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Genetic diversity within species of *Ophisops* (Reptilia, Lacertidae) from the Maghreb

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Abstract

Previous phylogeographic assessments within species of the lacertid genus *Ophisops* have consistently revealed high levels of genetic diversity, suggestive of multiple cryptic species complexes. The snake-eyed lizard *Ophisops elegans*, endemic to the Mediterranean region and Central Asia, is no exception, although specimens of the North African endemic *Ophisops occidentalis* from Libya were not distinct from sympatric *O. elegans* individuals based on mitochondrial DNA (mtDNA) markers. However, the most westerly populations of these two species from Morocco and Algeria were not assessed. Here, we analyzed partial 16S rRNA mtDNA sequences and partial MC1R nuclear sequences from Morocco, Algeria, and Tunisia for comparison with previously published data. Our estimates of relationships based on both markers support the hypothesis that all North African populations form a clade, consistent with an east-to-west colonization pattern across this region. Additional mtDNA sublineages were identified, further highlighting genetic diversity in remote areas. While the genetic data indicate that a single species should be recognized in North Africa, high diversity within *O. elegans* across the remainder of its range still requires further investigation prior to a taxonomic revision differentiating taxa within this species complex.

Key Words

Algeria, MC1R, Morocco, *Ophisops elegans*, *Ophisops occidentalis*, phylogeography, Tunisia, 16S rRNA

Introduction

Identifying and delimiting biodiversity hotspots is crucial for effective conservation planning. However, socioeconomic factors introduce biases in biodiversity assessments, influencing species discovery trends, with wealthier regions exhibiting higher inventory completeness (Meyer et al. 2015), while accessibility and remoteness are also associated with biodiversity shortfalls. This pattern is evident across the Mediterranean region, with considerable phylogeographic research conducted in the European portion of the West Palearctic, but notable deficiencies in knowledge regarding species distributions and diversity patterns in the corresponding North African part of the same biodiversity hotspot (e.g., Berrilli et al. 2025). Reptile diversity in particular is thought to be underestimated (Ficetola et al. 2013), although genetic assessments have recently led to the description of many cryptic species (e.g., Javanmardi et al. 2019; Miralles et al. 2020). One emerging biogeographic pattern is a common phylogeographic break between the western and eastern Maghreb (e.g., Salvi et al. 2017; Beddek et al. 2018). Among various Maghreb endemics, this break coincides with the arid valley of the Moulouya River in eastern Morocco. Some species with a much broader distribution outside the Maghreb, including snakes, skinks,

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and shrews, show a similar pattern, although not universally (reviewed in Salvi et al. 2017).

Snake-eyed lizards of the genus *Ophisops* represent one such broadly distributed group, with two species recognized in the region. *Ophisops elegans* is widely distributed across the eastern Mediterranean and Southeast Asia, with additional populations in Libya, Egypt, and Algeria, specifically in the Aurès region. *Ophisops occidentalis* occurs across northern Algeria, extending into eastern Morocco, and is also present in Tunisia, Libya, and Egypt. A broad phylogeographic study based on mitochondrial DNA sequences revealed substantial diversity and indicated that both species were polyphyletic (Kyriazi et al. 2008). Samples of both species from Libya were nearly identical and closely related to *O. occidentalis* from Tunisia, which together formed a sister lineage to *O. elegans* samples from Israel (Clade B sensu Kyriazi et al. 2008). However, as this study relied solely on mtDNA, the possibility of introgression between the species in Libya could not be excluded. Subsequent assessments of eastern lineages (Clade C) and samples from Anatolia also revealed high levels of genetic diversity and emphasized the need for a taxonomic revision (Montgelard et al. 2020; Bozkurt et al. 2022). Specimens from Saudi Arabia were also found to be genetically distinct, although more similar to those from Iran and clearly differentiated from the lineage in North Africa and Israel (Roobas et al. 2025). Montgelard et al. (2020), using both mtDNA and nuclear markers, proposed that at least the three main clades might warrant recognition as distinct species. Nonetheless, these assessments did not include any specimens from Algeria, where both *O. occidentalis* and *O. elegans* are found, or from Morocco.

