

Molecular Age Estimation of Rodent Remains as a Means to Date Late Pleistocene sites

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Molecular age estimation

Absolute dating of artefacts and sites is a cornerstone of palaeontological and archaeological research, enabling the reconstruction of evolutionary timelines, archaeological cultures, and faunal successions. **Radiocarbon dating** and **thermally/optically stimulated luminescence** are among the most widely used methods, but each has limitations—particularly regarding their applicable temporal range and preservation requirements. As an alternative or complement to these approaches, **molecular age estimation** infers the age of biological remains based on their phylogenetic placement. Because organisms accumulate DNA mutations over generations, the number of genetic differences between specimens can be used—within a calibrated phylogenetic framework—to estimate when an organism lived [1].

Here, we demonstrate that molecular dating of small mammal remains from palaeontological sites is a viable and valuable supplement to traditional dating methods, especially when collagen preservation or stratigraphic context prevents radiocarbon analysis.

Calibration datasets

- narrow-headed vole** (*Stenocranius sp.*) - mitogenomes, 11 radiocarbon dated, 6 modern samples [2](Baca et al., 2023a)
- field vole** (*Microtus agrestis*) - mitogenomes, 11 radiocarbon dated, 68 modern [3](Baca et al., 2025)
- root vole** (*Alexandromys oeconomus*) - mitogenomes, 10 radiocarbon dated, 32 modern [4](Żeromska et al., submitted)
- common vole** (*Microtus arvalis*) - 4.3 kb mtDNA fragment, 20 radiocarbon dated, 60 modern [5,6](Baca et al., 2020; 2023b)
- collared lemming** (*Dicrostonyx torquatus*) - mitogenomes, 10 radiocarbon dated, 60 modern [7] (Lord et al., 2022)

These species constitute the majority of small mammal assemblages at most of the Late Pleistocene sites - abundant material for sampling

