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Recent advances in phylogeny and taxonomy of Near and Middle Eastern Vipers – an update

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Abstract

The number of recognized viper species in the Near and Middle East has been raised significantly in the last 25 years (Table 1). While some smaller genera remained more or less stable, the genus *Vipera* has been subdivided into four genera on the basis of molecular genetic data. Of these genera, *Daboia* contains the former *Vipera palaestinae* and *D. russelii*, *Macrovipera* the species *M. lebetina*, *M. schweizeri* and an undescribed, basal species from Iran, and *Montivipera* the former *Vipera xanthina* and *V. raddei* complexes. While the genetic diversity in the *M. raddei* complex is fairly low, it is high in the *M. xanthina complex*. This may give reason to synonymize several taxa in the *M. raddei* complex, while new taxa can be described in the Turkish *M. xanthina* complex.

The number of known species in the Middle Eastern Saw-scaled vipers (genus *Echis*) must be raised from 2 to 6. These species belong to 3 different species complexes (an Asian, an African and an Arabian complex). A particularly high diversity of *Echis* is found in southern Arabia.

Antivenom producers should pay particular attention to new species in the medically important genera *Echis* and *Macrovipera*.

Keywords

Phylogeny, taxonomy, Viperidae, Near and Middle East

Table 1. Progress in taxonomical knowledge about Near and Middle East vipers during the last 25 years. Numbers of species in each genus occurring in the area of the Near and Middle East. The genus *Vipera* has been subdivided into 4 genera. Asterisks (*) indicate genera that only occur at the northern margin of the area.

Genus	Joger 1984	Today (2009)
Bitis	1	1
Cerastes	2	3
Echis	3	6
Eristicophis	1	1
Pseudocerastes	2	3
Daboia	2 (included in *Vipera*)	2
Macrovipera	1 (*Vipera lebetina*)	2–3 (one undescribed)
Montivipera	5 (*Vipera xanthina* group)	6–8
Vipera s.str.*	5	5
*Gloydius**	3	3
sum	25	32–35

Introduction

The taxonomy and phylogeny of Near and Middle Eastern vipers have been controversially discussed in the past. Until the eighties of the 20th century Palaearctic vipers, except *Eristicophis*, *Echis* and *Cerastes,* were subsumed under the genus *Vipera* (e.g. Schwarz 1936, Marx & Rabb 1965). Obst (1983) revalidated the genus *Daboia* for the large Asiatic taxa. Based on immunological comparisons of blood serum albumin Herrmann et al. (1992) resurrected the genus *Macrovipera* for *lebetina* and *mauritanica* and restricted the name *Daboia* to *russelii*. In 1999 Nilson et al. introduced the subgenus *Montivipera* for species of the '*xanthina* complex' and '*raddei* complex' which was raised to full rank by Joger (2005).

The number of recognized species in the region rose from 25 (Joger 1984) to 31 (David & Ineich 1999). The purpose of this contribution is to summarize the latest development, partly on the basis of own molecular phylogenetic analyses.

Molecular methods have transformed taxonomy and phylogenetics. First molecular analyses of Herrmann et al. (1999) and Lenk et al. (2001) found *Pseudocerastes* and *Eristicophis*, *Vipera* s. str., *Daboia*, *Macrovipera* and *Montivipera* to be monophyletic groups. *Echis* and *Cerastes* were found monophyletic by Joger & Courage (1999) but not by Lenk et al. (2001). Internal relationships and species concepts especially within *Montivipera* and *Macrovipera*, but also within *Echis*, have been debated controversially (e.g. Schätti et al. 1991, Cherlin1990). To elucidate the confusing taxonomy we used large samples and different molecular markers.

Species of marginal distribution

The Eurasian genus *Vipera* (s.str.) is represented in western and northern areas of Turkey (*Vipera kaznakovi*, *V. darevskii*, *V. anatolica*, *V. renardi eriwanensis*, *V. ammodytes*

transcaucasiana) the latter two probably in the extreme north of Iran. Mountain areas of northern Iran, Afghanistan and Pakistan are also populated by species of the genus *Gloydius* (formerly *Agkistrodon*), the only pitvipers (Crotalinae) in the area (see Joger 1984, Orlov & Barabanov 1999).

