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MITOGENOME ANNOUNCEMENT

First mitochondrial genome of the Caucasian squirrel *Sciurus anomalus* (Rodentia, Sciuridae)

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ABSTRACT

The Caucasian Squirrel, *Sciurus anomalus*, is the only representative of the Sciuridae family in the Eastern Mediterranean region. In this study, the mitochondrial genome of the *Sciurus anomalus* species was generated, and we investigate its phylogenetic position within the Sciuridae family. The generated mitogenome sequence is 16,234 bp. It is composed of a control region and a conserved set of 37 genes containing 13 protein-coding genes, 22 tRNA genes and 2 rRNA genes.

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The Caucasian squirrel, *Sciurus anomalus* (Güldenstädt, 1785), is a medium-sized squirrel. It is the only representative of the Sciuridae family in the Eastern Mediterranean region. This species is distributed in Iran, Iraq, Palestine, Jordan, Syria, Greece, through the Asian part of Turkey, Armenia, Georgia, Azerbaijan and Lebanon. Three subspecies of the Caucasian squirrel were reported: *Sciurus anomalus anomalus* (Gueldenstaedt, 1785), *Sciurus anomalus pallescens* (Gray, 1867), and *Sciurus anomalus syriacus* (Ehrenberg, 1829). The latter is the subspecies present in Lebanon, it differs from the other subspecies by its dark tail, feet and dorsal pelage (Bodenheimer 1935; Harrison and Bates 1991; Gavish 1993; Özkan 1999; Amr 2000; Ellerman 2009; Lewis et al. 2009; Oshida et al. 2009; Koprowski et al. 2016).

In this study, we sequenced the mitochondrial genome of *Sciurus anomalus syriacus* using historical DNA and we examined its phylogenetic position within the family Sciuridae. The specimen was obtained in 2007 from the Qobayat region. The generated sequence was submitted to GenBank database (accession number MW027641). Ours is the first study to present the mitochondrial genome of this species.

A tissue sample was obtained from the footpad of a preserved specimen (voucher number MOQ17) from the Museum of Birds, Mammals and Butterflies of Qobayat-Lebanon (34°34′00″N, 36°16′45″E); it was collected under sterile conditions using disposable scalpel blades and gloves. DNA was extracted using a modified silica-column extraction protocol (McDonough et al. 2018) in a clean, PCR-free laboratory dedicated to ancient DNA processing at the Smithsonian Center for Conservation Genomics (CCG) in Washington, DC where the DNA is stored. After DNA quantification using Qubit® fluorometer (Life Technologies) and 1 × dsDNA HS assay kit and fragment size estimation using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) with High Sensitivity DNA kits, we applied the Illumina blunt-end single-tube library preparation method for degraded DNA described by Carøe et al. (2018). We used qPCR to determine the number of indexing PCR cycles to perform, and performed dual indexing PCR with TruSeq-style indices (Meyer and Kircher 2010) using Kapa HiFi Uracil+ (Kapa Biosystems). The library was sequenced with 2 × 150 bp paired-end reads using an Illumina MiSeq® platform at the CCG.

PCR duplicates and poor-quality reads were removed from the raw sequence data with prinseq-lite-0.20.4; adapter contamination was removed using TrimGalore v0.4.1. The mitogenome assembly, consensus generation, and annotation were performed with Geneious v9.1.2. Quality-filtered reads were mapped to previously published mitogenome of the red squirrel, *S. vulgaris* (KC993006) using Geneious mapping algorithm. The generated consensus sequence was aligned to the reference sequence using the *MAFFT v7.450* plug-in (Katoh and Standley 2013).

The generated mitogenome sequence of *S. anomalus* is 16,234 bp, which covers 97.5% of the reference sequence.

