Huelsenbeck, 2003) using the ability of MrBayes to handle a wide variety of data types and models, as well as any mix of these models, based on the procedure described in MrBayes manual. The analysis was performed with four runs and eight for each run for 10^7 generations and the current tree was saved to file every 100 generations. This generated an output of $10 * 10^4$ trees. The $-\ln L$ stabilized after approximately 10^6 generations and the first 10^4 trees (10% "burn-in" in Bayesian terms) were discarded as a conservative measure to avoid the possibility of including random, sub-optimal trees. The percentage of samples recovering any particular clade in a BI analysis represents that clade's posterior probability (Huelsenbeck and Ronquist, 2001). A majority rule consensus tree ('Bayesian' tree) was then calculated from the posterior distribution of trees, and the posterior probabilities calculated as the percentage of samples recovering any particular clade (Huelsenbeck and Ronquist, 2001), where probabilities $\geqslant$95% indicate significant support. Two further independent Bayesian analyses were run so that global likelihood scores, individual parameter values, topology and nodal support could be compared to check for local optima.

Maximum likelihood analyses (Felsenstein, 1981) were conducted using PAUP∗ and RAxML-VI-HPC (v. 4.0.0; Available from: <http://phylobench.vital-it.ch/raxml-bb/>) (Stamatakis, 2006). In PAUP, heuristic ML searches were performed with 10 replicates of random sequence addition and TBR branch swapping, based on the successive-approximations strategy of Swofford et al. (1996) and Sullivan et al. (2005). In RAxML, maximum likelihood analysis was performed with DNA data partitioning (as in BI), under GTR + I + G model of evolution. Since a ML tree search with such a complex model would be computationally excessive in PAUP, the confidence of the nodes was assessed only in RAxML based on 100 bootstrap replicates, computed with the parallel message-passing-interface-based version of RAxML-VI-HPC.

Finally, an additional NJ analysis was carried out using all the available sequences of *Mesalina* in GenBank, increasing the ingroup dataset from 38 to 54 specimens and from 4 to 9 species (Appendix 1). Unfortunately, only the 16S gene was available for these extra specimens. So, this analysis was based only on a dataset that includes the 16S sequences. Our goal was to ascertain that the absence of other *Mesalina* species would not affect the resulting tree topology of this study.

2.6. Testing alternative hypotheses

We tested the hypothesis that *M. guttulata* is monophyletic. A tree with *M. guttulata* constrained to be monophyletic was generated with PAUP∗ under the likelihood criterion, and compared with our optimal topology using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) as implemented in PAUP∗ and employing RELL bootstrap with 1000 replicates.

2.7. Calibration of molecular clock and estimation of divergence times

A log-likelihood ratio test was used to examine the clock-like evolution of sequences of the ingroup in the combined data set by calculating a χ^2 statistic (Likelihood Ratio Test, LRT) based on ML values with and without rate constancy enforced ($\chi^2 = 2 \times [(-\ln L_{\mathrm{CLOCK}}) - (-\ln L_{\mathrm{UNCONSTRAINED}})]$, df = number of terminal nodes − 2) (Felsenstein, 1981). The LRT was negative (LRT = $2 * [3831.68 - 3796.82] = 69.72$, $df = 36$, $\alpha = 0.05$, $\chi_{\mathrm{critical}} = 50.99$, and $p = 0.0006$), therefore a clocklike evolution of the involved sequences could not be assumed.

For this reason, we retained in the analysis only the specimens of *Mesalina* that contain both genes and we included the corresponding sequences (cyt *b* and 16S) of three other lacertid species (*G. stehlini*, *G. symonyi* and *G. galloti*) and two more distant lizard species (*Chalcides viridanus* and *C. ocellatus*) (see Appendix 1). The divergence times and the confidence intervals of *Mesalina* lineages were estimated using the nonparametric rate smoothing (NPRS) analysis with the recommended Powell algorithm as implemented in the software r8s (v.1.7.1 for Mac) (Sanderson, 1997,2003), which relaxes the assumption of a molecular clock. For calibration point we used the previously estimated times of divergence between *G. stehlini* from *G. symonyi* and *G. galloti* [8.7–11.8 mya; Maca-Meyer et al. (2003) or ~9.5 mya after Arnold et al. (2007)]. Mean divergence times and confidence intervals were obtained for each node based on the procedure described in the r8s software manual (http://ginger.ucdavis.edu/r8s). We generated 100 bootstrap data matrices using Seqboot of Phylip (Felsenstein, 2004) and used these to generate 100 MP phylograms with the same topology but differing branch lengths in PAUP*, which were then read into r8s, estimating ages for all trees and summarizing age distributions for each node of interest (Eriksson, 2003).

