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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.

Keywords

Complete mitochondrial genome, Illumina sequencing, *Lacerta bilineata*, *Lacerta viridis*, Lacertinae, phylogeny

History

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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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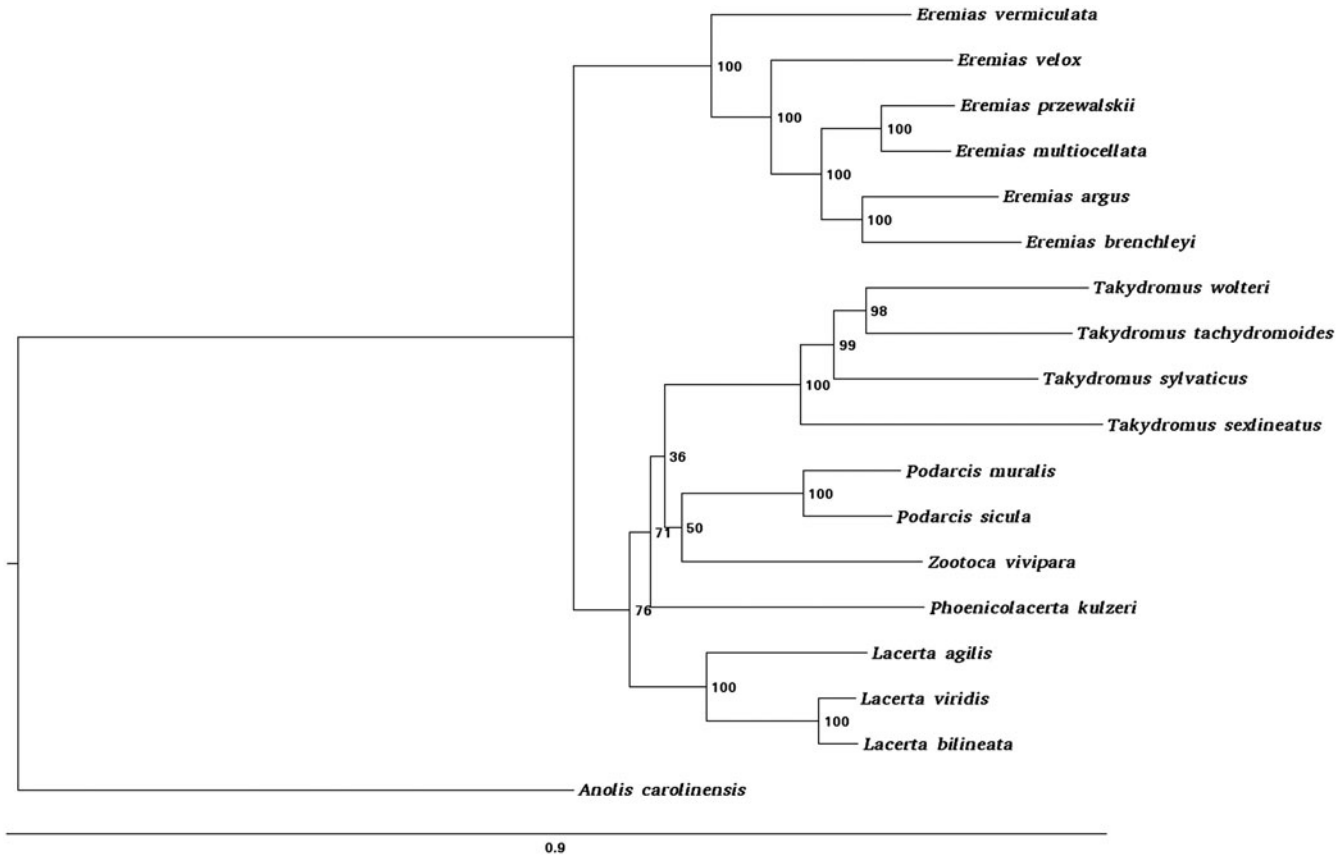