The aim of this study was to include specimens of *O. occidentalis* from Morocco, Algeria, and Tunisia, as well as *O. elegans* from the isolated Algerian populations, using 16S rRNA mtDNA and MC1R nuclear DNA sequences to compare with previously published data. This approach should provide further insight into the number of mtDNA lineages present in the region while also allowing a deeper assessment of phylogeographic breaks within the Maghreb. The combination of both markers may help rule out introgression as an explanation for the patterns observed in Libyan specimens based only on mtDNA. These data are also critical for any future taxonomic reassessment, especially considering that syntypes of *O. occidentalis* originate from eastern Algeria and Tunisia (Boulenger 1887).

Methods

Ten specimens of *Ophisops* species were collected in the field in Morocco, Tunisia, and Algeria (Fig. 1, Table 1). Across most of this region, only *O. occidentalis* is present. In Algeria, where *O. occidentalis* and *O. elegans* can occur in sympatry, they were distinguished based on the number of supraciliary granular scales (<17 corresponding to *O. occidentalis*, ≥ 17 to *O. elegans*), following Chirio and Blanc (1993). In the field, a small piece of tail tissue was preserved in 96% ethanol for genetic analyses.

DNA was extracted from these tissues using standard high-salt methods (Sambrook et al. 1989), followed by PCR amplification of a segment of the mitochondrial 16S rRNA gene using the 16SH and 16SL primers from Palumbi (1996) and the conditions described in Harris et al. (1998). To compare individuals of *O. elegans* and *O. occidentalis* using a nuclear marker, an additional PCR was conducted with primers MC1Rf and MC1Rr (Pinho et al.

Table 1. List of samples sequenced in this study for 16S rRNA. Samples marked with an asterisk (*) were also sequenced for the MC1R nuclear gene fragment.

Figure Code	Specimen Code	Coordinates
1	Rep005*	35.2591, 6.3329
2	Rep007	35.1266, 6.3242
3	Rep024*	35.2540, 6.1382
4	Rep025*	35.5862, 6.1779
5	Pap1*	35.5648, 6.2133
6	DB4036	35.1546, 8.4401
7	DB19128	35.8160, 9.3660
8	DB14683*	34.3423, -1.9890
9	DB1631*	36.2260, 8.8913
10	DB3973*	34.0250, -2.6056

