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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of the parthenogenetic Caucasian rock lizard *Darevskia unisexualis* (*Squamata*: *lacertidae*) contains long tandem repeat formed by 59 bp monomer

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ABSTRACT

The first complete mitochondrial genome sequence of parthenogenetic Caucasian rock lizard *Darevskia unisexualis* (*Lacertidae* family) is determined by hybrid assembly with Illumina HiSeq and PacBio RS II platforms. The circular 21.4 kbp mitogenome contains 13 protein-coding genes, 12S and 16S rRNA genes, 20 tRNAs, two pseudogenized tRNAs, and one long tandem repeats with 4.1 kbp length formed by 59 bp monomer repeated x70.6 times located before control region. This finding represents a new example of mitogenome variation in lizards of hybrid origin, providing fundamental data for following study of a unique hybridization system formed by parthenogenetic and bisexual species in the mountain steppe of central Armenia.

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Parthenogenetic lizard species represent a unique model of organisms for study of genetic and ecological bases of hybridogeneous speciation. *Darevskia unisexualis* is one of the seven parthenogenetic species originated from the interspecific hybridization of *D. raddei nairensis* (maternal species) and *D. valentini* (paternal species), which belong to different clades (Moritz et al. 1992; Murphy et al. 2000; Fujita & Moritz 2010).

Mitochondrial genomes of *Squamata* often have unusual and complicated structure (Amer and Kumazawa 2005; Dong & Kumazawa 2005; Amer & Kumazawa 2007; Fujita et al. 2007; Kumazawa et al. 2014). In this article we report a new kind of rearrangement in mitochondrial genome of asexual lizard *Darevskia unisexualis*: a long tandem repeat with 59 bp monomer located before the control region (Darevsky et al. 1985; Moritz et al. 1992; Murphy et al. 2000; Fujita & Moritz 2010).

DNA samples extracted from blood of *D. unisexualis* obtained previously between 1997 and 2006 from lizards of Armenian population Takyarlu (Artavaz, 40°37'20.2"N 44°34'51.4"E) were delivered to the Axeq/Macrogen (Korea) for genomic library preparation and sequencing with Illumina HiSeq 2000 and PacBio RS II.

To overcome complexity of control region assembly we used approach based on reads extraction with following greedy assembly previously successfully adopted in *Solenodon paradoxus* mitogenome assembly (Brandt et al. 2016). A subset of Illumina reads having common kmers with *Podarcis siculus* mitogenome (NC_011609; Podnar et al. 2009) was extracted with Cookiecutter software (Starostina et al. 2015), mapped back to the reference mitogenome with bowtie2 (Langmead & Salzberg 2012), and assembled into consensuses sequence, that was used as query for alignment against PacBio reads with Blasr aligner (Chaisson & Tesler 2012). Cookiecutter's kmer database was updated from 215 successfully found PacBio reads. Updated kmer database was used for accurate read extraction from Illumina HiSeq raw data. After that Illumina reads were mapped back to PacBio reads and assembled to the final sequence. The consistency of assembly was verified: (1) by consistency with PacBio reads that were mapped to it with MAFFT tool (Katoh & Standley 2013); (2) by consistency with previously published restriction map (Moritz et al. 1992) that has a perfect match with assembled mitogenome except two inverted restriction sites; (3) consistency of Illumina reads coverage. The assembled genome was annotated with MITOS WebServer (Bernt et al. 2013) with additional checking for predicted tRNA and protein-coding genes.

The circular assembled mitogenome size is 21,433 bp, with 13 predicted protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, and 20 tRNAs, two pseudogenized tRNAs, and one complex tandem repeats with 59 bp monomer repeated x70.6 times and located before control region. MITOS WebServer predict second ND4 in 17202–18491 regions inside found tandem repeat, however, verification by Blast alignment did not find any significant match in this region. The validation of phylogenetic position is shown in Figure 1.