An Afrotropical species, the puffadder *Bitis arietans*, reaches Southwest Arabia (southern Saudi Arabia, Yemen, and Dhofar in Oman).

These marginal taxa are not typical inhabitants of the Middle East, and therefore will not be focused on in the present paper.

Near and Middle Eastern vipers

Phylogenetic analysis of concatenated mtDNA (Fig. 1) using Bayesian inference (Metropolis-coupled Markov-Chain Monte-Carlo) produced a fully resolved bifurcating topology that strongly supports the monophyly of the genera *Pseudocerastes* and *Eristicophis*, *Daboia*, *Macrovipera* and *Montivipera*. Our data confirm the major topology of Lenk et al. (2001), except the basal position of *Vipera* s.str. All major branches are supported by robust posterior probabilities, except the monophyly of *Macrovipera xanthina*.

The deeply forked group of desert snakes, *Pseudocerastes* (Fig. 4) and *Eristicophis*, is the most basal group, followed by the Afro-Asiatic cluster of *D. russelii*, *D. palaestinae* (Fig. 5) and *D. mauritanica*. Both groups are characterised by a high number of apomorphic states. The *Pseudocerastes* have hornlike scale structures on top of their supraocular scales, a convergence to *Cerastes* (see below). There are three nominal species: *P. fieldi* (northern part of Arabian peninsula and Sinai), *P. persicus* (Oman, UAE, Iran, Afghanistan, Pakistan) and a newly described species from western Iran, *P. urarachnoides* Bostanchi et al. (2006). *Eristicophis* bears a horseshoe-shaped scale on top of its snout and has a prehensile tail. Its single species, *E. macmahoni*, is restricted to the deserts of Baloutchistan shared by Iran, Afghanistan and Pakistan. *Daboia* has a disjunct distribution, with one or two North African species (*mauritanica*, *deserti*) formery included in *Macrovipera*, the Levantine *D. palaestinae* (formerly *Vipera palaestinae*) and the South Asian *Daboia russelii*, which reaches the Middle East only peripherally in Pakistan (Joger 1984, Lenk et al. 2001). *Daboia* is characterized by a raised numbers of body scales.

Montivipera and *Macrovipera* are groups with several distinct geographic haplotypes. *Montivipera* (Fig. 2) consist of two sister lineages (Nilson & Andrén 1986): the '*xanthina* complex' with the nominal species *xanthina*, *bornmuelleri*, *wagneri* (Fig. 6) and *bulgardaghica* inhabiting Asia Minor, Syria and Lebanon, while the eastern counterpart, the '*raddei* complex', inhabits Armenia, Azerbaijan southwards to Zagros- and Alborz mountains (Iran) (Joger 1984, Nilson and Andrén 1986). The '*raddei* complex' includes the nominal species *raddei*, *albicornuta* and *latifii*.

Montivipera has been the subject of controversial interpretations of species concepts (Schätti et al 1991, 1992 and Nilson & Andrén 1992).

