# Ancient DNA clarifies the evolution of the field vole species complex

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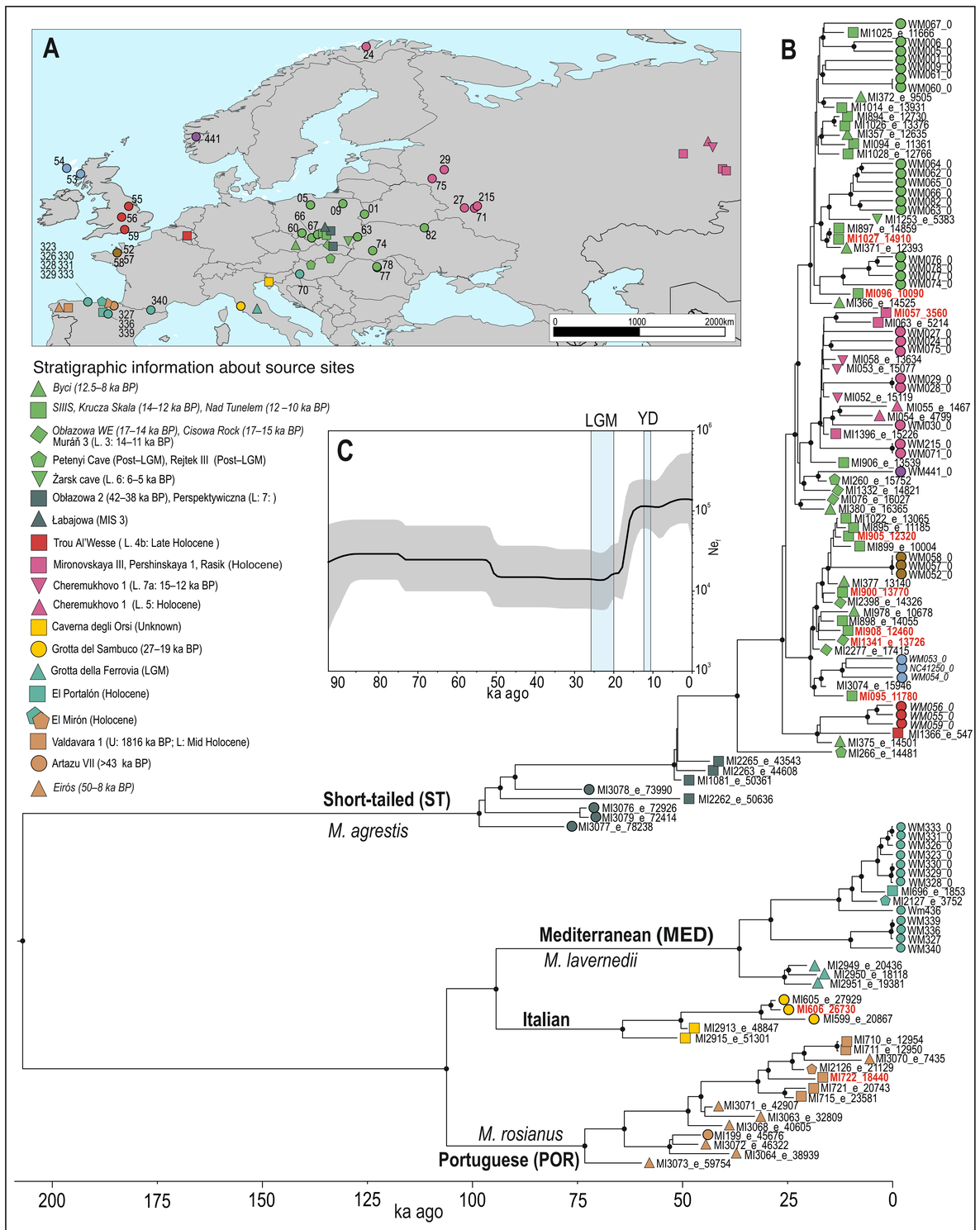


**Figure 1. Mitochondrial phylogeny of Last Glacial Period field voles. A**. Sampling locations of vole specimens and stratigraphic information for ancient samples. **B**. Tip-dated phylogeny of field voles based on mtDNA genomes reconstructed in BEAST 1.10.4. Tips are anotated with estimated ages of ancient specimens (_e_). Labels in red and bold indicate directly radiocarbon dated specimens. Dots at nodes indicat posterior probability above 0.95. **C**. Demographic trajectory of the short-tailed field voles reconstructed using Bayesian skyline approach.