3. Results

For the phylogenetic analyses, a data set of 879 nucleotide sites and 41 combined sequences were used. Including the outgroups, there were 331 (161 for cyt *b* and 170 for 16S) variable and 229 (128 for cyt *b* and 101 for 16S) parsimony informative sites. For cyt *b*, ingroup sequence divergence ranged from 0 to 22.2%, while for 16S sequence divergence ranged between 0 and 12%.

Bayesian inference under the GTR + I + G model for cyt *b* and 16S rRNA resulted in a topology (Fig. 2) with mean $\ln L = -5{,}018.361$. Identical topologies were recovered for each of the runs with the full dataset. Neighbor-joining analysis produced tree with the same topology. It is worth noticing that the tree topology is related to the major lineages of *Mesalina*. This means that when we claim that we have identical topologies between the analyses, we refer to the basic lineages of *Mesalina*. Equally weighted parsimony analysis of the 229 parsimony-informative characters (186 for the ingroup) produced 13 most-parsimonious trees with a length of 832 steps (HI = 0.438, RI = 0.793). None of the 13 MP trees was significantly worse than the others ($p = 1.000$), based on Kishino-Hasegawa test as implemented in PAUP* under the MP criterion. The topology of these trees was identical to BI tree.

Maximum likelihood analyses under the same model of evolution resulted in topologies with $\ln L = -4975.81$ in PAUP and $\ln L = -4898.786$ in RAxML, which are identical to BI tree. The final parameters estimates for the whole dataset (results from PAUP) were gamma distribution parameter (a) = 0.434, invariable sites (I) = 0.698, and rate matrix A/C = 3.071, A/G = 11.57, A/T = 3.88, C/G = 0.96, C/T = 30.47, and G/T = 1.000, whereas for the first partition (cyt *b*) (results from RAxML) were (a) = 1.214, I = 0.533, and rate matrix A/C = 9.53, A/G = 23.41, A/T = 2.63, C/G = 0.62, C/T = 72.45, and G/T = 1.000, and for the second partition (16S) were (a) = 0.321, I = 0.137, and rate matrix A/C = 2.83, A/G = 10.06, A/T = 5.15, C/G = 1.41, C/T = 21.69, and G/T = 1.000.

All phylogenetic analyses indicate that the species *M. guttulata* and *M. bahaeldini* form a monophyletic group, just as *M. brevirostris*. For the specimen with code 35 (*M. guttulata*), which appeared more closely related to *M. oliveiri* group, see below in Section 4. Three major lineages were identified (the lineage of *guttulata*, the lineage of *brevirostris*, and the lineage of *olivieri*). Within these two lineages three clades were identified, corresponding to different species and/or to separate geographic regions (clade A: *M. bahaeldini* and *M. guttulata* from Jordan and Sinai, clade B: *M. guttulata* from northern Africa, and clade C: *M. brevirostris* from Syria and Arabia). It is worth noticing that the same topology was obtained, based on the larger dataset with all the available in GenBank sequences of *Mesalina* (54 specimens and 9 species; Fig. 2B), where *M. bahaeldini* and *M. guttulata* consist of a clade and *M. brevirostris* form a distinct lineage, which belongs to the same group of species as *M. bahaeldini* and *M. guttulata*. Given that, the small number of representatives per species (one or two specimens) and the fact that only one of the two genes used in this study is available in GenBank led us to exclude these extra specimens from the main analyses.

According to the calibration reference points, the diversification of the lineages of *Mesalina* included in this study occurred at ~9 mya during the late Miocene, whereas the splitting between *M. brevirostris* and "*M. guttulata* complex" dated ~7 mya (Fig. 2A).

4. Discussion

Because many of the taxonomic questions also relate to the biogeography of the three focal species of this study, we outline the taxonomic implications of our phylogeny first, before discussing our biogeographic hypotheses.