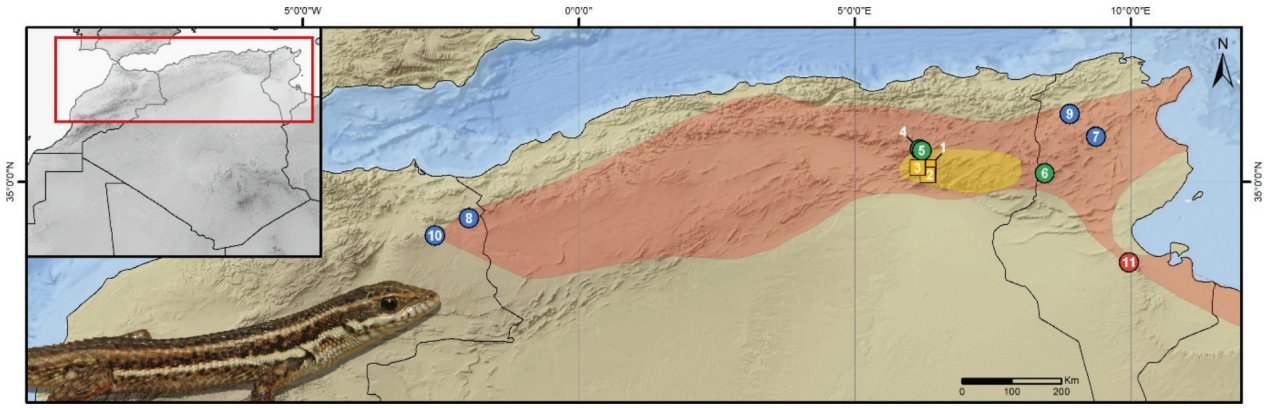

Figure 1. Distribution map of *Ophisops* mtDNA lineages in North Africa. Circles represent *O. occidentalis*, and squares represent *O. elegans*. Color codes correspond to the sublineages identified in Fig. 2. Numbers 1–10 indicate newly sequenced specimens (see Table 1), and locality 11 represents an approximate location from Kyriazi et al. (2008). Species photograph by Idriss Bouam.

2010) using the conditions described in Salvi et al. (2013). Positive PCR products were cleaned and sequenced at the facilities of BIOPOLIS.

The partial 16S rRNA sequences were aligned in BioEdit using ClustalW (Thompson et al. 2003), along with nine members of the North African/Middle Eastern clade (Clade B, from Kyriazi et al. 2008) and two more distantly related specimens: *O. elegans blandfordi* from Syria (Kyriazi et al. 2008) and *O. elegans* from Greece (Pavlicev and Mayer 2009). In total, this dataset comprised 21 specimens and 485 bp. For the MC1R marker, seven new sequences were compared with published data from the other two main clades within *O. elegans* (Montgelard et al. 2020), following the same approach. Two sequences from *Mesalina watsonana* (Simo-Riudalbas et al. 2019) and *Mesalina martini* (Sindaco et al. 2018) were included as outgroups. The dataset comprised 16 taxa and 584 bp.

Phylogenetic relationships derived from the 16S rRNA sequences were estimated using a maximum likelihood approach in PhyML 3.0 (Guindon et al. 2010), both to identify the most appropriate model of evolution under the AIC criterion (Lefort et al. 2017) and to infer the phylogeny. Node support was assessed using 1,000 bootstrap replicates. The model selected by PhyML and used in the analysis was GTR+I+G. Trees were visualized using FigTree v1.4.4. Because variation in the nuclear marker was extremely low, a haplotype network was constructed using the statistical parsimony method implemented in TCS (Clement et al. 2000).

All new sequences were submitted to GenBank (PV810783–PV810792).

Results

Based on the 16S rRNA sequences, six sublineages were identified within Clade B sensu Kyriazi et al. (2008): specimens of *O. occidentalis* from Morocco and Tunisia; specimens of *O. occidentalis* from Tunisia and Algeria; specimens of *O. occidentalis* from Tunisia; specimens of *O. elegans* from Algeria; specimens of both *O. occidentalis* and *O. elegans* from Libya; and specimens of *O. elegans* from Israel (Fig. 2). Three of these six sublineages had previously been identified (Kyriazi et al. 2008). Relationships among them are not strongly supported based on this single mtDNA gene fragment. However, the samples from Israel appear to be sister to all lineages found in North Africa, while within the region, the Libyan lineage is sister to the four Maghreb lineages (Fig. 2).

Based on the MC1R sequences, all seven North African individuals representing both *O. elegans* and *O. occidentalis* shared only two haplotypes (Fig. 3). Although relationships among the major groups sensu Kyriazi et al. (2008) and Montgelard et al. (2020) are weakly supported, the North African haplotypes are more closely related to those from individuals in Turkey and Azerbaijan (mtDNA Clade A) than to those from Iran (mtDNA Clade C).

