Russian J. Theriol. 22 (2): 97–101

The complete mitochondrial genome of endemic Taurus ground squirrel *Spermophilus taurensis* (Rodentia: Sciuridae)

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ABSTRACT. Complete mitochondrial genome of paratype specimen of Taurus ground squirrel *Spermophilus taurensis* was assembled for the first time. We provide data concerning the sequencing, assembly, and annotation of the obtained mitochondrial genome. The studied mitogenome being 16 447 bp in length, and containing 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, an origin of L-strand replication and a control region. Data obtained for the whole mitochondrial genome confirmed sister position of *S. taurensis* and the closely related species *S. citellus* on the phylogenetic tree, which was shown earlier on single genes.

How to cite this article: Matrosova V.A., Gündüz İ., Ermakov O.A., Demirtaş S., Simonov E. 2023. The complete mitochondrial genome of endemic Taurus ground squirrel *Spermophilus taurensis* (Rodentia: Sciuridae) // Russian J. Theriol. Vol.22. No.2. P.97−101. doi: 10.15298/rusjtheriol.22.2.02

KEY WORDS: Marmotinae phylogeny, mitochondrial DNA, Turkey.

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Полный митохондриальный геном эндемичного Таврического суслика *Spermophilus taurensis* (Rodentia: Sciuridae)

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РЕЗЮМЕ. Впервые был собран полный митохондриальный геном паратипового экземпляра таврического суслика *Spermophilus taurensis*. Мы предоставляем данные, касающиеся секвенирования, сборки и аннотирования полученного митохондриального генома. Исследованный митогеном имеет длину 16 447 п.н. и содержит 13 белок-кодирующих генов, два гена рибосомальной РНК, 22 гена транспортной РНК, точку начала репликации L-цепи и контрольный регион. Полученные данные по полному митохондриальному геному подтвердили показанное раннее на основании отдельных генетических маркеров сестринское положение *S. taurensis* и близкого вида *S. citellus* на филогенетическом дереве.

КЛЮЧЕВЫЕ СЛОВА: филогения Marmotinae, митохондриальная ДНК, Турция.

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Introduction

The Taurus ground squirrel *Spermophilus taurensis* Gündüz *et al.*, 2007 is a relatively recently described endemic species of the Old World ground squirrel (Fig. 1C). This rodent is distributed in a small area in the Taurus Mountains in southern Turkey and is sister to the European ground squirrel *S. citellus* (Linnaeus, 1766) with a divergence time of about 2.5 million years ago (Gündüz *et al.,* 2007). Only 23 localities of the species have been described, while the abundance has not been studied at all (Gündüz et al., 2007; Gür et al., 2018). Due to the lack of information, this species does not currently have a high international conservation status. Whole genome research is an important step in building a modern strategy for species protection.

In this study, we sequenced the complete mitogenome of *S. taurensis*. The individual OMUS-435♂ is

one of the eight paratypes collected in the village of Yarpuz in Akseki-Antalya, Turkey (Latitude: 37.13 N, Longitude: 31.88 E, Altitude: 1542 m) on August, 11 in 2005. Permission for specimen capture was kindly granted by the General Directorate of Nature Conservation and National Parks, Republic of Turkey Ministry of Agriculture and Forestry (Project No. OMU F-392).

Materials and methods

The research is based upon museum collection specimen and GenBank (NCBI) data. DNA was extracted from muscle tissue of the paratype specimen using the standard phenol–chloroform method (Sambrook *et al.,* 1989). The paratype specimen and its DNA were deposited in the collections of the Department of Biology, Faculty of Sciences, Ondokuz Mayıs University, Samsun, Turkey (https://bio-fen.omu.edu.tr/tr, subcollection İG, contact person and email: İ. Gündüz, gunduzi@omu.edu.tr) under the voucher number OMUS-435. DNA library preparation was carried out using the TruSeq Nano DNA Library Prep Kit (Illumina, San Diego, CA). Total genomic DNA was sonicated using the Covaris S220 (Covaris, Woburn, MA) according to the manufacturer's recommendations. After adapter ligation, the library was sequenced on the Illumina NovaSeq6000 platform (2 x 150 bp) (Illumina, San Diego, CA).

Reads were assembled *de novo* with NOVOPlasty 4.3.1 (Dierckxsens *et al.,* 2017) to obtain a full-length mitogenome with an average coverage of 467x. Read coverage depth map was created following the protocol by Ni *et al.,* 2023) (Fig. 1B). The mitogenome was annotated using MitoAnnotator (Iwasaki *et al.*, 2013).