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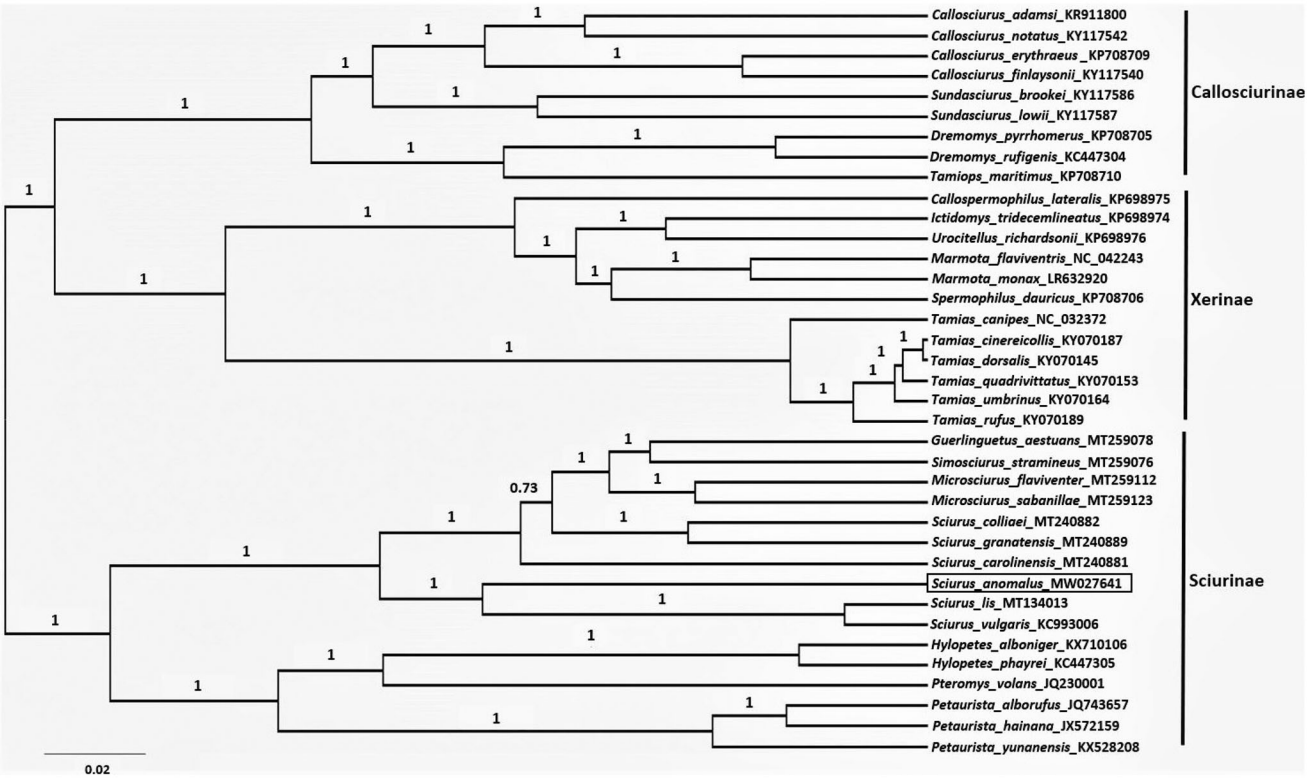

Figure 1. Bayesian phylogenetic tree of *Sciurus anomalus* and species of Sciuridae family based on complete mitogenome sequences. Values beside the nodes are Bayesian posterior probabilities. The tree is midpoint rooted.

The average sequencing depth was 22.2×. The sequence is composed of a control region and conserved set of 37 genes typically found in other squirrel species: 22 tRNA genes, 2 rRNA genes (*12S rRNA* and *16S rRNA*) and 13 protein-coding genes (PCGs) including ones for NADH dehydrogenase (*ND1, ND2, ND3, ND4, ND4L, ND5* and *ND6*), ones for cytochrome c oxidase (*COX1, COX2* and *COX3*), ATP synthase (*ATP6* and *ATP8*) and cytochrome b gene. The base composition was 30.6% A, 24.6% C, 12.8% G, 29.5% T and 2.5% N; the GC content was 37.4% which is consistent with other Sciuridae species (Kim et al. 2017). Due to the degraded nature of the DNA, some gaps remain in our assembled mitogenome particularly in the 16S ribosomal RNA and the NADH dehydrogenase subunit 2 genes.

Mitogenomes play an important and essential role in conservation studies, especially for phylogenetic analyses (Janke et al. 2002; Li 2019). Mitogenomes obtained from museum specimens such as this one demonstrated to be invaluable for understanding the evolutionary history and taxonomy of squirrels (de Abreu et al. 2020). To determine the position of *S. anomalus* within the Sciuridae family, a Bayesian phylogenetic tree was performed using BEAST v2.6.3 (Bouckaert et al. 2019) under optimal substitution model (GTR + G + I) selected by jModelTest v2.1.10 (Darriba et al. 2012) (Figure 1). The resulting tree was visualized in Figtree v.1.4.4 (Rambaut 2016). Our phylogenetic tree puts *S. anomalus* in a basal position to Old World species. This is consistent with the hypothesis of Atilla et al. (2008) based on cytogenetic features (chromosomal characteristics), *S. anomalus* is distantly related to the other Old World *Sciurus* species (*S. lis* and *S. vulgaris*). Our results are contradictory with those of Aghbolaghi et al. (2020) who suggest that speciation in the *Sciurus* genus began with the common ancestor of *S. vulgaris* and *S. lis*.

Previous analysis based on *cytb* gene sequences (Oshida et al. 2009) showed a close relationship between *Sciurus vulgaris* and *Sciurus lis* while *Sciurus anomalus* clustered with the New World *Sciurus* species but bootstrap values supporting this cluster were low. Studies with a higher number of New and Old World taxa are needed to decipher the unique phylogenetic position of *S. anomalus* in the evolutionary process of the genus *Sciurus*.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. MW027641. The associated BioProject, SRA, and BioSample numbers are PRJNA694869, SRX9969238, and SAMN17575819, respectively.

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