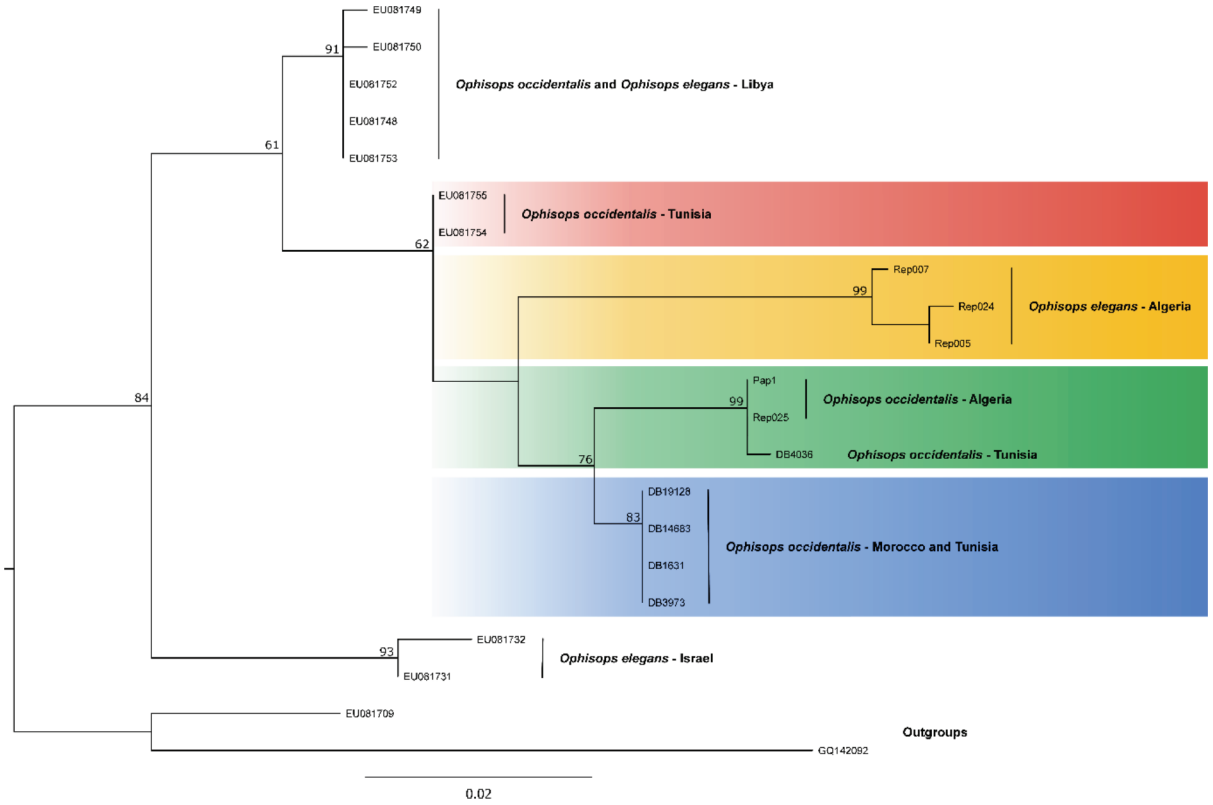

Figure 2. Estimate of relationships among *Ophisops* species occurring in North Africa, based on a maximum likelihood analysis of partial 16S rRNA sequences. Codes refer to Table 1 or GenBank accession numbers. Following Kyriazi et al. (2008), the tree was rooted using specimens from Greece and Syria. Numbers above nodes indicate bootstrap support values (1,000 replicates). Color codes and localities correspond to Fig. 1, Table 1.

