

Evolutionary history of *Microtus obscurus* reconstructed using modern and ancient mitogenomes

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Introduction

Altai gray vole (*Microtus obscurus*) is a rodent species present in Western Asia, Eastern Europe, and the Near East [1]. The taxonomic relationships with its sister species *M. arvalis* occurring in much of Europe is a matter of ongoing debate. Previous phylogeographic studies revealed presence of at least two main mtDNA lineages: the Middle-Eastern including West Iran [2] and South Caucasian [3], and Sino-Russian divided into two sublineages [3]. However, there is a lack of agreement regarding the dating of divergence of these lineages and the two sister species. Our aim was to further understand the evolutionary history of *M. obscurus*. This is a part of the bigger project with a goal of reconstructing species responses to climate change.

Main takeaway

Divergence of main mtDNA lineages of *obscurus* and *arvalis* were synchronous (ca. 100kya), and we estimated their TMRCA to be 0.13Mya, which is more recent than previously estimated. Several specimens from Dzudzuana Cave, Georgia represent a new, previously unknown mitochondrial lineage named Kartvelian that diverged 82kya. This suggest lineage/population turnover in the Caucasus at the end of the Late Pleistocene.

References

[1] Sibiriyakov, P.A., et al. (2018). Phylogeography of the Common Vole *Microtus arvalis*, the Obscurus Form (Rodentia, Arvicolinae): *Russ J Genet* 54.
[2] Mahmoudi, A., et al. (2017). New insight into the cradle of the grey voles (subgenus *Microtus*) inferred from mitochondrial cyt b sequences. *Mammalia* 81(6).
[3] Tougaard, C., et al. (2013). Exploring phylogeography and species limits in the Altai vole (Rodentia: Cricetidae). *Biol. J. Linn. Soc.*, 108(2).
[4] Wang, X., et al. (2023). Demographic history and genomic consequences of 10,000 generations of isolation in a wild mammal. *Curr Biol*.
[5] Baca, M., et al. (2022). Ancient DNA reveals interstadials as a driver of common vole population dynamics during the last glacial period. *J. Biogeogr.*
[6] Stewart, M., et al. (2017). Middle and Late Pleistocene mammal fossils of Arabia and surrounding regions: *Quat. Int.*

Results & Discussion

→ Figure 1.

We estimated TMRCA for *arvalis* and *obscurus* as 0.13 million years ago (Mya) contrary to 0.18Mya based on cyt b in Mahmoudi et al. [2] and 0.173Mya in Tougaard et al. [3]. We estimated the divergence time of main lineages of *obscurus* to be ca. 100kya. We identified new mtDNA lineage, Kartvelian (K), which diverged about 82kya: time of overall heightened climatic variability [6]. The divergence of main *arvalis* lineages also occurred ca. 100kya during MIS 5 interglacial characterized by the emergence of forests and reduction of open habitat suitable for this microtine species likely forcing fragmentation of population and divergence. In Dzudzuana cave (Georgia) Kartvelian and South Caucasian (SC) lineages are present with K specimens dating around 40kya and SC specimen dated at 3kya.

→ Figure 2.

Individuals from South Caucasian line show a population expansion starting 30kya, which is consistent with divergence of Middle Eastern line and may be the reason for presence of SC line in Dzudzuana, where Kartvelian line was previously present. This co-occur with the LGM (29-19kya) when some populations became isolated in the South Caucasus by the southernmost permafrost boundaries [see 3]. West Iran line diverge from SC around 70kya in here, yet mtDNA suggest much later divergence (30kya, see Fig. 1). *Arvalis* and *obscurus* divergence at ca. 100kya matches mtDNA analysis (see Fig. 1.). Both *arvalis* and *obscurus* specimens from Russia present similar demographic history suggesting local climate influence.

Figure 2. (right) PSMC analysis of nDNA of Altai and common vole. Correction for low coverage was applied to three samples with genomic coverage below 20x based on three downsampled sequences. Sample names arrangement: mtDNA-LINEAGE LOCALITY COUNTRY SHORTENED-SAMPLE-CODE[FNR correction].

