Differentiation within Syrian populations of the lizard *Mesalina brevirostris*

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To estimate phylogenetic relationships among the Syrian morphotypes of the Mesalina brevirostris *we analysed a stretch of 834 bp of mitochondrial cytochrome b of samples from four Syrian and two Jordan localities. The analysis reveals two main clades: the first one comprises the big part of samples from Syria and Jordan, the second one an undescribed cryptic form from central part of western Syria. Subdivisions within the first clade correspond to (1) the Lowland form occupying central and eastern Syria and traditionally associated with the name* M. b. brevirostris *(Blanford 1874), (2) the form from Jabal al Arab in southern Syria which can be assigned to the name* M. b. microlepis *(Angel 1936) and, finally, (3) an additional form of uncertain taxonomic status in eastern Jordan.*

Keywords: *Mesalina brevirostris*, mitochondrial DNA sequences, taxonomy, Syria, Jordan.

Introduction

Mesalina brevirostris (Blanford 1874) is considered morphologically variable polytypic species (or group of species) widely distributed in the Near and Middle East (*e.g.*, Arnold 1986, Anderson 1999, in den Bosch 2001). Traditionally, two morphotypes are distinguished within Syrian populations, usually associated with two names: *M. b. brevirostris* comprising eastern and central Syrian populations, and *M. b. microlepis* (Angel 1936), comprising western Syrian populations (*e.g.* Angel 1936; Haas & Werner 1969; Werner 1971; Disi 1991, 1996).

In a previous study (Moravec 2004), the re-evaluation of the distribution and morphological variation of Syrian *M. brevirostris* revealed the existence of three rather differentiated morphotypes within the Syrian borders: (1) Lowland form – mostly lower altitudes (up to 500-600 m) of the desert and desert-steppe regions of central and eastern Syria; (2) Western (intermediate) form – higher altitudes (600-800 m)

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of the western part of Syria; (3) Jabal al Arab form – eastern slopes of Jabal al Arab (J. Duruz) in southern Syria (ca 1000 m a.s.l). These morphotypes tend to differ in pholidotic characters, body shape and colouration. However, the morphological differences are not prominent enough to allow easy distinction of the individual forms in the field.

Therefore, the aim of this study was to investigate the phylogenetic relatedness among the specified morphotypes on the molecular level.

Material and Methods

The localities of samples are given in Table 1 and Fig. 1. Total genomic DNA was extracted from deep-frozen or ethanol preserved soft tissues, tongues and tail tips following a standard phenol-chloroform procedure (Sambrook *et al*. 1989). Preserved specimens are deposited in the National Museum Prague (NMP) and the Naturhistorisches Museum Wien (NHMW).

Amplifications of all PCR fragments were performed in 25 µl reaction mixtures containing PCR buffer with 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.4 µM of each PCR primer, 0.5 units of Taq polymerase (Amersham). Reaction conditions comprised an initial denaturation step of 2 min at 94 °C, 35 cycles of 10 s at 95 °C, 20 s

Table 1. Samples of *Mesalina brevirostris* used for molecular analysis (NHMW: Natural History Museum, Vienna; NMP: National Museum, Prague).

Specimen-**no.**	**Locality**	**Coord**	**Clade**
NMP 70305/1	SYR: Palmyra	3433/3817	**1**
NMP 70305/2	SYR: Palmyra	-"-	**1**
NMP 70211/2	SYR: J. Arab	32/36	**1**
NMP 70211/3	SYR: J. Arab	-"-	**1**
NMP 70439/3	SYR: Sadat	3416/3704	**1**
NMP 70439/4	SYR: Sadat	-"-	**2**
NMP 70439/5	SYR: Sadat	-"-	**2**
NMP 70440/7	SYR: Hawarin	-"-	**1**
NMP 70440/8	SYR: Hawarin	-"-	**1**
NMP 70224/1	JOR: Azraq	3150/3649	**1**
NMP 70224/3	JOR: Azraq	-"-	**1**
NMP 70629/1	JOR: Amman	3158/3558	**1**
NMP 70629/2	JOR: Amman	-"-	**1**
NHMW 32326:10	UAE: Abu Dhabi		**3**