4.1. Taxonomic considerations

4.1.1. Mesalina guttulata

The produced phylogenetic affiliations do not support the current taxonomical view regarding the widespread species of *M. guttulata*. In our analyses the specimens of *M. guttulata* and *M. bahaeldini* appear into two clades; A and B (Fig. 2A). Clade A, which is further divided into three subclades (A1, A2 and A3), includes specimens east of the Nile (i.e., Sinai and Jordan), while clade B comprises the populations that inhabit Egypt (west of the Nile), Libya, Tunisia and Morocco. However, the phylogenetic position of subclade A1 represents an unexpected and interesting problem for the taxonomy of *M. guttulata*. This subclade consists of specimens of *M. bahaeldini* collected from Sinai. Consequently, *M. guttulata* is a clearly paraphyletic taxon, since *M. guttulata* populations from Jordan and Egypt are more closely related to populations of *M. bahaeldini* from Sinai than they are to conspecific populations of *M. guttulata* from the rest of its distribution (North Africa). This result was further reinforced using nonparametric (Shimodaira-Hasegawa test, SH) bootstrapping, where we were able to reject the alternative hypothesis that *M. guttulata* is a monophyletic species ($p = 0.002$).

Paraphyly is observed in many species (Funk and Omland, 2003 and references therein) and has multiple potential causes, including: (a) introgressive hybridization through interspecific mating followed by backcrossing of hybrids into parental populations, (b) incomplete lineage sorting due to recent speciation events and (c) imperfect taxonomy caused by misidentification of intraspecific variation.

Assuming recent divergence within the clade A (between *M. bahaeldini* and *M. guttulata* east of Nile) as supported by the estimated divergence times (in late Pliocene and Pleistocene, see below), incomplete lineage sorting may explain species paraphyly in the haplotype tree. *M. guttulata* shows a comparatively wide distribution and polymorphic allelic lineages. Hence, it may represent the ancestral species of *M. bahaeldini* whereby all these taxa (Clade B, and subclades A2 and A3) retain common alleles. However, the most likely explanation for the observed paraphyletic pattern is, in our opinion, inaccurate taxonomy caused probably by the fact that species-level variation within the *guttulata* complex has been considered as intraspecific variation. Segoli et al. (2002) claim that