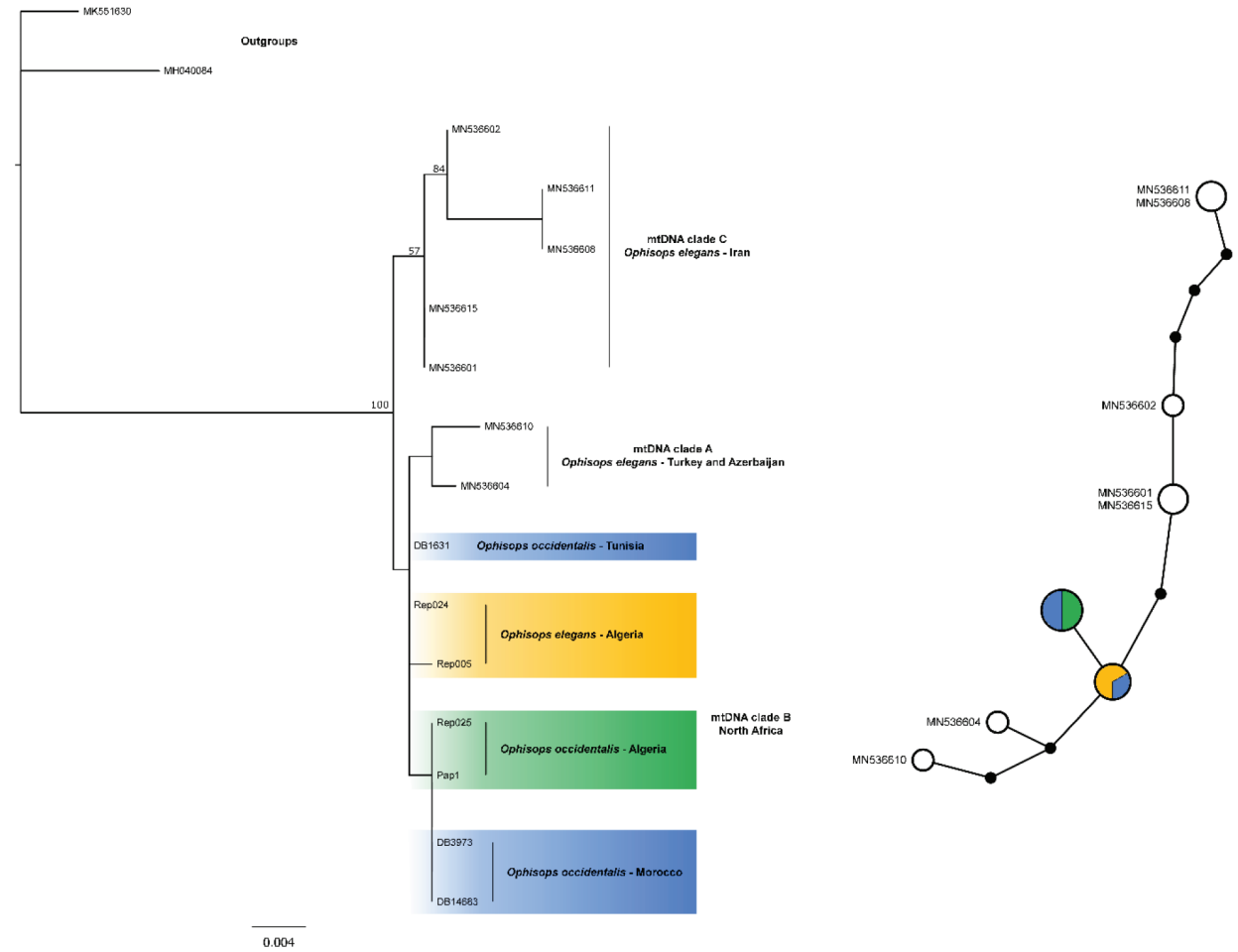

Figure 3. Estimate of relationships within the *Ophisops elegans* complex based on a maximum likelihood analysis of partial MC1R sequences. Codes refer to Table 1 or GenBank accession numbers. The tree was rooted using two species from the genus *Mesalina*. Numbers above nodes indicate bootstrap support values (1,000 replicates). The network derived from a statistical parsimony analysis is color coded identically, with black dots indicating missing haplotypes. Mitochondrial DNA lineages are delimited following Kyriazi et al. (2008) and Montgelard et al. (2020).

