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The complete mitochondrial genomes of Altai vole *Microtus obscurus* and common vole *Microtus arvalis* (Rodentia: Arvicolinae)

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ABSTRACT: Within the frame of the current study, we assembled and annotated seven mitochondrial genomes: three of them for the first time obtained for the Altai vole *M. obscurus*, four belong to the Eastern lineage of common vole *M. arvalis*. Obtained sequences enlarge amount of available genomic data for two sister vole species that creates the basis for further studies on phylogeny and mechanisms of adaptive mitochondrial DNA introgression, apparently took place within the group under study.

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Полные митохондриальные геномы алтайской полевки *Microtus obscurus* и обыкновенной полевки *Microtus arvalis* (Rodentia: Arvicolinae)

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РЕЗЮМЕ: В рамках настоящей работы мы собрали и аннотировали семь митохондриальных геномов: три из них впервые получены для алтайской полевки *M. obscurus*, четыре принадлежат восточной линии обыкновенной полевки *M. arvalis*. Полученные последовательности увеличивают объем доступных геномных данных для двух сестринских видов серых полевок, что создает основу для дальнейших исследований как их филогенетических взаимоотношений, так и механизмов адаптивной интрогрессии митохондриальной ДНК, вероятно, имевшей место в пределах изучаемой группы.

КЛЮЧЕВЫЕ СЛОВА: Серые полевки, митохондриальная ДНК, гибридизация.

Introduction

The Arvicolinae subfamily Gray, 1821 (Rodentia: Cricetidae) represents an extensive in the number of species group, whose diversification started back in Late Miocene having passed through the three waves of radiations produced more than 150 species from 28 genera (Abramson *et al*., 2021). Common voles of the genus *Microtus* Schrank, 1798 represent one of the youngest branches within Arvicolinae's radiation. Herein, we focused on two sister species within this genus: common vole *M. arvalis* (Pallas, 1778) and Altai vole *M. obscurus* (Eversmann, 1841). Common vole distribution area extends in Europe from Atlantic coasts in the west to the central Russia in the east, where it forms elongated secondary contact zone with the Altai vole, inhabiting vast territories from Ukraine and Asia Minor up to the Central Siberia. The last species long time considered as a karyomorph of *M. arvalis*: possessing identical diploid number ($2n = 46$) Altai vole differs from common vole in proportion of small acrocentric and metacentric chromosomes (*M. obscurus* $NF = 72$; *M. arvalis* $NF = 84$; Bulatova *et al*., 2010). Later, on the basis on differentiation in sequences for mitochondrial cytochrome b gene (*Cyt b*), shown 4.6% nucleotide substitutions between these two forms, it was suggested to considered it at least as semi-species (Lavrenchenko *et al*., 2009). Finally, based on phylogenetic analysis of comprehensive dataset including 43 individuals of *M. obscurus* and 125 of *M. arvalis*

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(but still on sequences of *Cyt b* only), authors suggested that both taxa should be ranked as species (Tougard *et al*., 2013).

Microtus arvalis and *M. obscurus* form stable hybrid zone, spans over 800 km in European Russia (Lavrenchenko *et al*., 2023). Common vole possess pronounced phylogeographical structure throughout its distribution in Europe, formed by means of independent evolution of several genetic lineages in isolated refugia during last glaciation (Stojak *et al*., 2015). Currently, these lineages come into secondary contacts with formation of several hybrid zones of different ages (Beysard & Heckel, 2014). Take this into account, one can speculate that *M. arvalis–M. obscurus* species pair represents a promising evolutionary model to studying both mechanisms of speciation and processes underlying hybrid zones' maintenance.

With the advent of high-throughput sequencing a gradual accumulation of genomic data, primarily on complete mitochondrial genomes, happening at an ever-increasing pace. However, within the group under consideration, the only complete and publicly available mitogenome is for *M. arvalis* individual from Germany (RefSeq No. NC_038176.1). To fill this gap, we sequenced and annotated 4 complete mitochondrial genomes of *M. arvalis* and 3 of *M. obscurus* sampled from Central Russia.

Material and methods

Individuals were sampled in 2015–2022 years from different localities in Central Russia (Table). Soft tissue samples were kept in 96% ethanol and stored in collection of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (Moscow, Russia). Total genomic DNA were extracted using the Jena Bioscience PP-208 DNA extraction kit (Jena Bioscience, Germany). Preparing Illumina libraries and sequencing on NOVASeq PE 150 lane was performed in NOVOGENE UK Sequencing Center (Cambridge, UK), that produced an average 2.5 GB of paired-ended 150 bp raw reads per sample. Quality of obtained raw reads were assessed in FastQC (Andrews *et al*., 2010). Assembly was performed in Mitoz software (Meng *et al*., 2019). Obtained FASTA sequences were aligned against available complete mitochondrial genome of *M. arvalis* (RefSeq: NC_038176.1) and manually edited to provide equal starting position for all linear sequences. Annotation of obtained sequences was performed in Mitoz v.3.6. Available data were deposited in GenBank database (Table).

