

Phylogeography and genetic diversity of *Psammophis schokari* (Serpentes) in North Africa based on mitochondrial DNA sequences

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The snake *Psammophis schokari* has a widespread distribution across North Africa, and in Morocco/Western Sahara is represented by three different morphotypes: striped, unicoloured and the Western-Sahara morph. ND4 mitochondrial DNA sequences from 28 specimens comprising 20 *P. schokari*, two *P. aegyptius*, one *P. elegans*, two *P. sibilans*, one *P. condanarus* and two outgroups were analysed. Within *P. schokari* we identified four genetic lineages (Morocco/Western Sahara, Mauritania, Algeria and Israel) with a genetic divergence ranging from 4–5%, less than that typically found between different species. Surprisingly, Moroccan/Western Sahara and Algerian lineages are the most divergent ones. This geographic substructuring may be due to severe climate changes in the Sahara desert between the Miocene and Pleistocene associated with expansion/contraction phases of this desert. *Psammophis aegyptius* is the sister-taxon of *Psammophis schokari* with a high level of genetic divergence between them (10.7%) supporting the recognition of *P. aegyptius* as a distinct species. The three Moroccan/Western Sahara colour morphotypes form one genetic lineage, indicating that colour pattern does not reflect a different phylogenetic history, and is probably an ecological adaptation to the local environment.

Key words: *Psammophis schokari*, North Africa, phylogeny, ND4 sequence, colour pattern.

INTRODUCTION

The genus *Psammophis* (Psammophiinae) includes 24 species, most of them with an African origin, but some also occur in the Middle East and Asia. *Psammophis schokari* is widespread in North Africa having a Saharo-Sindian distribution (Bons & Geniez 1996); it is also found in the Middle East, Arabia, Iran, a large part of Afghanistan, Uzbekistan and northwest India (Geniez *et al.* 2004). *Psammophis aegyptius* Marx, 1958, was formerly considered as a subspecies of *Psammophis schokari* but is currently recognized as a distinct species (Schleich *et al.* 1996). In Morocco/Western Sahara, three distinct morphotypes have been recorded for *P. schokari*: the striped form that occurs in the Atlantic Coast and occasionally in the High Atlas Mountains; the unicoloured form typically present in the High Atlas; and the Western-Sahara form with a slightly less slender body, weakly striped pattern and greyish belly (Bons & Geniez

1996). The first two co-occur in Southern and Central Morocco, while the Western-Sahara morph is the only form in this region. The occurrence of striped and unicoloured morphotypes has also been recorded in Israel and Sinai, Egypt (Kark *et al.* 1997).

During the Pleistocene, North Africa experienced alternative humid and drier periods that appear to have influenced genetic subdivisions in this region in terrestrial species ranging from snails (Guiller *et al.* 2001) to mammals (Cosson *et al.* 2005). Several reptiles also show deep subdivisions, including *Agama impalearis* (Brown *et al.* 2002) *Acanthodactylus* (Bons & Geniez 1995; Harris *et al.* 2004a) and *Uromastix* (Harris *et al.* 2007). The Moulouya River Valley, in Eastern Morocco, is often suggested to be a geographical barrier to gene flow for non-desert species (Álvarez *et al.* 2000). However, at least within the tortoise *Testudo graeca*, or the snake *Malpolon monspessulanus*, the Moulouya River Valley is not coincident with

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Table 1. Morphotype, locality, sample code and coordinates of the specimens sequenced for this study.