Discussion

Previous phylogeographic assessments of *Ophisops* in the Mediterranean (Kyriazi et al. 2008), Iran (Montgelard et al. 2020), and India (Agarwal and Ramakrishnan 2017) all highlighted extensive cryptic diversity. Initial diversification appears to have begun in India, followed by a back-dispersal into Saharo-Arabia during the Middle Miocene (Agarwal and Ramakrishnan 2017). Kyriazi et al. (2008) estimated that the sublineages within Clade B began to diversify around 5.58 Mya, beginning with the separation of the Israeli lineage from those in North Africa, followed by the divergence of the Libyan and Tunisian lineages around 4.9 Mya. Given this timeframe, it is unsurprising that we identified additional sublineages within North Africa in the present study, although the overall pattern remains consistent with an east–west dispersal. Interestingly, three sublineages occur in Tunisia and two in Algeria, indicating regional diversification.

From a taxonomic perspective, neither *O. elegans* nor *O. occidentalis* is monophyletic in the present study or in previous assessments (Kyriazi et al. 2008), despite the high levels of genetic diversity reported across studies of the genus (Kyriazi et al. 2008; Agarwal and Ramakrishnan 2017; Montgelard et al. 2020; Bozkurt et al. 2022). Although most lineages have been identified using mitochondrial markers alone, studies incorporating additional markers have often supported the conclusions drawn from mtDNA (e.g., Montgelard et al. 2020), and some lineages have subsequently been described as full species following morphological assessments (e.g., Agarwal et al. 2018). The finding that individuals with the *O. occidentalis* morphotype from across the Maghreb are unrelated to those in Libya but form distinct sublineages within *O. elegans* suggests that population origin, rather than morphotype, is associated with mtDNA diversity. Hemipenial characters are similar in *O. elegans* and *O. occidentalis* from Libya but differ from those in other *O. elegans* populations (Arnold 1986), further supporting the hypothesis of a single, geographically variable species rather than two species with introgression. Assessment of nuclear MC1R sequences also supports this conclusion: only two haplotypes were found in North Africa, with both species sharing one haplotype. We therefore suggest that a single

species of the *Ophisops elegans* complex occurs across North Africa. Montgelard et al. (2020) proposed that at least the three major mtDNA lineages should be referred to as candidate species, with lineage B (North Africa and Israel) corresponding to *O. occidentalis*, lineage A4 to *O. elegans*, and lineage C to *O. persicus*. However, given the deep diversity and phylogenetic structure observed, some sublineages may also correspond to additional species. Moreover, the identity of the Israeli specimens requires confirmation using nuclear markers.

Regarding notable phylogeographic breaks, *O. occidentalis* does not cross the Moulouya River valley but reaches its eastern margin—a known barrier for various reptile and amphibian species (Salvi et al. 2017). Since *O. occidentalis* exhibits an east–west expansion pattern, it may have reached this region after the barrier was already in place. Indeed, the 16S rRNA sequences from Moroccan individuals are very similar to some Tunisian sequences, suggesting that the expansion across western Algeria was relatively recent (Fig. 2). Based on Kyriazi et al. (2008), the divergence between Algerian and Tunisian subpopulations likely occurred during the Plio-Pleistocene, a period of major climatic fluctuations in the region that coincided with Sahara expansions and contractions (Le Houérou 1997). Other reptile sublineages in the region also date to this period (Machado et al. 2021), suggesting similar ecological responses to past climatic changes.

In conclusion, our additional data from the westernmost populations of both *O. elegans* and *O. occidentalis* further support the biogeographic hypothesis of an east–west expansion across North Africa. Plio-Pleistocene climatic fluctuations may have contributed to the emergence of distinct mtDNA sublineages in Algeria and Tunisia, while the expansion into Morocco may have occurred more recently. Current evidence indicates that a single taxon should be recognized across North Africa—*O. occidentalis*—although the deep divergence observed in the eastern part of the range of *O. elegans* underscores the need for a detailed taxonomic revision of the entire species complex.

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