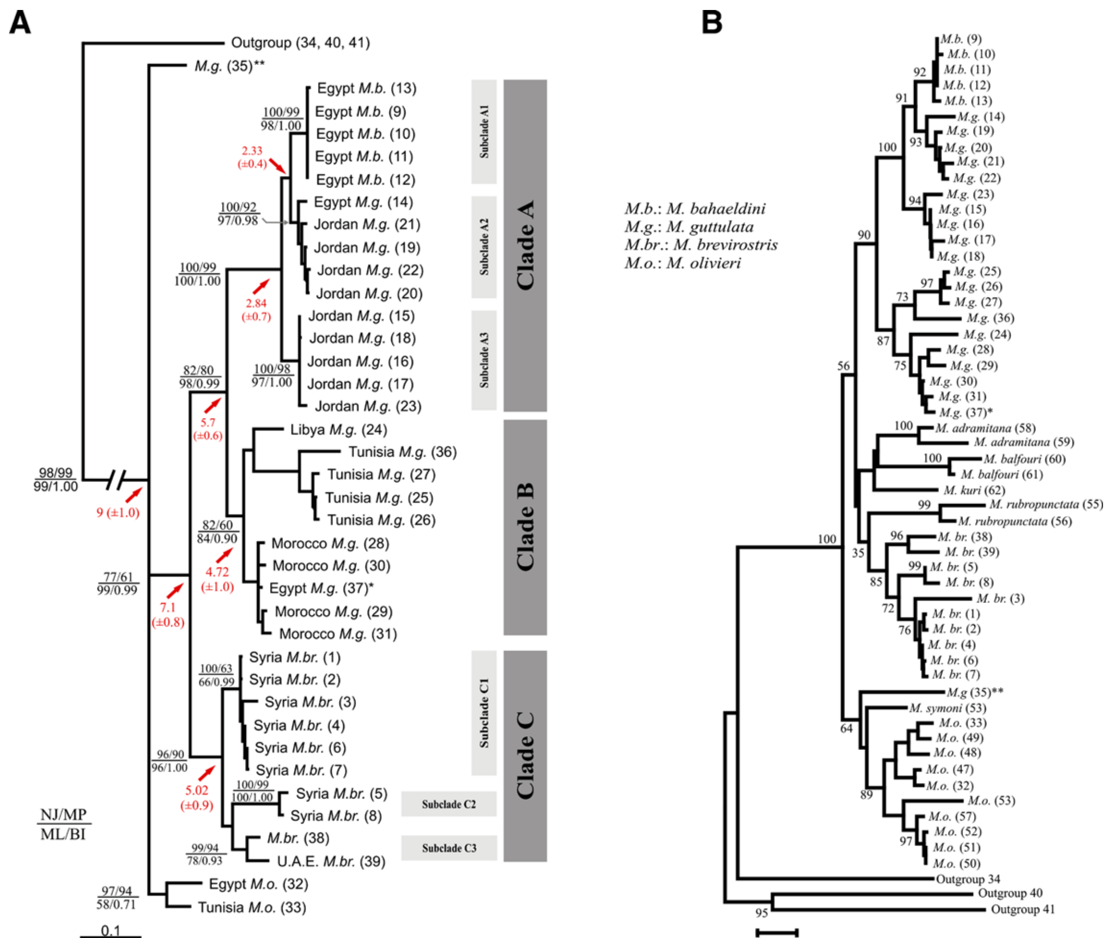

Fig. 2. (A) Phylogenetic relationships among the three focal *Mesalina* species included in the analyses. Individuals of *O. elegans*, *A. erythrurus* and *A. cantoris* were used as outgroup taxa. Phylogenetic analyses of neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) produced trees with the same topology with regard to the major lineages. Only the BI tree is presented. Numbers above the branches are bootstrap values (greater than 50% based on 1000 replicates) on NJ followed by MP, while under the branches the ML bootstrap (100 replicates) values are followed by those of the posterior probabilities of BI. Red arrows indicate the estimated time of divergences and the corresponding confidence intervals. Numbers in brackets indicate the code number in Appendix 1. * = West of the Nile river. **See Section 4 for comments on this specimen. *M.b.* = *M. bahaeldini*, *M.g.* = *M. guttulata*, *M.br.* = *M. brevirostris*, *M.o.* = *M. olivieri*. (B) Phylogenetic relationships of neighbor joining (NJ) among all the available *Mesalina* species (this study and GenBank). Numbers above the branches are bootstrap values (based on 1000 replicates) on NJ. Individuals of *O. elegans*, *A. erythrurus* and *A. cantoris* were used as outgroup taxa.

there is considerable geographic variation, mainly expressed in the coloration pattern, which varies from very faint to bold ocelli fusing to form transverse bands. This comes in agreement with Scleich et al. (1996) and Ross (1988) who mentioned the colour variation within this species, while the former also states that some populations are darkened to a glossy black. Baha El Din (2006) observed variation in pattern within the limits of Egypt (where clades A and B meet); in higher altitudes the populations tend to be darker and more strongly patterned than the lowlands, while in the western Mediterranean cost the populations appear pale greyish and patternless. Apart from the coloration another difference mentioned by Scleich et al. (1996) is that the dark borders in the palpebral disk is not present in all populations.

Due to the inconsistency of the present taxonomy of *M. guttulata* and the recovered relationships, the currently defined taxonomic status of this group is doubtful. Our results render the need for taxonomic reconsideration of this paraphyletic species and suggest that species limits in *M. guttulata* should be revised. The easiest solution to avoid the paraphyly of *M. guttulata* could be the consideration of *M. bahaeldini* as a synonym of *M. guttulata*. However, *M. bahaeldini* (subclade A1), which is a recently described species known only from Mount Sinai on the southern Sinai Peninsula of Egypt (from 600 to 1000 m asl.), appeared monophyletic (Fig. 2A) and includes specimens that differ morphologically from the rest specimens in clade A. Moreover, according to Segoli et al. (2002), who recorded both morphological and ecological differences between *M. bahaeldini* and *M. guttulata*, the two species occur in sympatry (syntopy not excluded). Although the genetic distance between *M. bahaeldini* and *M. guttulata* from Jordan and Egypt is low (4.6–5.7% in cyt *b* and 2.8–3.4% in 16S), the above evidence suggest that *M. bahaeldini* represents a distinct evolutionary lineage and could not be synonymised with *M. guttulata* in order to remove the paraphyly observed. The low level of genetic divergence could be explained by the relatively recent split of *M. bahaeldini* from *M. guttulata* of Jordan (~2 mya in late Pliocene—early Pleistocene; Fig. 2A).