To show phylogenetic position of the newly obtained genomes within the mitochondrial diversity of the common and Altai voles, we extracted *Cyt B* gene sequences (1140 bp) and aligned it with publicly available data from a number of published works (Baker *et al*., 1996; Haynes *et al*., 2003; Fink *et al*., 2004; Braaker & Heckel, 2009; Bužan *et al*., 2010; Barbosa *et al*., 2013; Martínková *et al*., 2013; Tougard *et al*., 2013; Stojak *et al*., 2015). Total alignment consists of 116 individuals. To show phylogenetic relationships among sequenced individuals and available complete mitochondrial genome of *M. arvalis*, we constructed alignment including 13 protein-coded genes, 22 tRNA's and 2 rRNA's sequences. The length of total alignment was 14 945 bp. The mitochondrial genome of *M. rossiaemeridionalis* (GenBank No. DQ015676) was used as an outgroup. Both phylogenetic trees were reconstructed by means of IQtree (Nguyen *et al*., 2015) with using 1000 bootstrap replications (Fig. 1) and visualized using FigTree v1.4.3 software (http://tree.bio.ed.ac.uk/software/figtree/).

Results

All obtained mitochondrial genomes were assembled into circular structure and revealed typical composition including 13 protein-coded genes, 22 transport

Table. Detailed information on newly assembled mitochondrial genomes.

Individual ID	Species	Coordinates	Raw data amount (Gb)	Coverage (Max/Min)	Total length (bp)	Genbank accession no.
19-208	*M. obscurus*	N 52.1339; E 39.5771	2.2	157 / 42	16 285	OR654969
20-052	*M. obscurus*	N 56.9151; E 43.7556	3.1	693 / 115	16 286	OR654973
1261	*M. obscurus*	N 55.4579; E 42.5799	2.3	833 / 312	16 284	OR654974
19-278	*M. arvalis*	N 52.1736; E 39.1501	2.4	277 / 79	16 282	OR654970
19-426	*M. arvalis*	N 52.0639; E 38.6966	2.4	677 / 336	16 279	OR654971
19-635	*M. arvalis*	N 57.2743; E 43.2443	3.0	477 / 157	16 280	OR654972
1088	*M. arvalis*	N 56.0355; E 40.5592	2.3	626 / 70	16 281	OR644944

