MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of *Lacerta bilineata* and comparison with its closely related congener *L. Viridis*

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Abstract

We sequenced the mitochondrial genome of the Western green lizard (*Lacerta bilineata*) using Illumina technology and additional Sanger sequencing. The assembled 17 086 bp mitogenome had a GC content of 40.32% and consisted of 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and one control region (CR), with a gene order identical to the chordate consensus. In addition, we re-sequenced the mitogenome of the closely related Eastern green lizard *L. viridis* using the same techniques as for *L. bilineata*. The mitogenomes of *L. bilineata* and *L. viridis* showed a sequence identity of 94.4% and 99.9%, respectively, relative to the previously published *L. viridis* mitogenome. The phylogenetic reconstruction based on 17 Lacertinae mitogenomes using *Anolis carolinensis* as the outgroup supported *L. bilineata* and its sister species *L. viridis* as distinct lineages.

The taxonomy and biogeography of the Western and Eastern green lizards, *L. bilineata* and *L. viridis* respectively, has been highly debated (Godinho et al., 2005; Rykena, 1991; Sagonas et al., 2014). Despite the lack of clear morphological differences between adults of the two taxa (Nettmann & Rykena, 1984), as well as relatively low genetic divergence when compared with other *Lacerta* species, some degree of sterility and inviability was detected in crosses between individuals of the two species (Rykena, 1991,1996,2001).

In order to help clarifying their phylogenetic relationships, we sequenced the genome of an adult female *L. bilineata* (Daudin, 1802) specimen collected near Mâlain in Eastern France ($4°28'2.01''E$, $47°21'16.27''N$) via whole-genome shotgun sequencing with Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) through paired-end libraries (96 bp) based on the protocol of Meyer & Kircher (2010). An automated pipeline consisting of base-calling using freeIbis (Renaud et al., 2013), read sorting, and adaptor trimming using leeHom (Renaud et al., 2014) was implemented. The mitogenome reads were baited using the reference of *L. viridis* (Böhme et al., 2007) with the split-read

option of Segemehl (Hoffmann et al., 2009) and *de novo* assembled using the Edena assembler (Hernandez et al., 2013). In addition, the additional Sanger sequencing of the CR ends confirmed the complete circular structure of the assembled mitochondrion. Depth of coverage (over 2000×) was used to inspect for assembly errors using Tablet viewer (Milne et al., 2010). The assembled 17 086 bp mitogenome (GenBank accession no. KT722705) had a CR of 1674 bp length and was located between *tRNA-Pro* and *tRNA-Phe* genes. Similar to other Sauropsids, the predicted origin of the leading-strand replication (OH) was located in the largest non-coding region between *COB* and *12S rRNA*, while that of the lagging-strand (OL) was in the small non-coding region between *NAD2* and *COX1* (Sahyoun et al., 2014).

Additionally, we sequenced the mitogenome of a *L. viridis* specimen from North Eastern Hungary near Tokaj ($21°38'35,9''E$; $48°10'18,9''N$) using identical methods as for the *L. bilineata* specimen. The mitogenome sequence of our *L. viridis* sample differed by 0.1% compared to the reference from Austria, available in the Genbank for this same species (AN: AM176577.1; Böhme et al., 2007).

The comparison between *L. viridis* and *L. bilineata* mitogenomes revealed an overall 4.6% divergence. The highest level of similarity in protein coding-genes between the taxa was for the *COX3* gene at 99%, whereas the lowest was for *NAD3* gene