The logical outcome of the reasoning presented is that all major subclades of clades A and B should also deserve species rank. On the other hand the populations studied here do not cover the entire distribution of *M. guttulata* which is not clearly delimited. This is especially true for *M. guttulata*'s eastern distribution limits after the description of *M. watsonana*. Given that, the description of these subclades as distinct species would be inaccurate. Thus we suggest, for the time being, to refer to *M. guttulata* as *M. guttulata** (Pennisi, 2001) in order to avoid taxonomic confusion at least until further input, mainly on morphology, becomes available.

This means that the current view of *M. guttulata* as monotypic (Disi et al., 2001) is superficial and it is in agreement with the observation of Baha El Din (2006), who considers *M. guttulata* a species complex requiring revision.

It is worth noticing that there is one *M. guttulata* specimen of unknown locality (code 35, see Appendix 1), retrieved from GenBank, which does not group with the rest *M. guttulata* specimens. It appeared as a sister taxon to *M. olivieri* and *M. simoni* (see Fig. 2B), which makes it more likely to be a misidentification of a species belonging to the group of *M. olivieri* (Arnold, 1986a) (i.e., *M. olivieri*, *M. simoni*, *M. pasteuri*, *M. martini*). However in case this specimen does actually correspond to a *M. guttulata*, then the point made here i.e., the need for taxonomic reconsideration of the taxon becomes even more evident, since we would be dealing with a case of polyphyly.

4.1.2. Mesalina brevirostris

Although monophyletic, *M. brevirostris* (clade C) consists of three very well supported subclades (C1, C2 and C3), the relationships of which could be consider unresolved. The first two (C1 and C2) contain specimens from Syria, while the third (C3) includes two haplotypes, one of unknown origin and the other from the United Arab Emirates. However, in the case of Syrian populations, there is not any accordance between the haplotypes grouping and the geographic origin of the specimens.

The haplotypes of C1 correspond to the three forms that Moravec (2004) distinguished based on pholidotic characters and named as "Jabal Al Arab", "W. Syria" and "Lowland". The subclade C2 includes specimens that Mayer et al. (2006) recognized as a new cryptic form (Sadat form) of *M. brevirostris*, which differs morphologically from all the other forms in having several roughly equal scales in the lower eye-lid. This study expands its distribution from Sadat locality (Mayer et al., 2006 and specimen 8 in this study) to East of the Euphrates (specimen 5 in this study) (Figs. 1 and 2A), confirming the general pattern of mixed distributions of the above forms, which could be attributed to the changes in the geographical distribution of the respective forms due to the current aridisation of the Near East (Mayer et al., 2006).

Of particular note, the genetic distance between C1 and C2 is 11.5% for cyt *b* and 3.7 for 16S rRNA (Table 1), which is considerably high for conspecific specimens occurring in sympatry and is higher than the corresponding distance between *M. bahaeldini* from Sinai and *M. guttulata* from Jordan and Egypt (5.7 for cyt *b* and 3.4 for 16S). The clear morphological and phylogenetic divergence between the two subclades of Syria (C1 and C2) renders the need of taxonomic reconsideration of this species and confirms the statement of several authors (Arnold, 1986b; Anderson, 1999; In den Bosch, 2001; Moravec, 2004; Mayer et al., 2006), who suggest that even though it is a monophyletic taxon, *M. brevirostris* is a polytypic species or even a species complex.

4.2. Historical biogeography

In historical biogeography, a reliable phylogenetic analysis and approaches that include divergence time estimation are essential to reconstruct precise hypotheses and interpret the pattern of distribution of modern taxa via dispersal–vicariance events. However, in interpreting our molecular phylogeny of *M. guttulata*, *M. bahaeldini* and *M. brevirostris* caution is needed because of the lack of the other *Mesalina* species in the present study. There is no doubt that a more robust hypothesis can be proposed once all missing taxa are included in the molecular analysis.