## Introduction

The field vole is a complex of three cryptic species. The short-tailed field vole (*Microtus agrestis*) is present over much of Eurasia, the Mediterranean field vole (*Microtus lavernedii*) is found in southern Europe, and the Portuguese field vole (*Microtus rozianus*) is limited to western Spain and Portugal [1]. Previous studies showed discordance of mitochondrial (mtDNA) and nuclear gene trees and suggested that the Portuguese field voles diverged from Mediterranean and short-tailed field voles ca. 70 thousand years (ka) ago, while the two latter separated about 25 ka ago during the Last Glacial Maximum (LGM) [2–3]. This was reinforced by the Ecological Niche Modeling which predicted three potential glacial refugial areas during the LGM [3].

Previous reconstructions of demographic history of the short-tailed field vole showed a species-wide bottleneck around the Younger Dryas (12.5–11.7 ka ago) [4–5]. However, little is known about earlier population dynamics during the Late Pleistocene.

Here, we decided to reassess the previous divergence estimates and to provide new information on evolutionary history of this species complex using mitochondrial and genomic data from ancient and modern field vole specimens.

## Workflow and dataset

- Target enrichment of vole mtDNA used to generate high coverage mitogenomes of **75** ancient and **47** modern specimens ranging in age between ca. 70 and 0 ka ago (**Fig. 1A**).
- Ten ancient specimens directly radiocarbon dated to calibrate the molecular clock - collagen pretreatment in MPI EVA + AMS in ETH Zurich and Aix-en-Provence.
- Phylogenetic analyses, molecular age estimation of not dated specimens and demographic reconstruction performed using BEAST 1.10.4 [6].
- Shotgun sequencing of **11** ancient (coverage: 0.3–2.57×; median: 0.97×) and **14** museum and modern genomes (coverage: 0.73–36.8×; median: 8.8×).

## Mitochondrial phylogeny

- Three previously recognised field vole mtDNA lineages and a new one (Italian) confined to the Late Pleistocene specimens from Italian Peninsula.
- Divergence of main mtDNA lineages estimated to Marine Isotope Stage (MIS) 7 and MIS 5 (**Fig 1B**) suprisingly similar to the divergence of main mtDNA lineages in some cold adapted small mammals: narrow-headed voles, collared lemmings and common voles [7].
- Short-tailed field vole suffered a bottleneck around LGM and and post-LGM recovery (**Fig. 1C**) earlier than previously estimated, but mtDNA diversity was higher during Boling-Allerod (14.7–12.5 ka ago) than today.

## Genomic analyses

- MDS plot revealed three clusters corresponding to main mtDNA lineages. The oldest specimens (ca. 70 and 40ka old) are only slightly shifted towards the centre of the plot providing *terminus ante quem* for the divergence of field vole species (**Fig. 2B**).
- PSMC support the divergence of Portuguese field vole ca. 200 ka ago and subsequent separation of Mediterranean and short-tailed field voles <100 ka ago (**Fig. 2C**).
- Short-tailed and Mediterranean field voles suffered a bottleneck around LGM and a recovery around Pleistocene-Holocene transition (**Fig. 2C**).
- D statistics suggest geneflow both between MED and POR and MED and ST (**Fig. 2D**).

## Conclusions

- The divergence for field-vole species took place much earlier than previously estimated around 200ka (MIS7) and 100ka (MIS5), periods recently suggested to be important hotspots for differentiation [7].
- Discordance of mtDNA and genomic phylogenies - evidenced by different order of divergence - the case of ancient mtDNA capture ?
- Geneflow between MED and both ST and POR - geographically structured, occured after post-LGM expansion?
- Distinct mtDNA lineage on Italian peninsula - conforms with theory of Mediterranean peninsulas as a source of endemism [7].
- Both mtDNA and genomic data supports a bottleneck in ST and MED around LGM.
- In Central Europe the mtDNA diversity 15–10 ka ago was higher than today - where there another bottlneck around YD?

## To be done

- Use modeling on the genomic data to estimate the timing of bottleneck more precisely and check whether there was one or two subsequent bottlnecks.
- Estimate the time of genomic divergence more precisely.
- Estimate the direction and timing of geneflow between field vole species.

