Next-generation sequencing yields the complete mitochondrial genome of the endangered Milos viper *Macrovipera schweizeri* (Reptilia, Viperidae)

Evanthia Thanou\textsuperscript{a,b} and Panagiotis Kornilios\textsuperscript{a,b,c}

\textsuperscript{a}Department of Biology, University of Washington, Seattle, WA, USA; \textsuperscript{b}The Molecular Ecology Backshop, Loutraki, Greece; \textsuperscript{c}Institute of Evolutionary Biology (CSIC – Universitat Pompeu Fabra), Barcelona, Spain

**ABSTRACT**

The Milos viper, *Macrovipera schweizeri*, is an endangered vipersid snake found on four Aegean islands (Greece). Its complete mitochondrial genome, the first reported for the genus *Macrovipera*, was assembled through next-generation sequencing. Its total length is 17,152 bp and includes 22 tRNAs, two ribosomal RNA genes, 13 protein-coding genes and two control regions, showing the typical gene-arrangement for Viperidae. Eight tRNAs and ND3 are encoded on the light strand, while all other genes are encoded on the heavy strand. A mitogenomic phylogeny that included *Macrovipera schweizeri* and 13 other vipersid genera returned an unresolved relationship among the genera *Macrovipera*, *Daboia* and *Vipera*.

The mitogenome was assembled de novo from the off-target sequences with NOVOPlasty 2.7.1 (Dierckxsens et al. 2017). Genes were annotated using the MITOS WebServer (Bernt et al. 2013) and checked manually. Alignment with other vipersid mitogenomes, one from every genus currently available in GenBank, was done with MAFFT (Katoh et al. 2017). This dataset, after removing the two poorly aligned control regions, was used to reconstruct a Maximum Likelihood (ML) tree with IQ-TREE\textsuperscript{1.4.3} (Nguyen et al. 2015), using 1000 ultrafast bootstrap alignments (Minh et al. 2013). The Milos viper mitogenome (Genbank accession number MH717075) has a length of 17,152 bp and includes 22 transfer RNA genes (tRNA-Leu and tRNA-Ser are duplicated), two ribosomal RNA genes, 13 protein-coding genes and two control regions, showing the typical gene-arrangement for Viperidae (IIIB type in Qian et al. 2018). The putative L-strand replication origin (OL\textsuperscript{36}) is 36 bp long and located between tRNA-Asn and tRNA-Cys. Eight tRNAs and ND3 are encoded on the light strand, while all others are encoded on the heavy strand. The two control regions have similar length with 1020 bp (CRI\textsuperscript{12.5%}) and 1030 bp (CRII\textsuperscript{12.5%}). The overall composition of the heavy strand is A (32.1%), T (27.1%), C (28.3%) and G (12.5%).

The unrooted ML phylogeny based on complete mitochon- drial genomes of vipersid genera distinguishes the three subfamilies as monophyletic groups (Figure 1). *Macrovipera* is nested within Viperinae but it is not clear whether it is closely related to the genus *Vipera* or *Daboia*. The relationship between the latter two and *Macrovipera* and *Montivipera* have historically been unclear in molecular phylogenies.
Lenk et al. 2001; Garrigues et al. 2005; Stumpel and Joger 2009; Alencar et al. 2016). It seems that even complete sequences of the mitochondrial DNA are not able to resolve these relationships which could point to a rapid radiation from the common ancestor of this group.

Acknowledgments

The sequence-capture work took place in the Leaché Lab (Department of Biology, University of Washington). This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant agreement no. 656006 (Project Acronym: CoPhyMed).

References


Figure 1. The unrooted maximum-likelihood tree based on the mitogenomes of Macroviper a schweizeri and 13 other viperid genera. Numbers next to nodes indicate bootstrap support values (bs): closed circles = 100 bs; no circles < 70 bs; open circles > 70 bs.


Garrigues T, Dauga C, Ferquel E, Choumet V, Failloux AB. 2005. Molecular phylogeny of Vipera Laurenti, 1768 and the related genera Macro vire pa (Reuss, 1927) and Daboia (Gray, 1842), with comments about neurotoxic Vipera aspis populations. Mol Phylogenet Evol. 35:35–47.