If we consider that the scenario for the origin of Saharo-Sindian species (described in the Section 1) is correct, our mtDNA analyses advocate for a late Miocene speciation of *Mesalina* somewhere in southwest Asia. This event resulted in the three different lineages (Fig. 2A and B) which later invaded North Africa. The first lineage branched off before 9 (±1) mya and led to the lineage recognized today as *olivieri*, whereas the second major divergence event dated at 7.1 (±0.8) mya and led to the other two major lineages (*brevirostris* and *guttulata*). These speciation events may have been correlated to the change of climate in these areas (North Africa and southwest Asia). Palaeobotanical data suggest that northern Africa was occupied by a subtropical woodland savanna with a sclerophyllous evergreen forest until the late Miocene (Quezel, 1978; Caujape-Castells et al., 2001). Since the late Miocene, northern Africa has become progressively more arid (Duellman and Trueb, 1986). In fact, a long-lasting arid period during the upper Miocene with only minor climatic oscillations should have allowed for range expansion of any xeric group, including *Mesalina*. This is evidenced by the fact that the greatest divergence of African lacertids is associated with adaptations to arid habitats (Fu, 2000).

Given the poor sampling on the lineages of *brevirostris* and *olivieri* in our dataset, we will focus on the *guttulata* lineage. Based on the estimated time of divergence, we supposed that during the upper Miocene, an ancestral form of the *guttulata* lineage might have been distributed widely throughout the regions of North Africa and southwest Asia. The separation of Eastern (Clade A) and Western (Clade B) populations took place during the late Miocene [5.7 (±0.6) mya], which coincide with the flooding of the Nile, in early Pliocene, due to the uplift of the Mediterranean sea-level (Goudie, 2005). Based on this, we can assume that the diversification of this lineage in the two clades east and west of Nile was probably the result of the former event, which divided the populations of *M. guttulata* and did not allow them to come in contact ever since. The further diversification within the two clades (east

Table 1
Sequence divergences (%) among the main mtDNA subclades of *Mesalina* for cyt *b* (below diagonal) and 16S rRNA (above diagonal), based on Tamura & Nei model of evolution No values were calculated (n/c) where no data was available

Clades-subclades	1	2	3	4	5	6	7	8	9	10	11
1. A1_*M.b.*		2.8	3.4	6.5	7.9	7.6	8.1	9.1	9.2	20.1	23.7
2. A2_*M.g.* (Jordan-Egypt)	4.6		3.7	7.2	9.6	9.2	9.7	10.8	10.2	20.4	23.6
3. A3_*M.g.* (Jordan)	5.7	4.6		6.5	7.8	8.0	8.0	10.5	8.9	21.0	24.4
4. B_*M.g.* (N. Africa)	13.8	13.7	15.6		8.1	8.0	8.0	11.2	9.8	19.8	24.8
5. C1_*M.br*	17.7	17.8	17.4	14.7		3.7	4.3	9.4	9.1	19.7	24.3
6. C2_*M.br*	20.9	18.4	19.7	18.5	11.5		5.1	8.6	9.9	19.0	25.6
7. C3_*M.br*	n/c	n/c	n/c	n/c	n/c	n/c		10.0	11.2	19.3	25.7
8. *M. guttulata* (AF080362)**	n/c	n/c	n/c	n/c	n/c	n/c	n/c		6.6	22.7	22.6
9. *M. olivieri* (N. Africa)	17.4	16.8	17.8	16.8	14.5	17.5	n/c	n/c		20.5	21.8
10. *Ophisops*	23.9	27.5	26.2	23.4	27.7	29.0	n/c	n/c	24.2		23.8
11. *Acanthodactylus*	32.6	30.1	31.6	29.7	26.8	28.6	n/c	n/c	30.5	33.7	

M.b. = *M. bahaeldini*, *M.g.* = *M. guttulata*, *M.br.* = *M. brevirostris*.
** See Section 4 for comments on this specimen.

and west of Nile) could be associated with the aridification of the Sahara in Mid-Upper Pliocene (Le Houerou, 1997) and the significant climatic changes of Pliocene and Pleistocene glaciation cycles (Caujape-Castells et al., 2001). The distribution of *guttulata* lineage was probably disrupted considerably by the climatic upheavals in North Africa during the late Pliocene and Pleistocene glaciations, leading to the different small groups of haplotypes (i.e., Tunis, Morocco in the west clade) that we observed within each clade.

Especially in the clade east of the Nile, the specimens of *M. guttulata* from Jordan appear in two subclades [A2: Egypt (Sinai) and Jordan; and A3: Jordan], the genetic divergence of which is similar as each from A1 (*M. bahaeldini*), forming a polytomy. To explain the triple polytomy, we envisage a hypothesis of a vicariant event which separated the (initially uniform) population of *M. guttulata* east of the Nile to three 3 subpopulations (i.e., one to the east of Wadi Araba, one on the mountains of Sinai and a last on the lowlands west of Wadi Araba. The latter is also mentioned by Arnold (1987) as a potential barrier for several reptile species (including *M. guttulata*) during moister periods whereas today they may be found across it.