Phylogenetic analyses

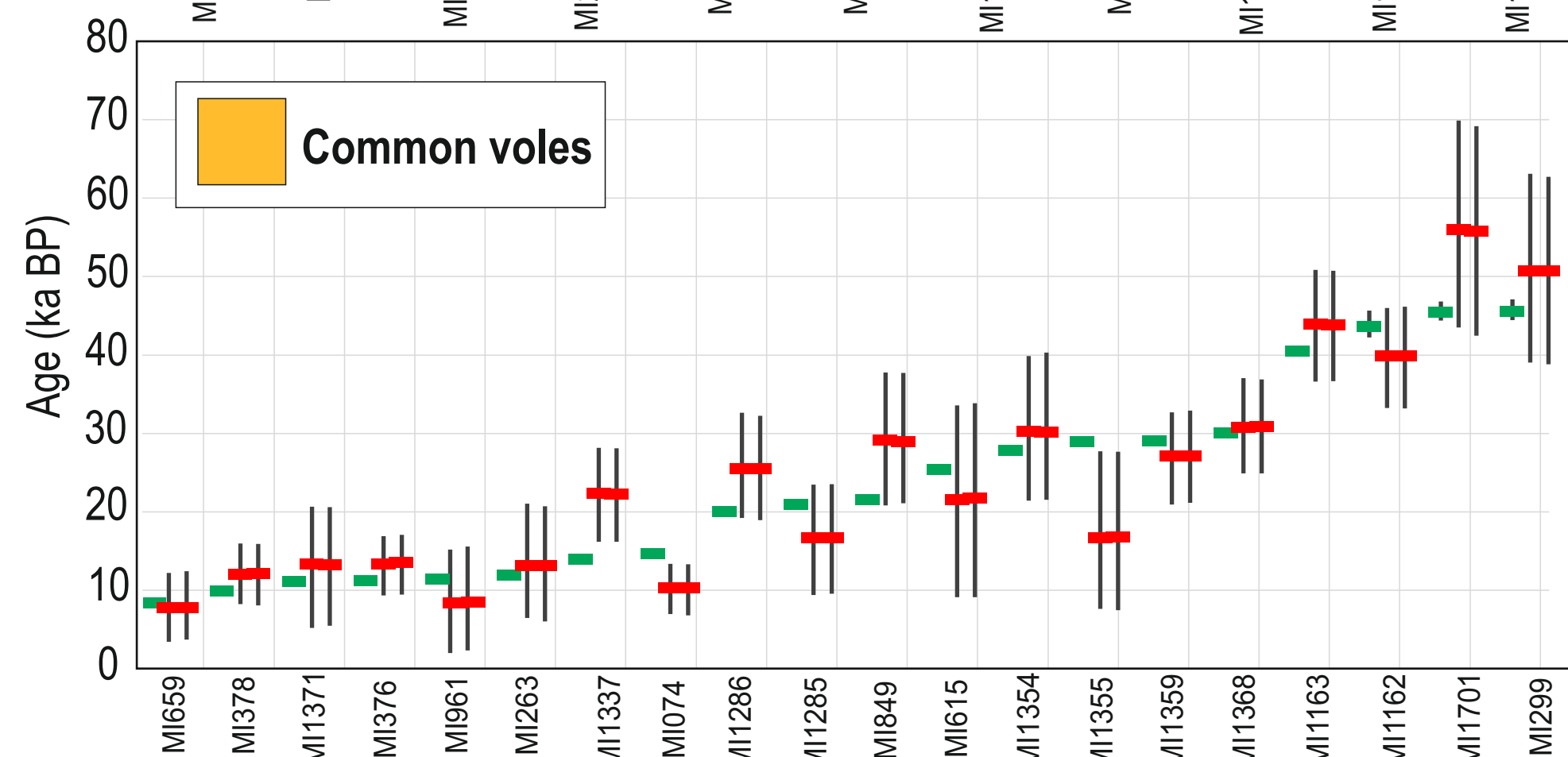
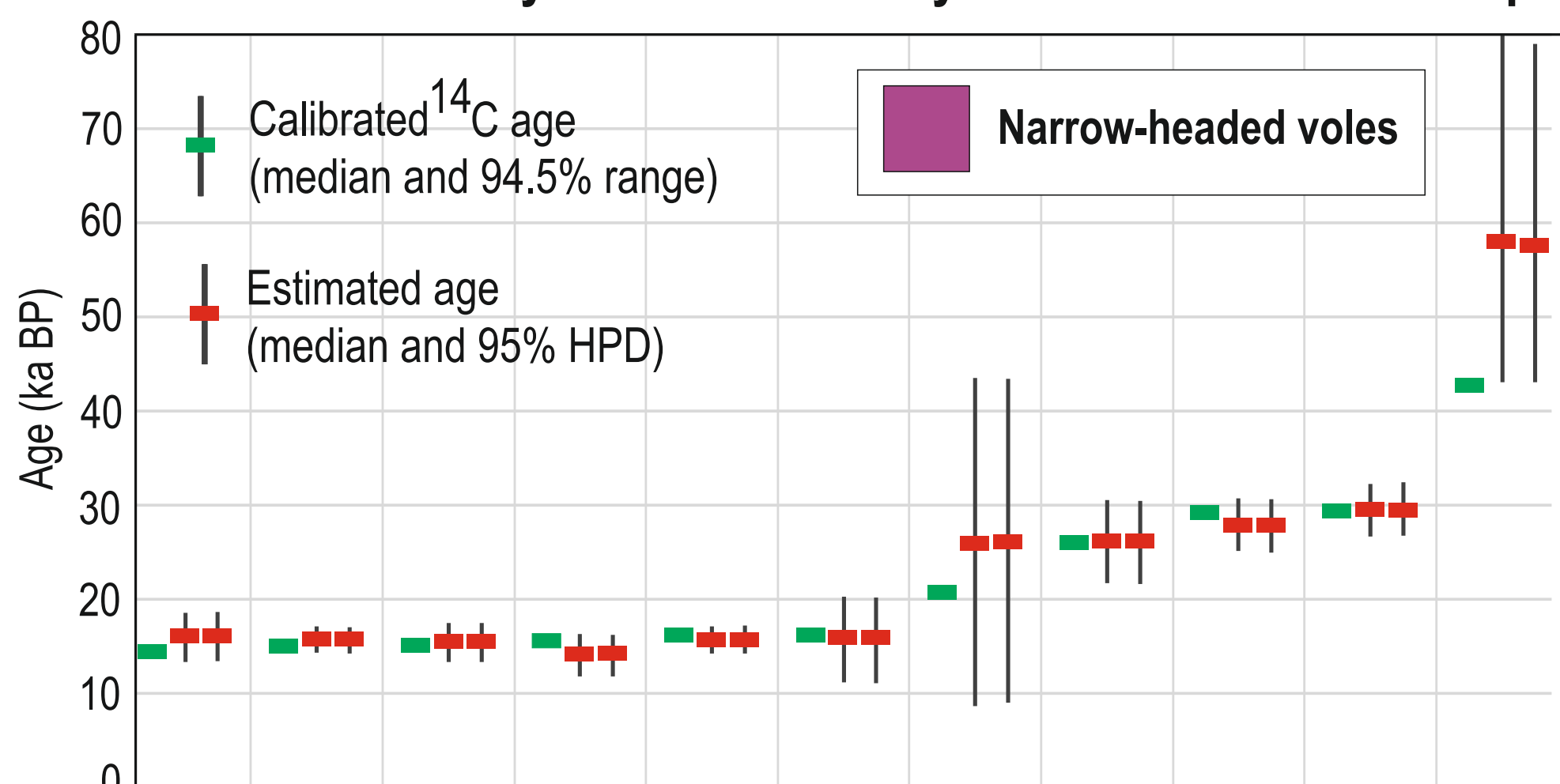
- Molecular ages estimated using BEAST 1.10
- Radiocarbon dated and modern samples used to calibrate the molecular clock
- Appropriate clock, tree prior selected using MLE, partitioning scheme and substitution model selected using PartitionFinder2
- Each undated sample was tip-dated in a separate BEAST run using a wide gamma prior on the age and operator weight increased to 5