Workflow

- DNA extraction and library preparation optimized for ancient DNA for 14 specimens and modern DNA for 6 specimens: Laboratory of Paleogenetics, University of Warsaw.
- Addition of 3 modern *obscurus* mtDNA sequences from Wang et al. [4] and *arvalis* dataset with 22 directly radiocarbon dated samples from Baca et al. [5].
- Phylogenetic analyses and molecular age estimation on 4.2kb fragment of mtDNA using BEAST 1.10.4, 4 partitions identified using Partition Finder 2.
- Tip-dating of each undated specimen separately with uniform prior.
- Joint phylogenetic analysis of all sequences with ages of samples estimated in previous step used as normal priors on sample age.
- PSMC 0.6.5. analyses on 6 modern nuclear DNA sequences of *obscurus* adjusted for low coverage if needed.

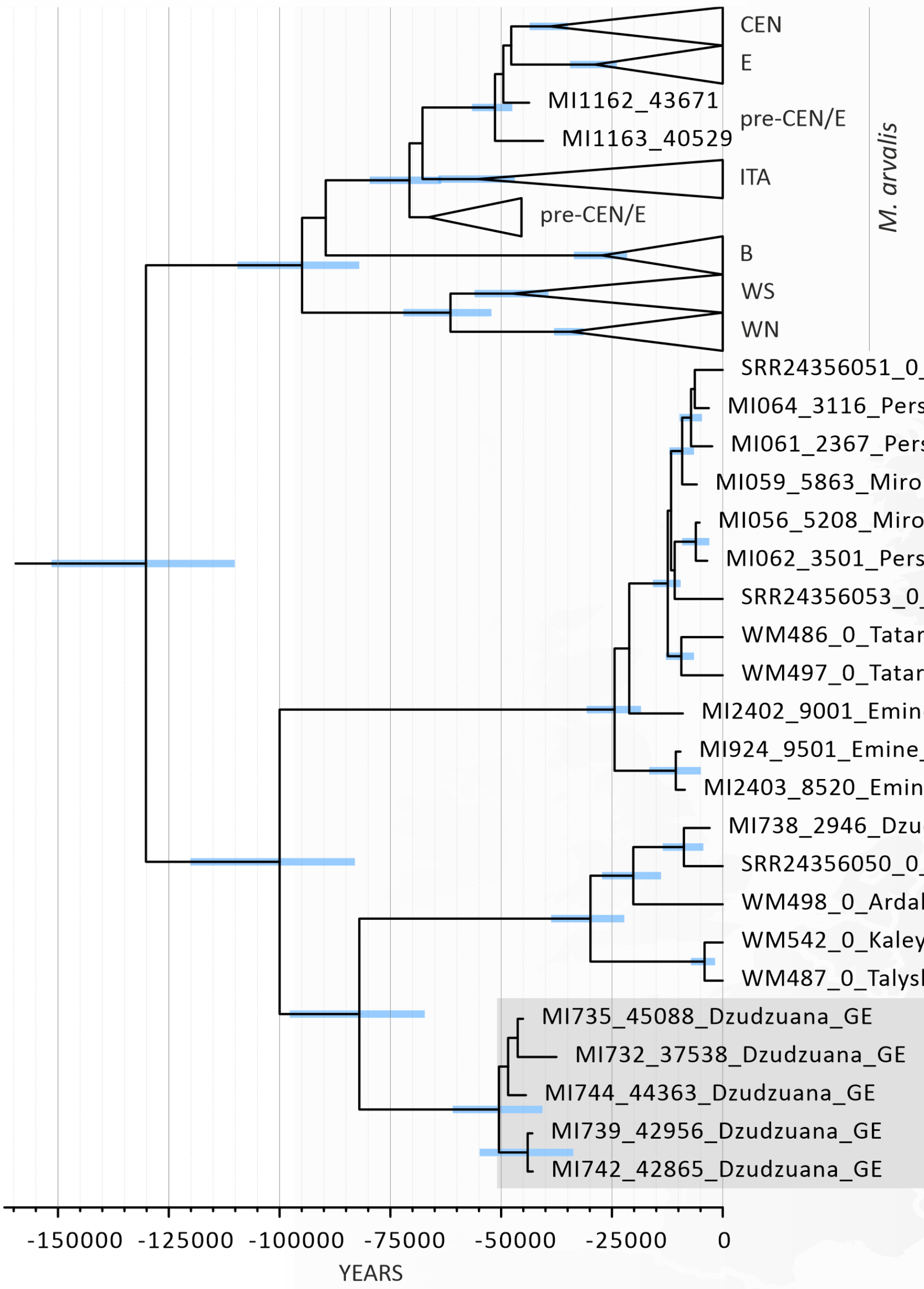
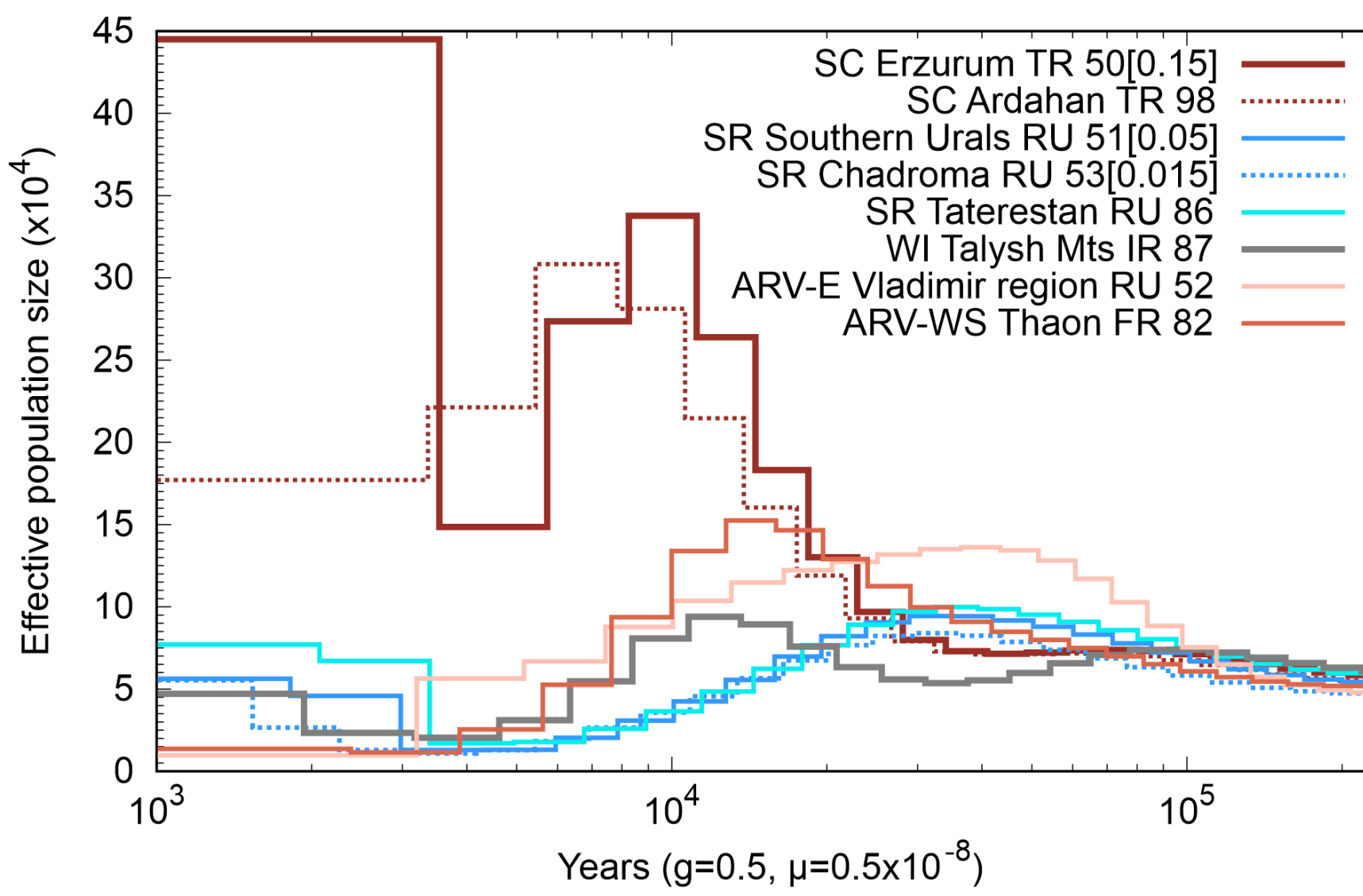


Figure 1. (top and right) Mitogenomic phylogeny of Altai vole. Top: Tip-calibrated phylogeny of Altai vole based on 4.2kb fragment of mtDNA. Blue bars represent 95% HPD intervals of divergence time and indicate posteriori probability >0.95. Tips are annotated with sample number, median of estimated age, and locality. Grey marks a previously unknown lineage. Right: Sample locations of *M. obscurus* specimens.

ancient

modern