For phylogenetic analyses we used complete mitochondrial genomes from other members of the Marmotini tribe available in GenBank. The mitochondrial

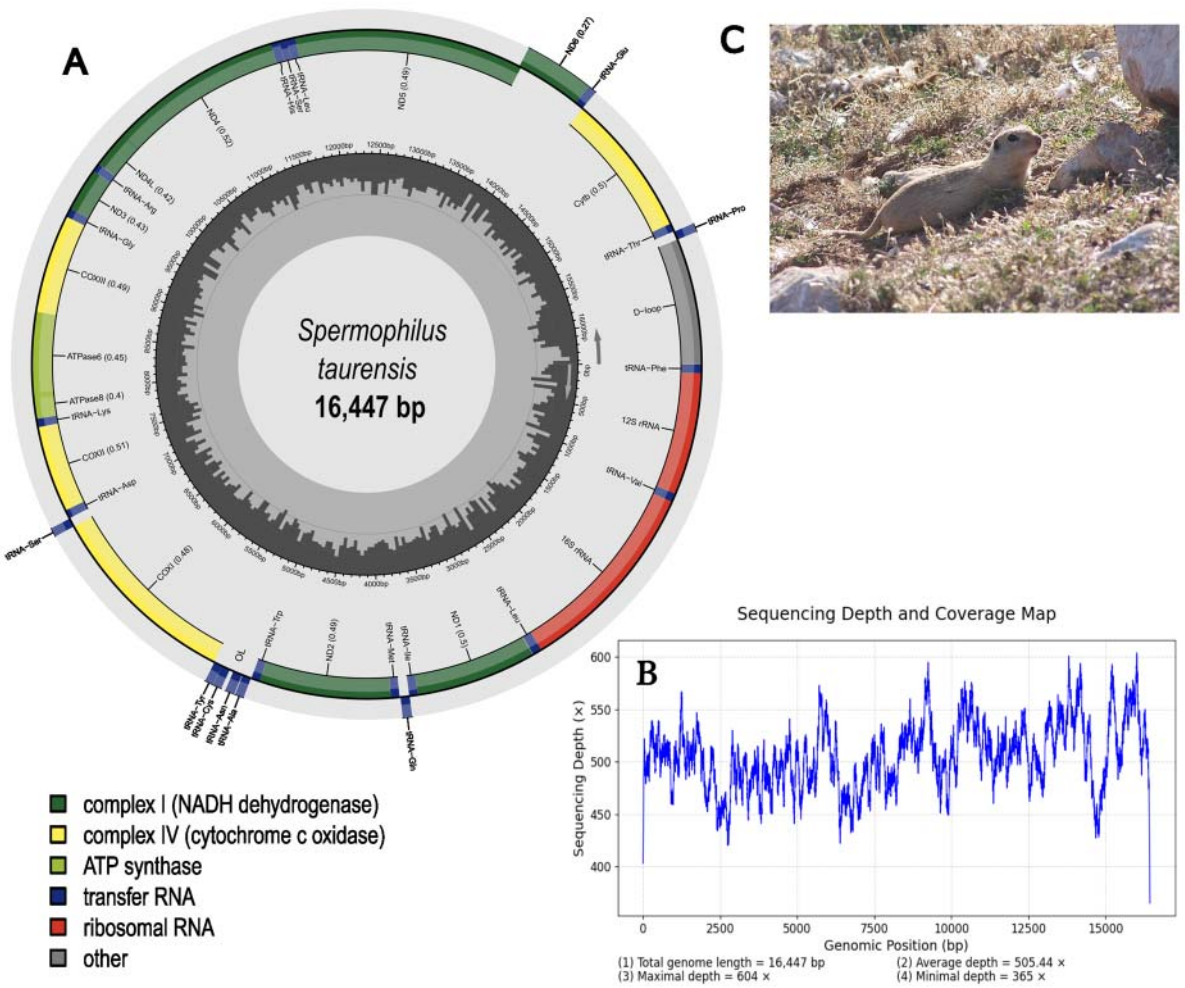

Fig. 1. (A) Circular map of the mitogenome of *Spermophilus taurensis*. The outer circle denotes gene composition, the inner circle shows local GC density. (B) Sequencing coverage graph of mtDNA. (C) An adult Taurus ground squirrel *Spermophilus taurensis*, with characteristic reddish brown dorsal pelage for this species. Photograph was taken in the Taurus Mountains during 2010, by İslam Gündüz.

Table 1. *S. taurensis* mitochondrial genome composition and characteristics.