This study revealed that dispersal–vicariance events between North Africa and Southwest Asia throughout the Miocene, Pliocene, and Pleistocene resulted in the present distribution of the species of *Mesalina* under study. As a whole, the examination of mtDNA lineages in the three species of the genus *Mesalina* used in this study may contribute substantially to the refining of their taxonomic status. Phylogenetic information can now be added to the knowledge of their morphology and distribution, producing a more accurate taxonomy for those species. The present results also confirm that the molecular information in conjunction with paleogeographical and paleoclimatic data can be used to resolve questions about the phylogeography of a species. However, our results suggest two future lines of research in *Mesalina*. First, the sampling of the rest species from North Africa and southwest Asia should be expanded. These samples may be critical for understanding their evolutionary history. Second, an assessment of the phylogenetic relationships using a nuclear DNA region is needed. This would result in independent divergence time estimates and provide a second molecular data set for assessing relationships among these species.

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Appendix 1

List of the specimens used in molecular analyses. Showing map code; species name; samples localities (in decimal degrees: Latitude/Longitude); NHMC: Natural History Museum of Crete; Acc. No: GenBank accession numbers. Sequences retrieved from GenBank are indicated by bold. Asterisk indicates specimens that used in the estimation of the divergence times. Sequences 47–62 were used only in the NJ analysis based on the 16S dataset (see Section 2).

Code	Species	Country: LATDD/LONDD	Museum No.	Acc. No.	
				Cyt *b*	16S
1	*M. brevirostris*	Syria: 34,3619/38,1740	NHMC80.3.69.1	EF555260	EF555302
2	*M. brevirostris*	Syria: 34,6000/37,8315	NHMC80.3.69.2	EF555261	EF555303
3	*M. brevirostris*	Syria: 34,6000/37,8315	NHMC80.3.69.3	EF555262	EF555304
4	*M. brevirostris*	Syria: 34,8142/38,7897	NHMC80.3.69.6	EF555263	EF555305
5	*M. brevirostris*	Syria: 35,4174/40,3198	NHMC80.3.69.9	EF555264	EF555306
6	*M. brevirostris*	Syria: 35,4268/40,0278	NHMC80.3.69.10	EF555265	EF555307
7	*M. brevirostris*	Syria: 35,4268/40,0278	NHMC80.3.69.11	EF555266	EF555308
8	*M. brevirostris*	Syria: 34,2931/36,7655	NHMC80.3.69.12	EF555267	EF555309
9	*M. bahaeldini*	Egypt: 28,5408/33,9810	NHMC80.3.108.1	EF555243	EF555285
10	*M. bahaeldini*	Egypt: 28,5408/33,9810	NHMC80.3.108.2	EF555244	EF555286
11	*M. bahaeldini*	Egypt: 28,5408/33,9810	NHMC80.3.108.3	EF555245	EF555287
12	*M. bahaeldini*	Egypt: 28,5408/33,9810	NHMC80.3.108.4	EF555246	EF555288
13	*M. bahaeldini*	Egypt: 28,7064/33,7480	NHMC80.3.108.5	EF555241	EF555283
14	*M. guttulata*	Egypt: 29,9651/33,1606	NHMC80.3.72.22	EF555242	EF555284
15	*M. guttulata*	Jordan: 31,9116/36,6168	NHMC80.3.72.14	EF555275	EF555317
16	*M. guttulata*	Jordan: 31,9116/36,6168	NHMC80.3.72.16	EF555277	EF555319
17	*M. guttulata*	Jordan: 31,9116/36,6168	NHMC80.3.72.17	EF555278	EF555320
18	*M. guttulata*	Jordan: 31,9116/36,6168	NHMC80.3.72.15	EF555276	EF555318
19	*M. guttulata*	Jordan: 31,2531/35,6135	NHMC80.3.72.10	EF555251	EF555293
20	*M. guttulata*	Jordan: 31,2531/35,6135	NHMC80.3.72.11	EF555252	EF555294
21	*M. guttulata*	Jordan: 30,7022/35,5841	NHMC80.3.72.13	EF555253	EF555295
22	*M. guttulata*	Jordan: 31,2531/35,6135	NHMC80.3.72.20	EF555250	EF555292
23	*M. guttulata*	Jordan: 29,5704/35,4113	NHMC80.3.72.24	EF555279	EF555321
24	*M. guttulata*	Libya: 30,4659/24,5366	NHMC80.3.72.8	EF555254	EF555296
25	*M. guttulata*	Tunisia: 33,5225/9,9925	NHMC80.3.72.1	EF555268	EF555310
26	*M. guttulata*	Tunisia: 33,5225/9,9925	NHMC80.3.72.2	EF555269	EF555311
27	*M. guttulata*	Tunisia: 33,1502/10,2899	NHMC80.3.72.7	EF555270	EF555312
28	*M. guttulata*	Morocco: 32,0472/-4,4088	NHMC80.3.72.5	EF555255	EF555297
29	*M. guttulata*	Morocco: 31,4018/-5,7276	NHMC80.3.72.9	EF555256	EF555298
30	*M. guttulata*	Morocco: 31,0882/-6,4673	NHMC80.3.72.18	EF555257	EF555299