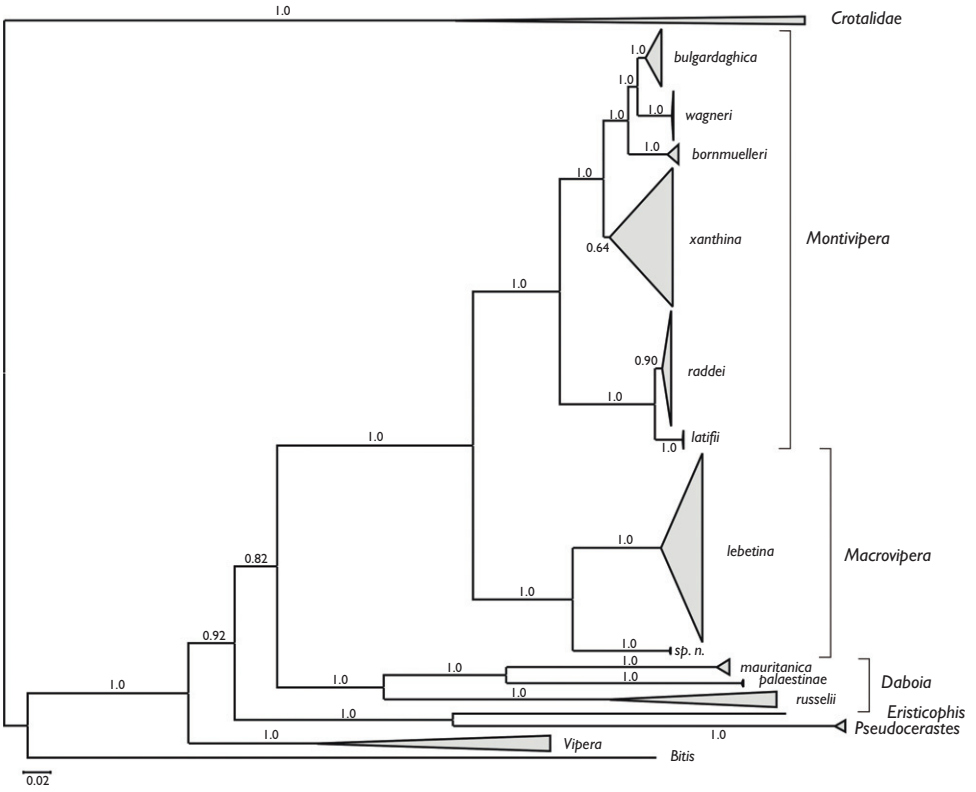

Figure 1. Bayesian 50% majority-rule consensus tree of 176 specimens with posterior probability values showing the phylogenetic relations among west palearctic vipers. The genealogy was inferred from three protein coding mitochondrial genes (Cytb, COI, ND5). Sequences were aligned separately using ClustalW (Thompson et al. 1994) implemented in Bioedit 7.0.9 (Hall 1999) and concatenated into a single amino acid alignment with 2566 positions. Phylogenetic relations were inferred using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2001) under the best fit model (GTR+I+G) selected using MrModeltest 2.2 (Nylander 2004). Two independent runs with one cold and three heated chains (MC3) were run for three million generations sampling every 100 generations and discarding the first 25% of the trees as burnin. Convergence was estimated in Tracer v1.4.1 (Rambaut & Drummond 2007). A more detailed description of material and methods will be published elsewhere (in prep).

Following Nilson's and Andrén's species concept, populations of the '*xanthina* complex' were geographically isolated along the "Anatolian Diagonal". These vicariance events resulted in the speciation of isolated populations. Consequently, they separated the eastern populations from the west Anatolian *xanthina* and evaluated three new species *albizona*, *bulgardaghica* and *wagneri* (Nilson & Andrén 1984, 1985a, 1990). In 1991, Schätti et al. present a well founded but oppositional study about the morphological variation of the '*xanthina* complex'. In their opinion, the new species are conspecific and reflect merely differences between distant and polymorphic populations of *xanthina*.

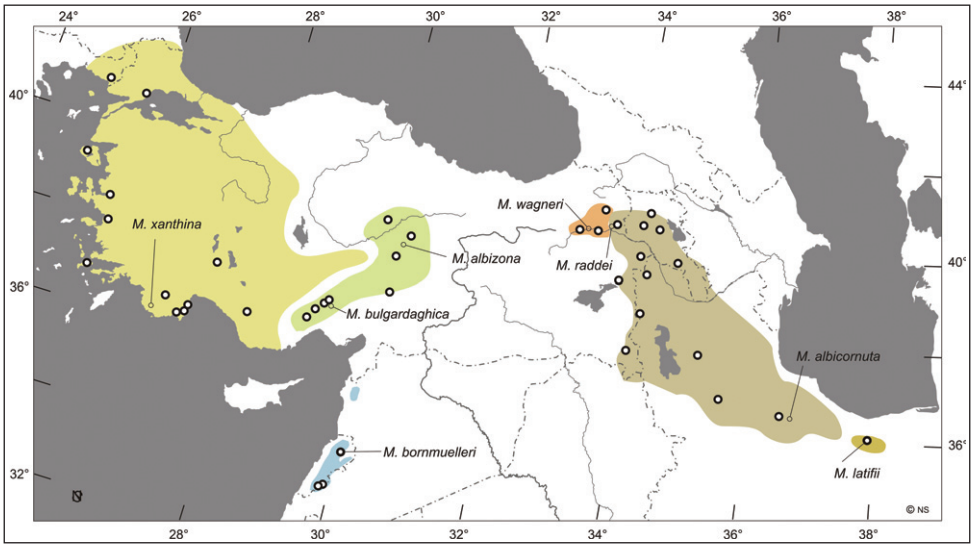

Figure 2. Approximate distribution of *Montivipera* in Asia Minor, Iran, Levantine and adjacent regions. Geographic origin of sampling locations are indicated by open circles. Nominal taxa are given with corresponding terrae typicae.