Gene	Position Number		Size (bp)	Codon		Strand	Intergenetic Nucleotide
	Start	Stop		Start	Stop	H	
$tRNA^{Phe}$	1	70	70			H	0
rRNA 12S	71	1041	972			H	0
$tRNA^{Val}$	1042	1110	69			H	0
rRNA 16S	1111	2676	1566			H	0
$tRNA^{Leu}$	2677	2750	74			H	0
ND1	2754	3709	956	ATG	TA-*	H	+3
$tRNA^{Ile}$	3710	3778	69			H	0
$tRNA^{Gln}$	3776	3847	72			L	–2
$tRNA^{Met}$	3855	3923	69			H	+7
ND2	3924	4965	1042	ATT	T--*	H	0
$tRNA^{Trp}$	4966	5033	68			H	0
$tRNA^{Ala}$	5037	5105	69			L	+3
$tRNA^{Asn}$	5111	5183	73			L	+5
$tRNA^{Cys}$	5215	5281	69			L	+31
$tRNA^{Tyr}$	5282	5347	66			L	0
COI	5356	6897	1542	ATG	TAA	H	+8
$tRNA^{Ser}$	6900	6968	69			L	+2
$tRNA^{Asp}$	6972	7040	69			H	+3
COII	7041	7724	684	ATG	TAA	H	0
$tRNA^{Lys}$	7728	7794	61			H	+3
ATPase8	7796	7999	204	ATG	TAG	H	–43
ATPase6	7957	8637	681	ATG	TAA	H	–1
COIII	8637	9420	784	ATG	T--*	H	0
$tRNA^{Gly}$	9421	9490	70			H	0
ND3	9491	9837	347	ATA	TA-*	H	0
$tRNA^{Arg}$	9838	9904	67			H	0
ND4L	9906	10202	297	ATG	TAA	H	+1
ND4	10196	11573	1378	ATG	T--*	H	–7
$tRNA^{His}$	11574	11642	51			H	0
$tRNA^{Ser}$	11643	11701	59			H	0
$tRNA^{Leu}$	11702	11771	69			H	0
ND5	11772	13589	1818	ATT	TAA	H	0
ND6	13573	14097	525	TAC**	TCT**	L	–17
$tRNA^{Glu}$	14098	14166	68			L	0
Cyt b	14171	15310	1140	ATG	AGA	H	+4
$tRNA^{Thr}$	15311	15376	66			H	0
$tRNA^{Pro}$	15380	15446	67			L	+3
D-loop	15447	16447	1001				0

*TAA stop codon is completed by the addition of 3' A residues to the mRNA.
** The gene is encoded on the L- Strand (complement 5'->3').

genomes were aligned using MAFFT 7.453 (Katoh & Standley, 2013) in a high accuracy mode (with parameters -- maxiterate 1000 --globalpair). All gaps and poorly aligned positions were removed using Gblocks 0.91b (Castresana, 2000), including the whole control region, resulting in 15 241 bp length alignment. A maximum likelihood phylogenetic tree was generated using IQ-TREE 2.2.0 (Minh *et al.,* 2020). The best-fitting evolution model (GTR+F+R3) was selected with ModelFinder (Kalyaanamoorthy *et al.,* 2017) and the branch support was assessed with 1000 standard nonparametric bootstrap replicates.

Results and discussion

The genome sequence data generated in this study are openly available in GenBank of the NCBI (https://www.ncbi.nlm.nih.gov/) under the accession number