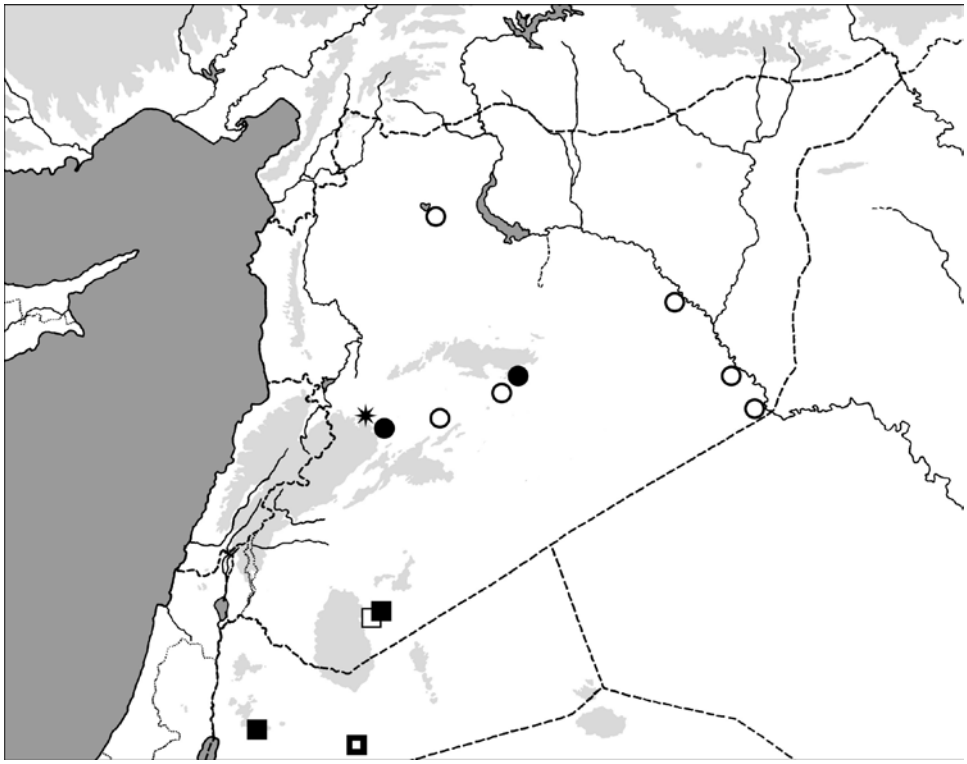

Fig. 1. Distribution of the individual forms of *Mesalina brevirostris* in Syria and northern Jordan according to examined samples. Open circles = not sequenced Lowland form samples; closed circles = sequenced Lowland form samples (Hawarin, Palmyra); asterisk = sequenced sample (Sadat) involving both Lowland and Cryptic form; open square = not sequenced sample of the J. Arab form (Jabal al Arab); closed squares = sequenced samples of the J. Arab form (Jabal al Arab, Amman); square with a light centre = sequenced sample of the desert population from eastern Jordan (Azraq).

Table 2. Primers used in the study.

Primer	Sequence	Use	Reference
L15050	5'-tat cta cat att gga cga ggc c-3'	PCR	this paper
L14775	5'-cag aca aaa ttc cat tcc acc-3'	PCR, Seq	this paper
H15425	5'-ggt tta caa gac cag tgc ttt-3'	PCR	Podnar *et al.* (2005)
H15465	5'-ttg ctg ggg tga agt ttt ctg ggt c-3'	PCR, Seq	this paper

at annealing temperature, 90 s at 72 °C, and a final extension step of 7 min at 72 °C. Negative and positive PCR controls were included in all PCR amplifications. Primers used in the study are given in Table 2. Two overlapping sections of cytb gene were amplified with the primers L15050 and H15465, and L14775 and H15150, respectively. Sequencing was performed by MWG (Ebersberg, Germany).

Results

MrBayes (Huelsenbeck & Ronquist 2001) and Neighbor-Joining (Saitou & Nei 1987) analyses resulted in trees of the same topology revealing three main clades with divergence of about 10% (Fig. 2). The first clade includes the representatives of all three morphotypes (*sensu* Moravec 2004) and is further divided into weakly divergent subclades: (i) Lowland form extending far to the west; (ii) J. Arab form of southern Syria and northern Jordan; (iii) Desert populations from the eastern Jordan (Azraq area). The second main clade includes a morphologically cryptic, so far undescribed form occurring syntopically with the Lowland form. The sample from Abu Dhabi represents a third main clade.