Species	Morphotype	Locality	Sample code	Coordinates
<i>Psammophis schokari</i>	Unicoloured	Merzouga, Ouarzazatte, Morocco	20	31°7'N, 6°24'W
	Unicoloured	Merzouga, Ouarzazatte, Morocco	90	31°7'N, 6°24'W
	Striped	15 km S Saka, Morocco	126	34°30'N, 2°20'W
	Striped	South of Nouakchott, Mauritania	D491	17°24'N, 16°4'W
	Western Sahara	280 km S Dakhla, Western Sahara	D538	22°5'N, 16°41'W
	Western Sahara	150 km S Dakhla, Western Sahara	D543	23°11'N, 16°9'W
	Western Sahara	Dakhla, Western Sahara	D545	23°8'N, 16°4'W
	Western Sahara	120 km S Boujdor, Western Sahara	D551	25°3'N, 14°48'W
	Western Sahara	60 km N Boujdor, Western Sahara	D557	26°26'N, 13°60'W
	Striped	Ouarzazatte, Marrakech, Morocco	D705	31°22'N, 7°24'W
	Western Sahara	155 km S Boujdor, Western Sahara	D797	24°45'N, 14°52'W
	Western Sahara	110 km S Boujdor, Western Sahara	D799	25°12'N, 14°49'W
	Unicoloured	Awrir i-Bedden, Ghardaia, Algeria	D872	32°29'N, 3°39'E
	Striped	10 km NE Chetma, Biskra, Algeria	D913	34°53'N, 5°54'E
	Unicoloured	10 km NE Chetma, Biskra, Algeria	D914	34°53'N, 5°54'E
	Striped	20 km NE Chetma, Biskra, Algeria	D915	34°60'N, 6°2'E
	Striped	7 km SW Rhoufi, Biskra, Algeria	D916	35°3'N, 6°7'E
	Striped	Costal Plain: Yavenh sands, Israel	21327	31°53'N, 34°42'E
	Striped	Costal Plain: Yavenh sands, Israel	21328	31°53'N, 34°42'E
	Striped	Costal Plain: Nizzanim sands, Israel	21445	31°46'N, 34°38'E
<i>Psammophis aegyptius</i>		Dirkou oasis, Niger	D340	18°59'N, 12°54'E
		Bilma oasis, Niger	D341	18°41'N, 12°55'E
<i>Psammophis elegans</i>		Didieni, Bamako Kayes, Mali	D434	13°44'N, 8°1'W
<i>Psammophis sibilans</i>		Fada N'Gourma-Bogande, Burkina Faso	D421	12°13'N, 0°18'E
		Fama, Segou Bamako, Mali	D432	12°46'N, 7°12'W

genetic subdivision (Carranza *et al.* 2006; Harris *et al.* 2003). Considering *Agama* (Brown *et al.* 2002) and the stripe-necked terrapin *Mauremys leprosa* (Fritz *et al.* 2005; 2006) the barrier appears to be the Atlas Mountain chain, which formed during the mid to late Miocene. High levels of genetic subdivision have indicated that some widespread forms may actually be species complexes, including *Tarentola mauritanica* (Harris *et al.* 2004b,c) and *Lacerta perspicillata* (Harris *et al.* 2003b), while the snake *Macroprotodon* also shows sub-structuring within Morocco between the Rif and the Atlas Mountains (Carranza *et al.* 2004).

The aim of this study was to compare the genetic diversity between the three different morphotypes of *Psammophis schokari* using mtDNA sequence data from North African specimens. An additional aim was to determine if geographic barriers were co-incident with genetically distinct units, and to compare phylogeographic variation within *Psammophis schokari* with that reported for other North African reptiles.

MATERIALS & METHODS

Tissue samples from 20 *Psammophis schokari*, two

Psammophis aegyptius, two *Psammophis sibilans*, and one *Psammophis elegans* were collected in Morocco, Western Sahara, Niger, Burkina-Faso, Mali, Mauritania, Algeria and Israel (geographic locations of the specimens are given in Table 1 and Fig. 1). Tail tips were collected from live specimens. All snakes were photographed and released. The majority of specimens were roadkills. All specimens from Israel came from a museum collection, with code numbers given in Table 1. Genomic DNA was extracted following standard high-salt protocols (Sambrook *et al.* 1989). A fragment including the terminal portion of the ND4 gene and the tRNAs for serine, histamine and leucine was amplified by PCR using the primers published by Arévalo *et al.* (1994). Amplification conditions were the same as described by Pinho *et al.* (2006). Sequences from both strands were obtained on an automated sequencer (ABI 310). All sequences were submitted to GenBank (accession numbers EF128005 to EF128029). Alignment was performed manually using Bioedit v. 5.0.9. (Hall 1999). One specimen of *Psammophis condanarus* was included from GenBank (AY058987). *Malpolon monspessulanus* and *Psammophylax variabilis* from Genbank were included as