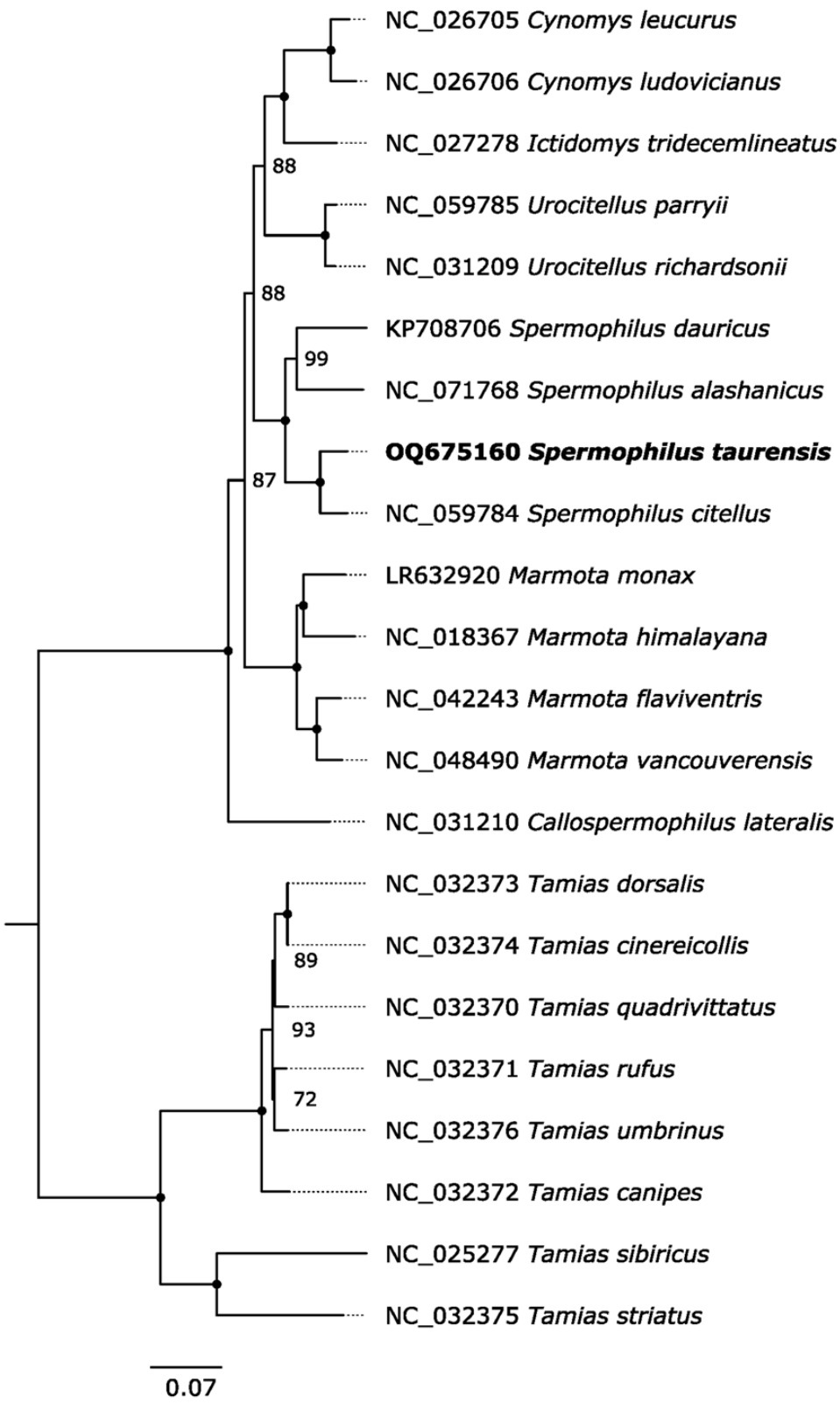

Fig. 2. Maximum likelihood phylogeny of 22 species of tribe Marmotini (Rodentia: Sciuridae) based on their complete mitogenomes. The mitogenome sequence generated in this study is labeled in bold. GenBank accession numbers for each species are shown before the name of the species. The bootstrap support values below 100% are shown by the numbers on the branches, while black dots designate 100% support.

OQ675160. The associated BioProject, SRA, and BioSample numbers are PRJNA971162, SRR24502290, and SAMN35020973, respectively. The complete mitogenome of *S. taurensis* consisted of 16 447 bp and had a typical structure consisting of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA), an origin of L-strand replication (O_L), and a control region (Fig. 1A; Table 1), which was consistent with other known species of *Spermophilus*. All mitochondrial genes were encoded on the H chain, with the exception of the ND6 gene and the eight tRNA genes ($tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$, $tRNA^{Glu}$, and $tRNA^{Pro}$) which were encoded on the L chain (Fig. 1A; Table 1). In the mitogenome, there were 12 gene spacers (from one to 31 bp in size). The longest gap was found between $tRNA^{Asn}$ and $tRNA^{Cys}$. In addition, there were five overlapping regions (from one to 43 bp in size). The longest overlapping region (43 bp) was located between ATPase 8 and ATPase 6 genes.

The overall base composition of the mitogenome in descending order was 32.7% — A, 32.0% — T, 23.0% — C, 12.4% — G, with an A + T bias (64.7%).

We made phylogenetic analysis of all Marmotini species with available mitogenome sequences (Fig. 2). The phylogenetic reconstruction supports the close relationship of *S. taurensis* and *S. citellus* (Gündüz *et al.*, 2007) and the monophyly of the Old World ground squirrels (genus *Spermophilus*) (Harrison *et al.*, 2003).

Taurus ground squirrel is a unique component of Turkish mountain ecosystems. It's known distribution is small and the species displays low genetic diversity (Gündüz *et al.*, 2007). Thus, there is a clear need to institute measures to ensure its conservation. Currently, ground squirrels have no legal protection and are viewed as pests by farmers (Özkurt *et al.,* 2005). Further studies of the ecology and the state of the population are urgently required.

ACKNOWLEDGMENTS. We thank Svetlana Kapustina for her help in laboratory work.

This study was supported by the Russian Science Foundation (RSCF), project number 22-24-01070.

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