Mitochondrial DNA sequences from 70 *xanthina* complex individuals with 39 haplotypes support the monophyly of two diverging haplogroups with (1) *M. xanthina* from West Anatolia and (2) *M. bornmuelleri* (Levant), *wagneri* (East Anatolia) and *bulgardaghica* (Taurus). Note that these haplogroups are concordant with the disjunct geographic distribution. Haplotypes of *bulgardaghica* are nested within *albizona* and do not support the validity of *albizona*. Within the West Anatolian *xanthina* (s. str.) cluster, there is substantial well supported phylogenetic structure suggesting the presence of taxonomically unrecognised genetic diversity.

The '*raddei* complex' is a genetically homogenous lineage represented by 5 haplotypes in 35 individuals, indicating their historically young radiation. *M. raddei kurdistanica* from Yüksekova (TR) and Qotur (IR) as well as specimens from *M. albicornuta* are paraphyletic. These results contradict the validity of *M. albicornuta* (Nilson & Andrén 1985b) and *M. r. kurdistanica* (Nilson & Andrén 1986). Both taxa are conspecific with *M. raddei*.

The genus *Macrovipera* (Fig. 3) has its main distribution area in Asia. It is found in semi-deserts and steppe habitat of the Levantine countries (Jordan, Syria, Turkey, Iraq, Iran and Azerbaijan) northeastwards to Middle Asia (Turkmenistan, Uzbekistan, Tadzhikistan, Kirgistan and Afghanistan) (Joger 1984, Bruno 1985). Together with *Echis*, *Macrovipera* is responsible for the majority of serious, often-lethal clinical problems in western Asia (e.g. Fatehi-Hassanabad and Fatehi 2004). Nevertheless, the taxonomic status of some taxa is still debated. Joger (1984) accepted only the subspecies *lebetina* and *obtusa* (Fig. 7), of which the latter includes the synonyms *euphratica* and

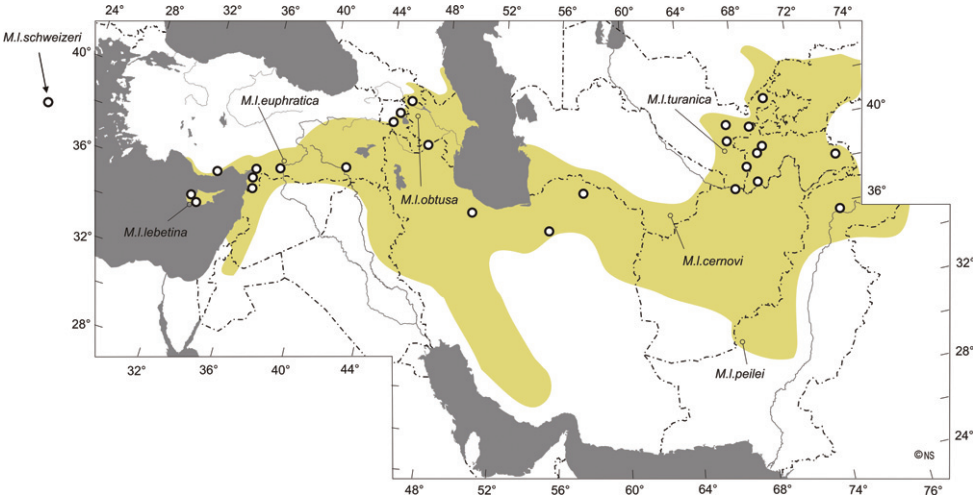

Figure 3. Approximate distribution of *Macrovipera* in the Middle East. Geographic origin of sampling locations are indicated by open circles. Nominal taxa are given with corresponding terrae typicae.

Figure 4. *Pseudocerastes persicus*

turanica. Nilson and Andrén (1988) raised the isolated island population *M. l. schweizeri* of the Greece Cyklades to species status and described a new subspecies *transmediterranea* from Algeria and Tunisia. Central Asian populations were treated as valid subspecies *cernovi* by Chikin and Szczerbak (1992). In our analysis haplotypes of *M. lebetina* segregate into four major lineages which support the validity of the allopatric subspecies *lebetina*, *obtusa*, *turanica* and *cernovi*.