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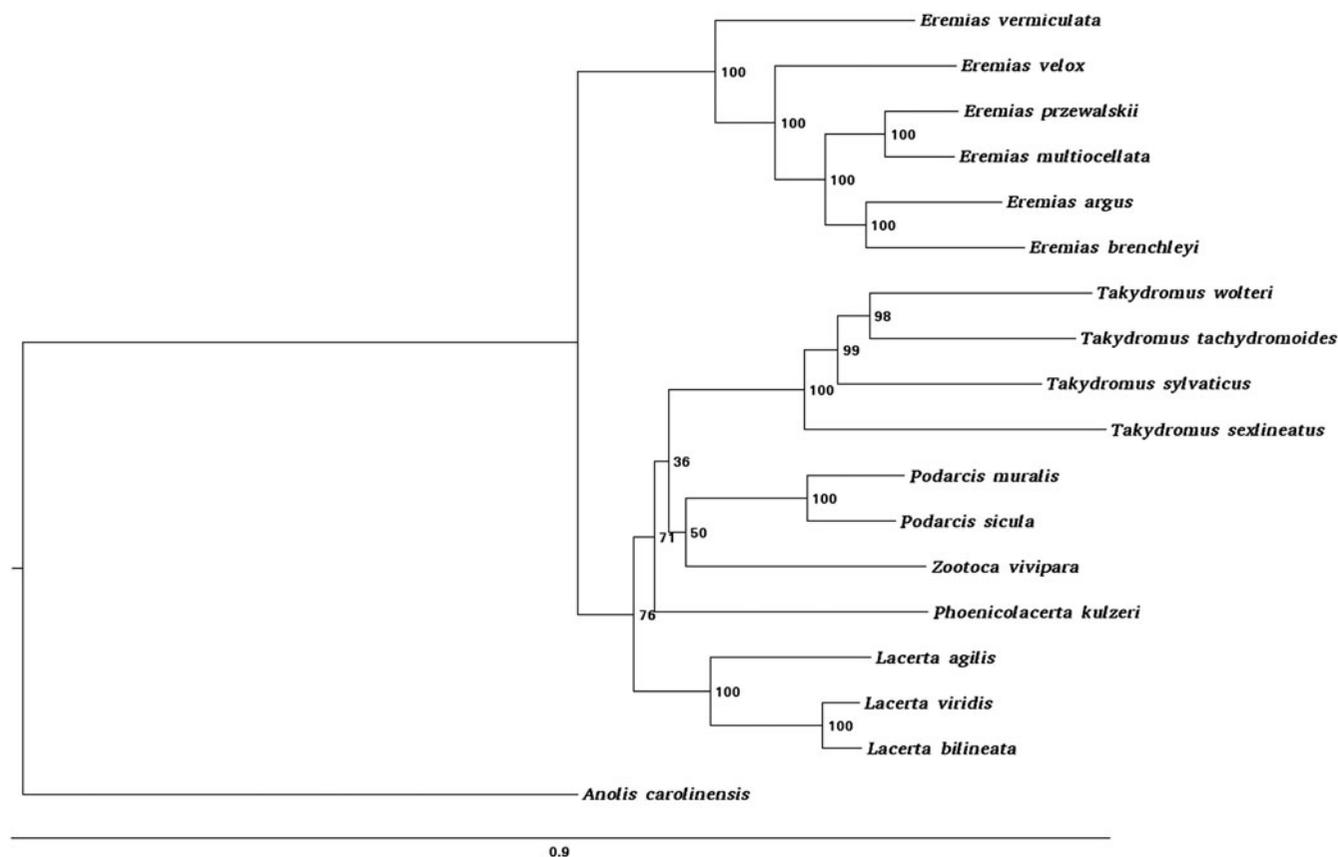
Figure 1. Phylogenetic tree of the complete mitochondrial genomes of 17 Lacertinae species including *Lacerta bilineata* (KT722705), *Lacerta viridis* (NC_008328.1), *Lacerta agilis* (NC_021766.1), *Phoenicolacerta kulzeri* (NC_011606.1), *Zootoca vivipara* (NC_026867.1), *Podarcis sicula* (NC_011609.1), *Podarcis muralis* (NC_011607.1), *Takydromus sexlineatus* (NC_022703.1), *Takydromus sylvaticus* (JX290083.1), *Takydromus tachydromoides* (AB080237.1), *Takydromus wolteri* (NC_018777.1), *Eremias brenchleyi* (NC_011764.1), *Eremias argus* (NC_016755.1), *Eremias multiocellata* (KJ664798.1), *Eremias przewalskii* (NC_025929.1), *Eremias velox* (KM359148.1), *Eremias vermiculata* (NC_025320.1) with *Anolis carolinensis* (NC_010972.2) as the outgroup generated through the maximum-likelihood approach with RA × ML. The branch-lengths represent number of nucleotide substitutions per site.

with 92.5%. Besides single-nucleotide variants, we also identified indels in the *12S rRNA*, *16S rRNA*, *tRNA-Phe*, *tRNA-Ser2*, *tRNA-Val*, *tRNA-Leu1*, *tRNA-Glu* and *tRNA-Pro*. Furthermore, compared with *L. viridis,* we found two 34 bp deletions of a repeated motif within the CR of *L. bilineata*. Unlike other lacertids, the first 770 bp sequence of the CR in *L. bilineata* consisted of stem-like structures similar to *12S rRNA*. However, this prediction was of low statistical significance (*e*-value = 0.01) as determined with Mitos (Bernt et al., 2013).

We performed a phylogenetic analysis using 17 complete mitochondrial genomes of the subfamily Lacertinae (Squamata, Acrodonta, Lacertidae), as well as *Anolis carolinensis* (Squamata, Iguania and Polychrotidae) as the outgroup. We concatenated the protein-coding genes and employed the maximum-likelihood approach with RAxML (Stamatakis, 2014) applying rapid bootstrapping and the GTR + GAMMA model (Figure 1). The resulting phylogenetic tree showed that *L. bilineata* and *L. viridis* are more closely related to each other than to *L. agilis*, confirming previous findings (Godinho et al., 2005; Pyron et al., 2013; Sagonas et al., 2014). In addition, even though the bootstrap supports for clades above the genus level were not as high as within genera, the tree reflected the taxonomic grouping into two distinct tribes, Eremiadini and Lacertini, with Lacertini consisting of the genera *Takydromus, Podarcis, Zootoca* and *Lacerta*.

Acknowledgements

Declaration of interest

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