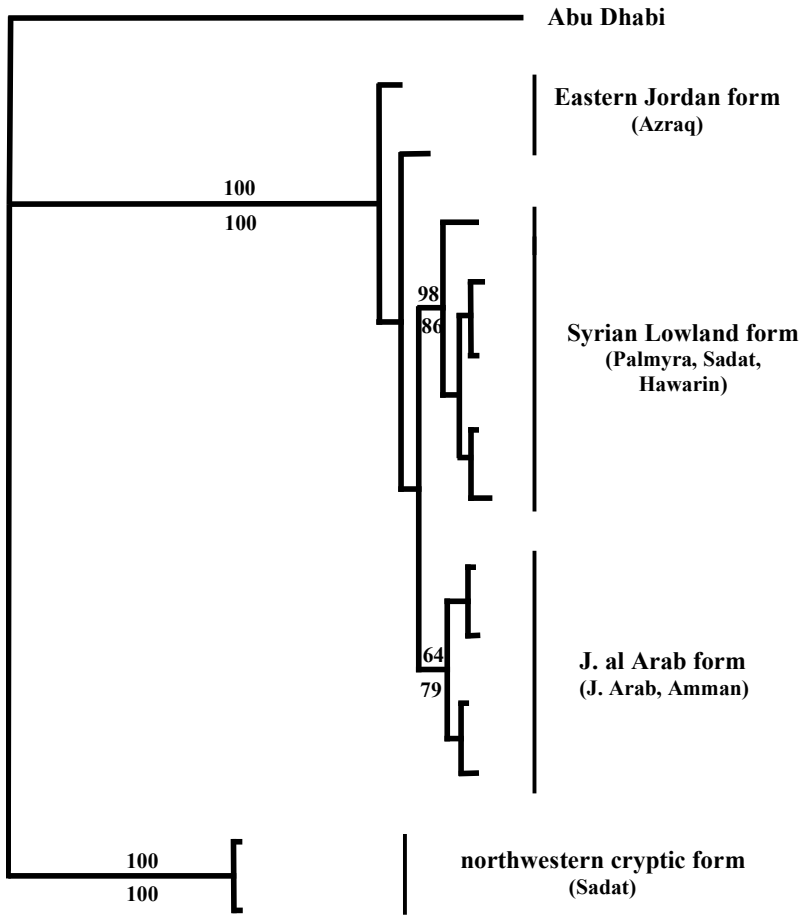

Fig. 2. Bayesian tree. Bayesian posterior probabilities are given above branches and NJ bootstrap values below branches, respectively).

Discussion

The results obtained suggest the following:

(1) The Syrian Lowland and J. Arab morphotypes may represent two different taxonomic units of *Mesalina brevirostris*, which evolved in relatively short time. The former can be associated with the traditional name *M. b. brevirostris*. The latter can be assigned to the name *M. b. microlepis*. These forms differ in size, number of gular, dorsal and preanal scales, as well as in body shape (Moravec 2004). However, this notion is not consistent with the type locality of *M. b. microlepis* (town of Hawarin ca.