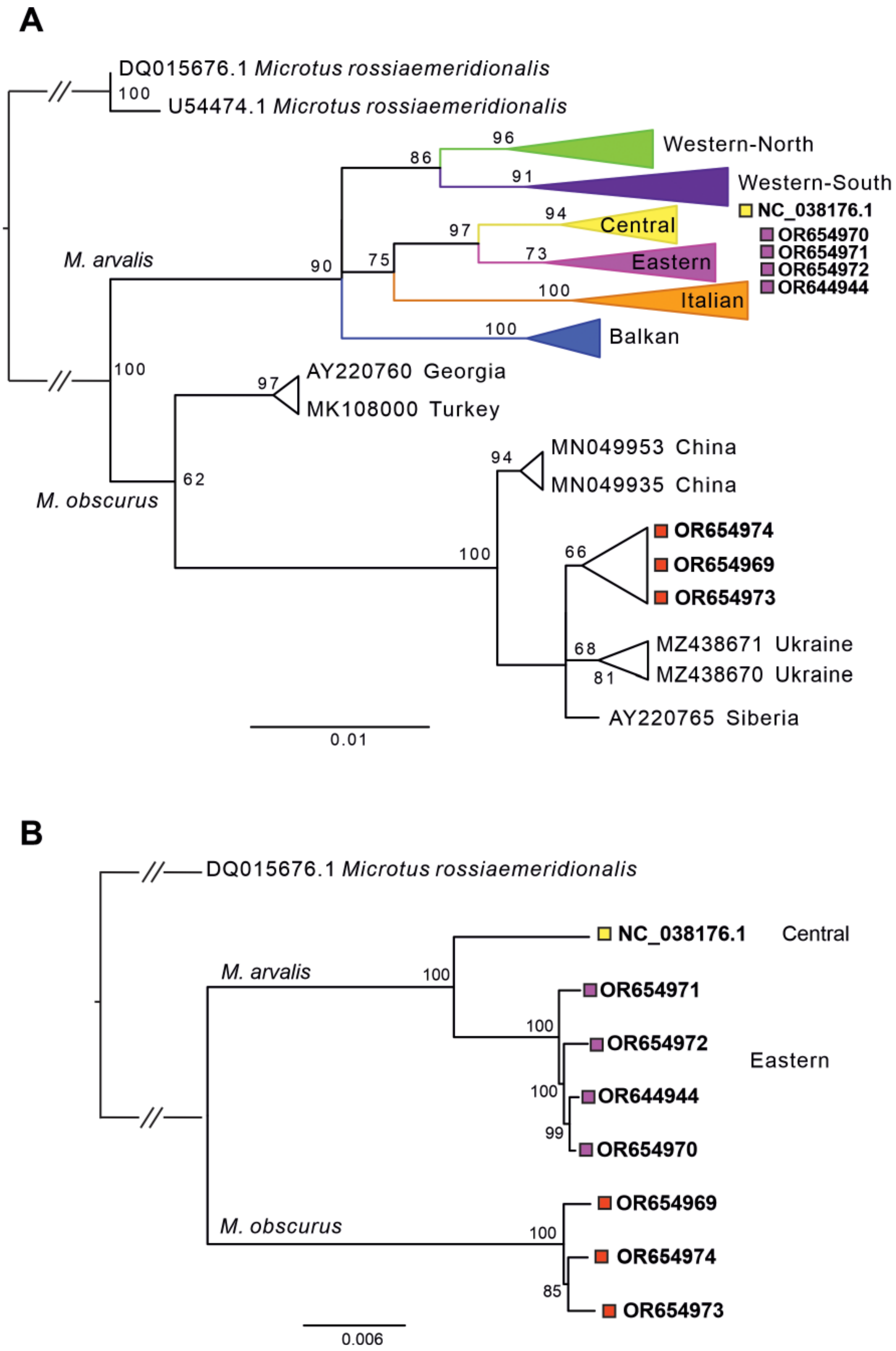

Fig. 1. A. Maximum likelihood phylogenetic tree based on *Cyt b* sequences from 116 individuals of *M. arvalis* and *M. obscurus*. *M. rossiaemeridionalis* represents the outgroup. In case of *M. arvalis*, colors of different mitochondrial lineages are given according to Stoyak et al., 2015. Individuals for which complete mitochondrial genome is available are in bold. NC_038176.1 was downloaded from GenBank, the other sequences obtained within the current study. B. Maximum likelihood phylogenetic tree based on 14 945 bp alignment.

RNA's, 2 rRNA's and control region. Detailed information including individual sample coordinates, coverage and GenBank accession numbers are presented in Table. Phylogenetic assignment of *Cyt b* sequences extracted from the obtained mitochondrial genomes revealed that sequenced individuals of *M. arvalis* from Central Russia belong to Eastern mitochondrial lineage, whereas previously available common vole mitochondrial genome from Germany (RefSeq No. NC_038176.1) is a representative of Central lineage (Fig. 1). Genetic data from other *M. arvalis* lineages (Western-North, Western-South, Italian and Balkan) are currently presented by available in GenBank database sequences of *Cyt b* gene only. In case of *M. obscurus* three assembled mitochondrial genomes are the only available complete mitochondrial genome data for this species to date. Sequences of *Cyt b* gene extracted from individuals from Central Russia expectedly fall on the phylogenetic tree into the so-called Sino-Russian lineage, grouping together with samples from Ukraine and Siberia.

Discussion

Our results for the first time recovered complete mitochondrial genome for the three individuals of Altai vole *M. obscurus* and four individuals of the Eastern genetic lineage of common vole *M. arvalis* (Table). Although it is still not enough to cover all mitochondrial diversity of these species at the genomic level, our data capture the shallowest (eastern and central lineage of *M. arvalis*) and deepest (*M. arvalis* and *M. obscurus*) levels of this species complex' diversity. Previous studies, assessing genetic differences between these two vole forms and placed *M. obscurus* in its own species, were based on the *Cyt b* gene sequences. Complete mitochondrial genomes assembled within the frame of the current study significantly increase the reliability of the obtained phylogenetic inferences and allow to confirm species rank of *M. obscurus*.

Apart from it, it is worth to note, that hybrid zone between *M. arvalis* and *M. obscurus* is characterized by pronounced shift of mitochondrial DNA cline contrary to clines obtained from nuclear loci (Lavrenchenko *et al*., 2023). Such pattern can be explained by the two alternative hypotheses. In the first case, asymmetrical introgression of *M. arvalis* mtDNA could be explained by the *M. obscurus* expansion into *M. arvalis* area and, as a consequence, adoption into gene pool of species-invader genetic material of the resident species (see detailed description of this process in study of Currat & Excoffier (2004), modeling human expansion into Europe). An alternative hypothesis based on preferential introgression of particular mtDNA owing to it adaptive advantages. Adaptive introgression of mtDNA is itself a fairly common phenomenon: encoding 13 genes involved in production of up to 95% of cellular energy (Da Fonseca *et al*., 2008), interspecific introgression of mitochondrial genome may serve as a relatively fast way towards adaptation to environments, where certain modifications of oxidative phosphorylation system turn out to be useful (Melo-Ferreira *et al*., 2012; Toews & Brelsford, 2012). It is an open question, to what extent the mentioned phenomenon occurs in the case of hybridization of gray voles. In some sense similar evolutionary situation, where interspecific introgression of mtDNA, apparently possessing adaptive nature, was shown in case of hybridization between two species of Eurasian bank voles (Boratyński *et al*., 2014). Anticipating further studies, publication of complete mitochondrial genomes allows to use this data to decipher possible adaptive nature of mtDNA introgression between *M. arvalis* and *M. obscurus*.

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