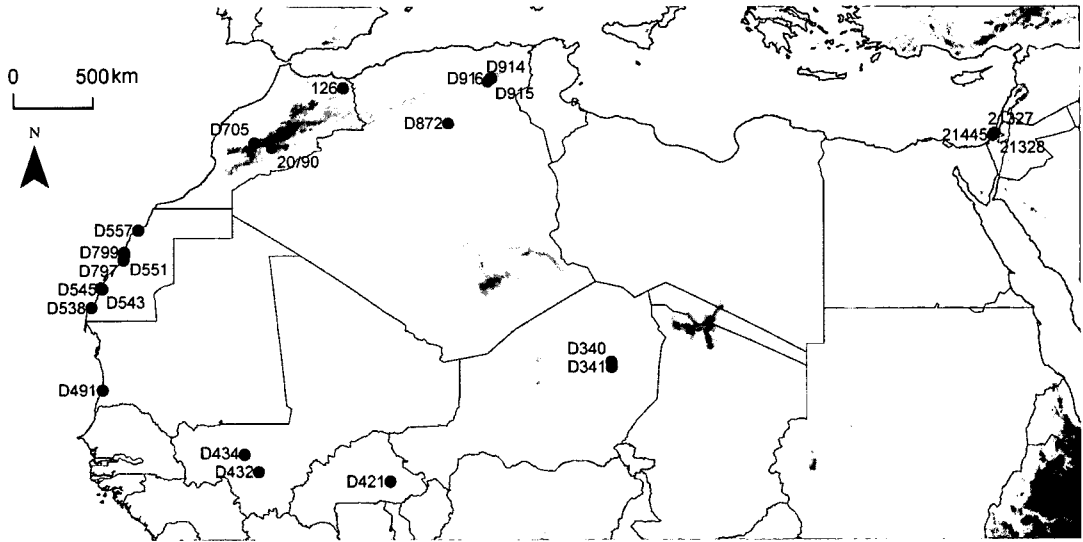


Fig. 1. Map showing sampling localities. Codes correspond to Table 1.

outgroups as both genera are known to be closely related to *Psammophis* (Kelly *et al.* 2003; Lawson *et al.* 2005; Nagy *et al.* 2005; Carranza *et al.* 2006). Sequences were imported into PAUP* 4.0b10 (Swofford 2003) and for the phylogenetic analysis we used maximum likelihood (ML) analysis with random sequence addition (10 replicate heuristic search). Maximum parsimony (MP) was also carried out with random sequence addition (100 replicate heuristic searches) and support for nodes was estimated through the bootstrap technique (Felsenstein 1985) with 100 (ML) and 1000 (MP) replicates, respectively. The AIC criteria in Modeltest 3.06 (Posada & Crandall 1998) were used to choose the model of evolution employed in the ML analysis. Bayesian analysis was also implemented using Mr.Bayes v.3.1 (Huelsenbeck & Ronquist 2001) with parameters estimated as part of the analysis and four incrementally heated Markov chains with the default heating values. All analysis started with a randomly generated tree and ran for 10^6 generations, saving one tree per 100 generations. The log-likelihood values of the sample point were plotted against the generation time and all the trees prior to reaching stationary were discarded, ensuring that burn-in samples were not retained. Combining the remaining trees, a 50% majority consensus tree was generated. The frequency of any particular clade of the consensus tree represents the posterior probability of that clade (Huelsenbeck & Ronquist 2001). Two independent replicates were conducted and inspected for consistency to avoid being

trapped in local optima (Huelsenbeck & Bollback 2001).

RESULTS

Including the outgroups, 28 individuals were analysed with an aligned length of 765 bp. The most appropriate model of evolution for this dataset was the TVM, with an estimate of invariable sites and a discrete approximation of the gamma distribution. The ML analysis recovered a single tree ($-\ln 3311$, Fig. 2). A total of 48 MP trees were recovered (523 steps) that differed from the ML tree only by being unresolved within regions (within Morocco/Western Sahara and within Algeria), and in the position of *P. elegans* which is considered to be the sister taxon of *P. sibilans* (Fig. 1). The Bayesian analyses recovered the same tree as the ML analysis.

CONCLUSIONS

The sampled species of *Psammophis* formed a monophyletic group, and all *Psammophis* species for which multiple samples were available formed well-supported monophyletic units. *Psammophis aegyptius* is the sister-taxon of *Psammophis schokari*, and since the level of genetic divergence between these two taxa (10.7%) is similar to that between the other species, these results are not in conflict with the recognition of *Psammophis aegyptius* as a distinct species. Within *P. schokari* four genetic lineages (Morocco/Western Sahara, Mauritania, Israel and Algeria) can be identified that are geographically coherent. The lineages from Morocco/

(Prentice *et al.* 2000). Apparently, all these climatic fluctuations from desert to vegetated land and *vice versa* were very rapid, and in some cases they lasted no more than a few hundred years (Sarnthein 1978). The consequences of these climate changes must have been dramatic to the fauna and flora of the region, leading to isolation/expansion among *Psammodromus schokari* populations and rapid range changes. This, associated with the contraction/expansion phases of the Sahara desert could have caused several events of isolation and differentiation, followed by connectivity and admixture over short periods of time. This may explain the distinct geographic units recovered, both in *P. schokari* and in other semi-desert species such as *Uromastyx* (Harris *et al.* 2007). With such a large range across the Middle East still unsampled, more phylogeographic conclusions cannot be drawn. However, our results clearly indicate that colour pattern is not reflecting phylogenetic history in this complex species.

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