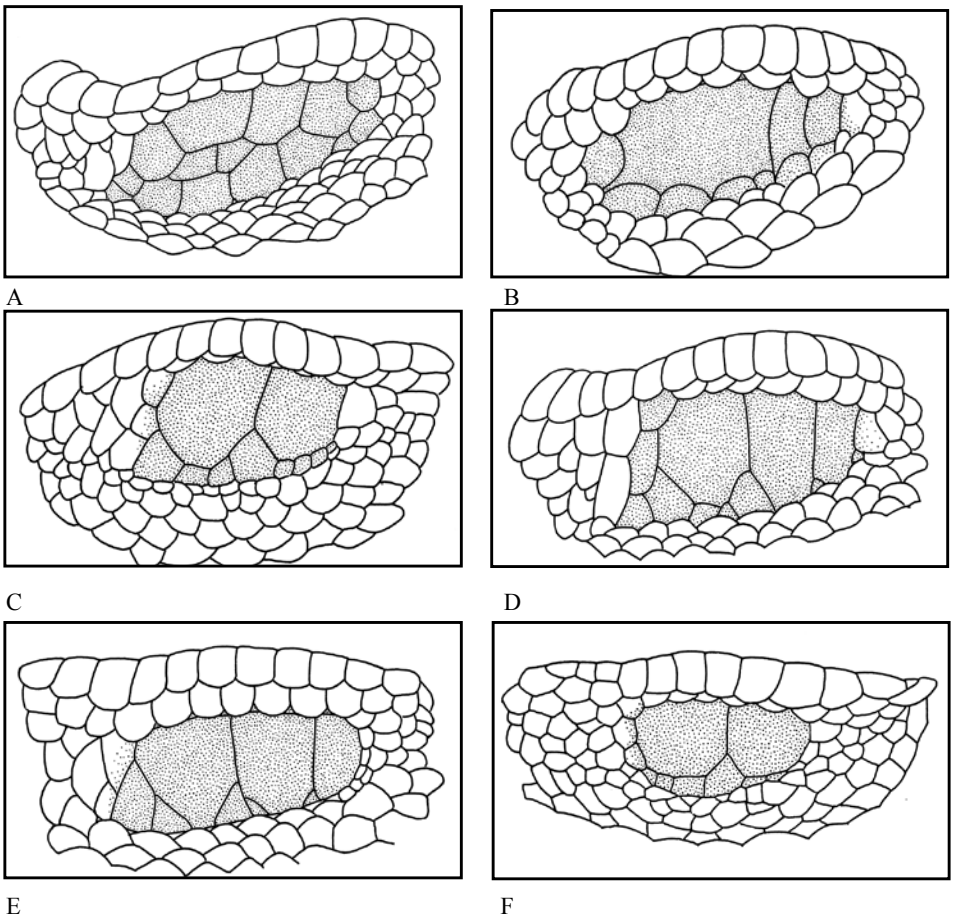

Fig. 3. Structure of the semitransparent window in different Syrian populations of *Mesalina brevirostris*. (A) Sadat, W Syria (Cryptic form); (B) Sadat, W Syria (Lowland form syntopic with the Cryptic form); (C) Palmyra, central Syria (Lowland form); (D) Hawarin, W Syria (present topotype of *M. b. microlepis*); (E) Jabal al Arab, S Syria (J. Arab form); (F) Azraq, E Jordan (Azraq form).

55 km SE of Homs, western Syria ["Haouarine – à 55 kilomètres au S.-E. de Homs"; Angel 1936]). Although the holotype of *microlepis* morphologically resembles J. Arab-form rather than any other morphotype, the molecular data of the present topotypes show that the type locality of *microlepis* lies within the range of the Lowland form (*M. b. brevirostris*).

Nevertheless, this situation could be attributed to the changes in the geographical distribution of the respective forms due to current aridisation of the Near East. The expansion of the Lowland (desert) form to higher elevations of western Syria could lead to replacement or assimilation of the original population, which the type belonged to.

(2) The "Western form" is an artificial unit consisting of two sympatric and morphologically very similar forms. The first one belongs to the Lowland form (*M. b. brevirostris*). The second one represents an undescribed *taxon* ("Sadat form"), which differs morphologically in being slightly larger in size, displaying clearly ocellated colour pattern and in having semitransparent window of the lower lid consisting of several roughly equal scales (Fig. 3). The last characteristic seems to distinguish this otherwise cryptic form from all other morphotypes examined in this study (the window consists of 1-3 larger scales).

Future investigation of nuclear markers, extended sample size and intensified sampling of the presumptive contact areas between suggested population groups is necessary to test whether the "Sadat form" and the "western Lowland form" hybridize, i.e., whether they represent different biological species or not.

(3) The desert population from eastern Jordan (samples from Azraq) is divergent from the J. Arab form inhabiting the Jordan Irano-Turanian steppe zone. Morphological comparison shows difference in size and colouration (Disi *et al*. 2001). The data is thus indicative of yet another undescribed *taxon*.

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References

Anderson S.C. 1999. The lizards of Iran. Contributions to Herpetology (SSAR) 15: 1-442, Ithaca, New York.

Angel F. 1936. Reptiles et batraciens de Syrie et de Mésopotamie récoltés par M.P. Pallary. Bulletin de l'Institut d'Egypte 18: 107-116.

Arnold E.N. 1986. A key and annotated check list of the lizards and amphisbaenians of Arabia. Fauna of Saudi Arabia 8: 385-435.

Bosch in den H.A.J. 2001. *Mesalina brevirostris* Blanford, 1874 (Reptilia: Laceridae) in Lebanon, with data on reproduction. Zoology in the Middle East 23: 31-46.

Disi A.M. 1991. A contribution to the herpetofauna of Jordan. 4. Lizards of Jordan. Zoology of the Middle East 5: 25-35.

Disi A.M. 1996. A contribution to the knowledge of the herpetofauna of Jordan. VI. The Jordanian herpetofauna as a zoogeographic indicator. Herpetozoa 9 (1/2): 71-81.

Disi A.M., Modrý D., Nečas P. & Rifai L. 2001. Amphibians and reptiles of the Hashemite Kingdom of Jordan. An atlas and field guide. Chimaira, Frankfurt a. M., 408 pp.

Haas G. & Werner Y.L. 1969. Lizards and snakes from Southwestern Asia, collected by Henry Field. Bull. Mus. Comp. Zool. 138: 327-406.

Huelsenbeck J.P. & Ronquist F.R. 2001. MrBayes: Bayesian inference of phylogeny, version 3.0b4. Biometrics 17: 754-755.

Moravec J. 2004. Distribution and morphological variation of the lizard *Mesalina brevirostris* in Syria. In: Peréz-Melado V., Riera N. & Perera A. (eds). The biology of Lacertid lizards. Evolutionary and ecological perspectives. Institut Menorquí d'Estudis. Recerca 8: 245-257.

Podnar M., Mayer W. & Tvrtković N. 2005. Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. Mol. Ecol. 14: 575-588.

Saitou N. & Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

Sambrook J., Fritsch E.F. & Maniatis T. 1989. Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press.

Werner Y.L. 1971. Lizards and snakes from Transjordan, recently acquired by British Museum (Natural History). Bull. British Mus. Nat. Hist. (Zool) 21: 215-256.