Figure 1. Phylogenetic tree of the complete mitochondrial genomes of 17 Lacertinae species including *Lacerta bilineata* (KT722705), *Lacerta viridis* (NC_008328.1), *Lacerta agilis* (NC_021766.1), *Phoenicolacerta kulzeri* (NC_011606.1), *Zootoca vivipara* (NC_026867.1), *Podarcis sicula* (NC_011609.1), *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.*

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The project was funded by the German Science Foundation (FZT 118). Capture permit (No. 36) for the French specimen was issued by the Prefet de la Cote-d'Or. R. F. is financed by FCT under the Programa Operacional Potencial Humano – Quadro de Referência Estratégico Nacional from the European Social Fund and the Portuguese Ministério da Educação e Ciência through the postdoctoral fellowship SFRH/BPD/89313/2012. K. N. is supported by the Volkswagen Foundation through the initiative ''Evolutionary Biology''.

References

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, et al. (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69:313–19.

Böhme MU, Fritzsch G, Tippmann A, Schlegel M, Berendonk TU. (2007). The complete mitochondrial genome of the green lizard *Lacerta viridis viridis* (Reptilia: Lacertidae) and its phylogenetic position within squamate reptiles. Gene 394:69–77.

Godinho R, Crespo EG, Ferrand N, Harris DJ. (2005). Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Laceridae) based on mitochondrial and nuclear DNA sequences. Amphibia-Reptilia 26:271–85.

Hernandez D, Tewhey R, Veyrieras JB, Farinelli L, Osterås M, François P, Schrenzel J. (2013). De novo finished 2.8 Mbp *Staphylococcus aureus* genome assembly from 100 bp short and long range paired-end reads. Bioinformatics 30:40–9.

Hoffmann S, Otto C, Kurtz S, Sharma CM, Khaitovich P, Vogel J, Stadler PF, Hackermueller J. (2009). Fast mapping of short sequences with mismatches, insertions and deletions using index structures. PLoS Comput Biol 5:e1000502.

Milne I, Bayer M, Cardle L, Shaw P, Stephen G, Wright F, Marshall D. (2010). Tablet-next generation sequence assembly visualization. Bioinformatics 26:401–2.

Meyer M, Kircher M. (2010). Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harb Protoc 2010: pdb.prot5448.

Nettmann HK, Rykena S. (1984). *Lacerta viridis* (Laurenti, 1768) – Smaragdeidechse. In: Böhme W, editor. Handbuch der amphibien und reptilien Europas. Bd. 2/I: Echsen II (Lacerta). Wiesbaden: Aula. p 129–80.

Pyron RA, Burbrink FT, Wiens JJ. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol Biol 13:93.

Renaud G, Kircher M, Stenzel U, Kelso J. (2013). freeIbis: An efficient basecaller with calibrated quality scores for Illumina sequencers. Bioinformatics 29:1208–9.

Renaud G, Stenzel U, Kelso J. (2014). leeHom: Adaptor trimming and merging for Illumina sequencing reads. Nucleic Acids Res 42:e141.

Rykena S. (1991). Kreuzungsexperimente zur Prüfung der Artgrenzen im Genus *Lacerta* sensu stricto. Mitt Mus Naturkde Berlin, Zool Reihe 67: 55–68.

Rykena S. (1996). Experimental interspecific hybridization in the genus *Lacerta*. Israel J Zool 42:171–84.

Rykena S. (2001). Experimental hybridization in green lizards (*Lacerta* s. str.), a tool to study species boundaries. Mertensiella 13:78–88.

Sagonas K, Poulaki N, Lymberakis P, Parmakelis A, Pafilis P, Valakos ED. (2014). Molecular systematics and historical biogeography of the green lizards (*Lacerta*) in Greece: Insights from mitochondrial and nuclear DNA. Mol Phylogenet Evol 76: 144–54.

Sahyoun AH, Bernt M, Stadler PF, Tout K. (2014). GC skew and mitochondrial origins of replication. Mitochondrion 17:56–66.

Stamatakis A. (2014). RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–13.