Figure 5. *Daboia palaestinae*

Figure 6. *Montivipera wagneri*

Figure 7. *Macrovipera lebetina obtusa*

Specimens from Turkish Mediterranean coast (Mersin) and the Greece Cyclade island Milos share the same haplotype. Their low genetic distance to Blunt-nosed Vipers from Cyprus indicate a common biogeographic history in the Eastern Mediterranen. Hereby we treat them as conspecific with *M. lebetina*. A recently discovered endemic population in Iran might be the relict of a common *Macrovipera* ancestor and represents a new ancestral species (in prep.). The high diversity in *Macrovipera* should be brought to the attention of antivenom producing institutes, as these snakes have great medical importance.

Cerastes and *Echis*

These two genera have been united in a monophyletic group based on the shared character of serrated lateral scales which they use for producing a warning sound like a rattle-snake (Groombridge 1986). It is considered an adaptation to desert conditions. However, as the harmless colubrid *Dasypeltis* and some species of African bushvipers (genus *Atheris*) possess the same type of serrated scales, a convergent development is possible (Joger & Courage 1999). A new phylogenetic analysis (Pook et al., in press) confirms the monophyly of *Echis* and *Cerastes*.

Cerastes, the Horned vipers, do not always bear horns on top of their supraorbital scales. There is a hornless species, *Cerastes vipera*, which is found in sand dune areas in North Africa, Egypt and Israel/Palestine. *Cerastes cerastes*, a less specialized species,

maybe horned or hornless (Figs 8, 9) and occupies, in a number of subspecies, desert and semi-desert areas in North Africa and western Arabia (Werner et al. 1991, Werner and Sivan 1992). The typical desert snake of most of Arabia and Khuzistan province (Iran) is *Cerastes gasperettii* Leviton & Anderson, 1967 (Gasperetti 1980), the sister species of *C. cerastes*, but which nearly always bears horns.

Echis, the Saw-scaled vipers, are found in a very large area from West Africa to India, including most of the Middle East countries. Their systematics and taxonomy have been a discussed for decades. Klemmer (1963) recognized only two species: *Echis carinatus* in most of the range and *E. coloratus* in Arabia. Joger (1984, 1987) added *E. pyramidum* for southwestern Arabia. Cherlin (1990) described a number of new species and subspecies and increased the total number of *Echis* species significantly. A new species within the *E. coloratus* group was described by Babocsay (2004). Pook et al. (in press), using molecular genetic methods, have now clarified the complicated situation. On the basis of their results and additional data, we recognize the following six species in the Near and Middle East:

Echis carinatus group (Asian group):
E. (carinatus) sochureki (Oman, UAE, Iran, Central Asia, Afghanistan, Pakistan)
Echis coloratus group (Arabian group):
E. coloratus (Egypt, Arabian Peninsula)
E. omanensis (Oman, UAE)
Echis pyramidum group (one of two African groups):
E. pyramidum (Egypt, Sudan, East Africa)
E. khosatzkii (western Oman, Yemen)
E. sp. (cf. *borkini*) (Yemen, SW Saudi Arabia)

E. borkini was originally described as a subspecies of the East African *E. varia* by Cherlin (1990). As we did not find a close phylogenetic relationship between *Echis* populations from Yemen and Ethiopia, we consider *borkini* a separate species.

There is a strong zoogeographical division in Arabian *Echis*, *E. sochureki* and *E. omanensis* being found in the eastern part of the peninsula only, whereas southwestern Arabia (including Dhofar province, Oman) is inhabited by *E. coloratus*, *E. khosatzkii* and *E.* cf. *borkini* (see also Joger 1987).

As *Echis* bites frequently cause death and successful bite treatment depends on choosing a species-specific antivenom (if available), it is of great importance to know which species of *Echis* occur in which area. There is still need for additional research in this genus. It is also time for an effort to study the venom of species like *E. khosatzkii* and *E.* cf *borkini*, and start production of antivenom against their bites.

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Figure 8. *Cerastes gasperettii* (horned)

Figure 9. *Cerastes gasperettii* (hornless)

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