## References

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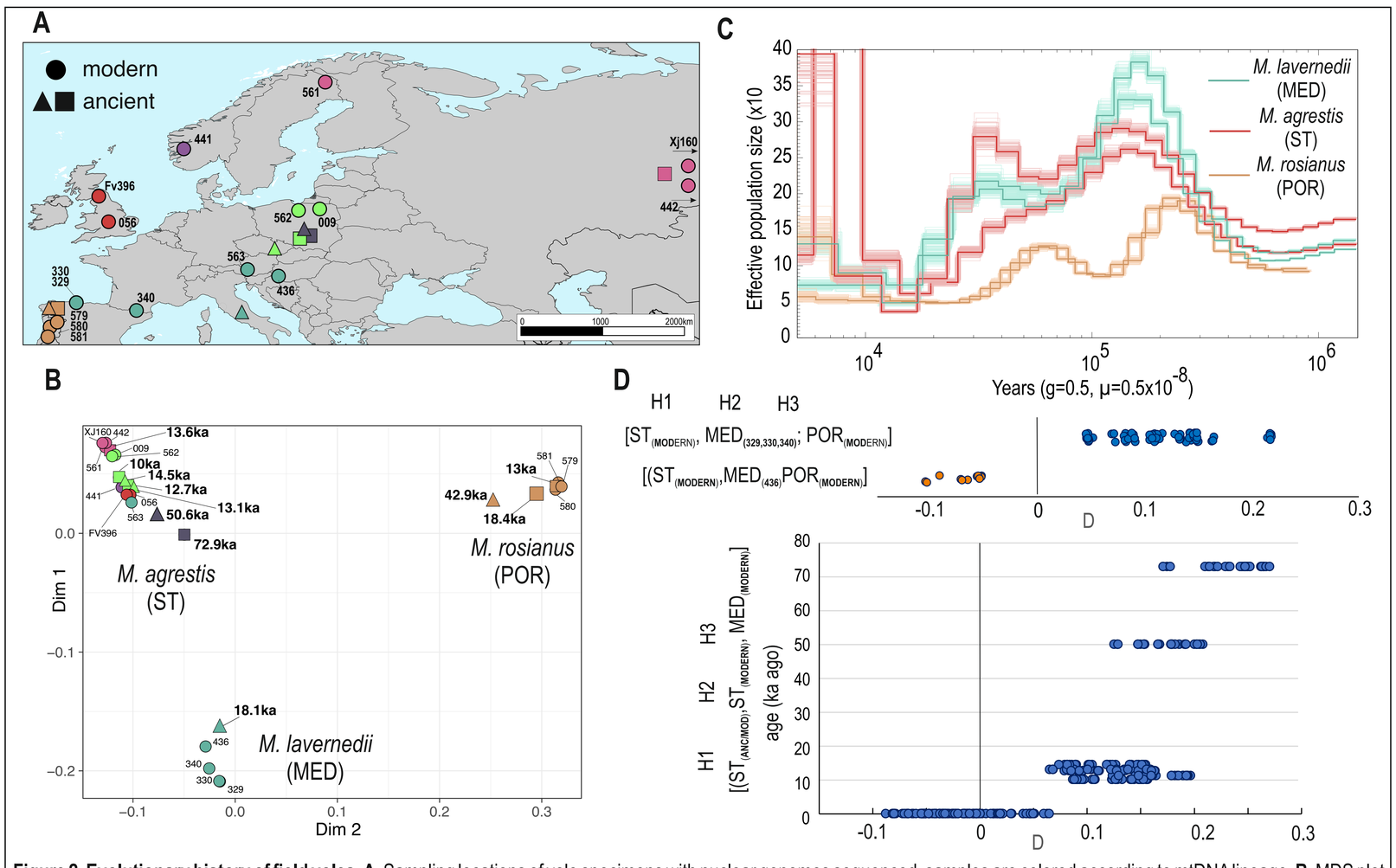


**Figure 2. Evolutionary history of field voles. A**. Sampling locations of vole specimens with nuclear genomes sequenced, samples are colored according to mtDNA lineage. **B**. MDS plot based on IBS matrix (12M variable positions) generated using random read sampling in Angsd. Ancient samples are annotated with their estimated age. **C**. PSMC plots of two samples from each species downsampled to 20× genomic coverage, faint lines represent 100 bootstrap replicaes. **D**. D-statistics based on random base sampling computed in Angsd, reads were mapped to the outgroup MicOch1.0 genome.

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