Appendix 1 (*continued*)

Code	Species	Country: LATDD/LONDD	Museum No.	Acc. No.	
				Cyt *b*	16S
31	*M. guttulata*	Morocco: 31,7146/-4,9221	NHMC80.3.72.21	EF555258	EF555300
32	*M. olivieri*	Egypt: 29,9651/33,1606	NHMC80.3.119.19	EF555248	EF555290
33	*M. olivieri*	Tunisia: 32,1287/10,5638	NHMC80.3.119.10	EF555272	EF555314
34	*O. elegans*	Syria: 34,3619/38,1740	NHMC80.3.70.70	EU081644	EU081716
35	*M. guttulata*	Unknown Harris J. (1997) Ph.D.	—	—	**AF080362**
36	*M. guttulata*	Tunisia: Tamerza	—	—	**AY035842**
37	*M. guttulata*	Egypt: Harraat al Harrah	—	**AY217815**	**AY217969**
38	*M. brevirostris*	Unknown	—	—	**AF206606**
39	*M. brevirostris*	United Arab Emirates: Abu Dhabi	—	—	**AY035841**
40	*A. erythrurus*	—	—	**AF206536**	**AF197498**
41	*A. gongrorhynchatus*	—	—	**AF080342**	**AF080343**
42	*G. simonyi*∗	—	—	**AF101224**	**AF101208**
43	*G. galloti*∗	—	—	**U51301**	**AF019651**
44	*G. stehlini*∗	—	—	**AY154899**	**AF149936**
45	*C. viridanus*∗	—	—	**Z980037**	**AF232667**
46	*C. ocellatus*∗	—	—	**Z98040**	**AF215234**
47	*M. olivieri*	Egypt: 29,9651/33,1606	NHMC80.3.119.16	—	EF555289
48	*M. olivieri*	Tunisia: 34,4076/7,9448	NHMC80.3.119.9	—	EF555313
49	*M. olivieri*	Tunisia: 33,7531/9,3350	NHMC80.3.119.14	—	EF555315
50	*M. olivieri*	Libya: 32,3912/21,2404	NHMC80.3.119.3	—	EF555323
51	*M. olivieri*	Libya: 32,3912/21,2404	NHMC80.3.119.2	—	EF555322
52	*M. olivieri*	Libya: 32,3912/21,2404	NHMC80.3.119.5	—	EF555324
53	*M. olivieri*	Egypt: 29,9797/32,1187	NHMC80.3.119.20	—	EF555291
54	*M. simoni*	Morocco: 31,9120/-7,5050	NHMC80.3.109.1	—	EF555301
55	*M. rubropunctata*	Egypt: 24,4000/33,01700	NHMC80.3.99.1	—	EF555316
56	*M. rubropunctata*	Egypt: Hurghada	—	—	**AY035840**
57	*M. olivieri*	Egypt	—	—	**AY035839**
58	*M. adramitana*	United Arab Emirates:Layn	—	—	**AY035843**
59	*M. adramitana*	Unknown Harris J. (1997) Ph.D.	—	—	**AF080360**
60	*M. balfouri*	Yemen	—	—	**AY035835**
61	*M. balfouri*	Yemen	—	—	**AY035834**
62	*M. kuri*	Yemen: Abd al-Kuri Island	—	—	**AY035836**

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