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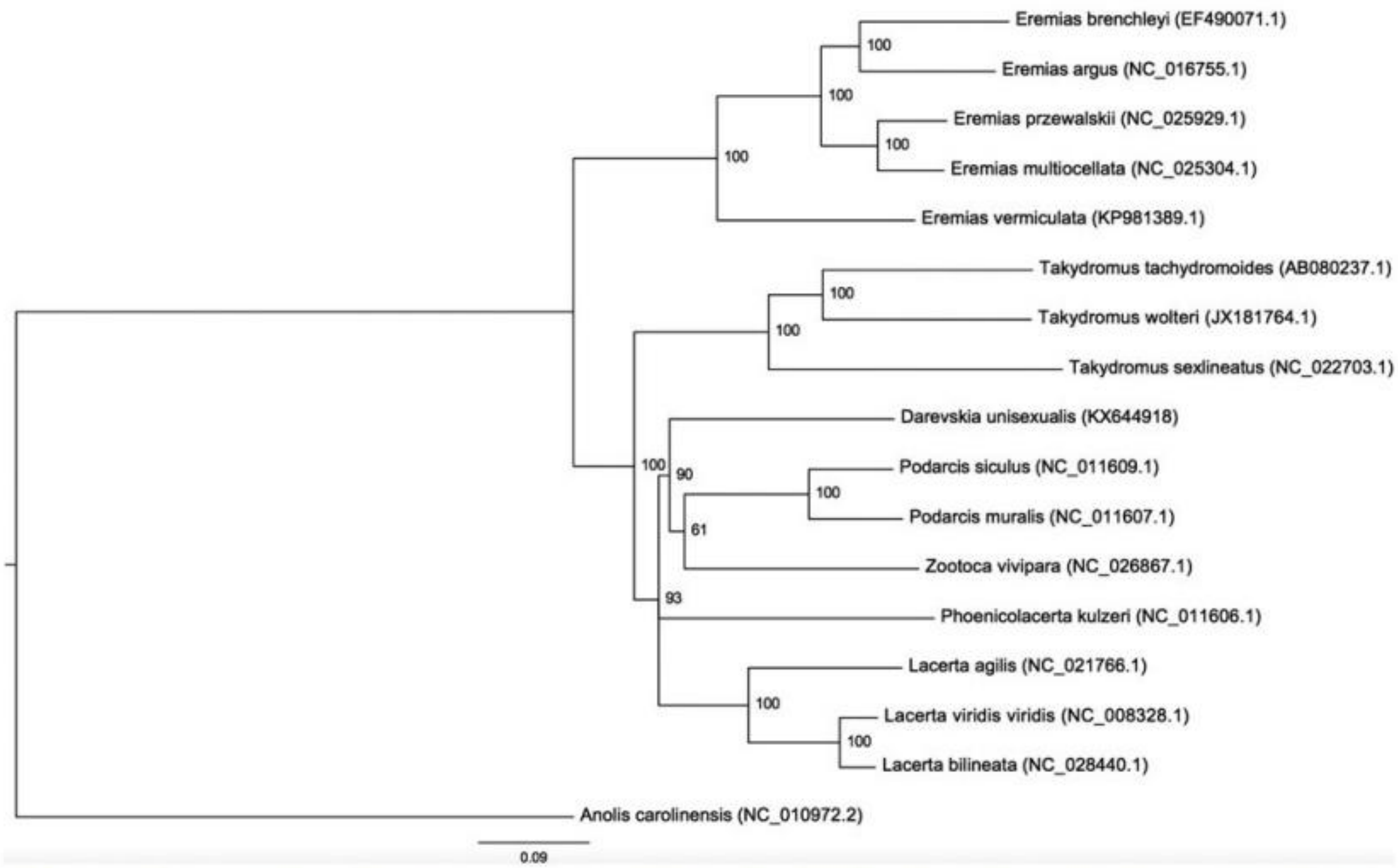

Figure 1. Molecular phylogeny of Daresvskia unisexualis and other Lacertidae species based on all mitochondrial protein-coding genes and rRNAs. Each of alignments was performed separately by MAFFT v7.187 (Katoh & Standley 2013), then alignments were concatenated. Tree was reconstructed by RAxML v8.0.22 (Stamatakis 2014) with 10,000 bootstrap replicates. All nodes with support lower than 50 were removed using ETE toolkit (Huerta-Cepas et al. 2010) and final tree was drawn by FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Unusual mitochondrial genome of parthenogenetic lizards containing long tandem repeat raises questions about stability and evolution of mtDNA after recent hybridization events. Moreover, its features provide unique opportunities to study mtDNA evolution not only in *Squamata* taxon, but in all animals.

Data availability

D. unisexualis mitogenome assembly is available from Genebank with accession number KX644918.

Disclosure statement

The authors declare that they have no conflicts of interest concerning this article.

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