Conclusions

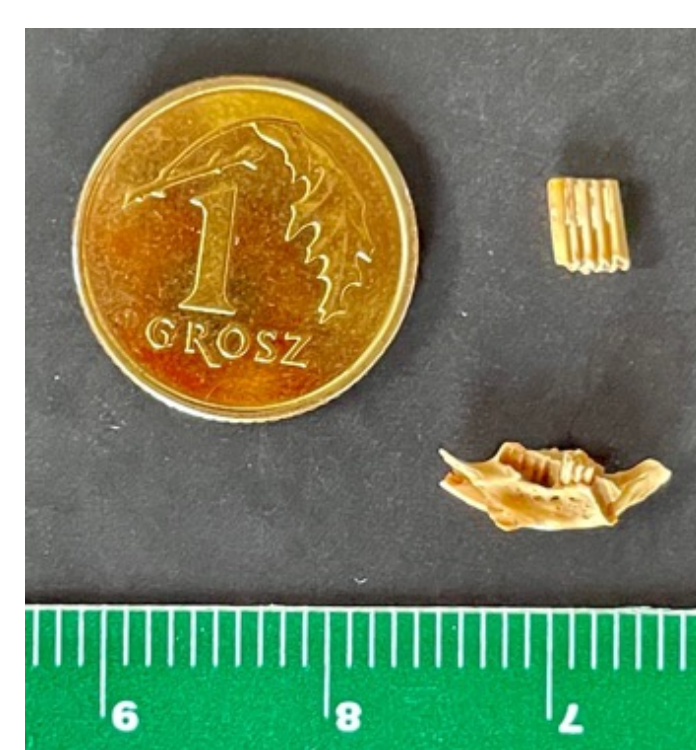
- Molecular age estimates of small mammal remains can complement other absolute dating methods.
- Often more accurate than molecular age estimates of large mammals.
- Multiple independent datasets help control for species-specific biases.
- MtDNA sequences can be retrieved from sites with poor collagen preservation.
- Better calibration datasets required to improve dating of Holocene samples

1. Shapiro B, et al. 2011 A bayesian phylogenetic method to estimate unknown sequence ages. Mol Biol Evol 28, 879–887 2. Baca M et al. 2023 Ancient DNA of narrow-headed vole reveal common features of the Late Pleistocene population dynamics in cold-adapted small mammals. PNAS 120, 3. Baca M et al. 2025 The evolutionary history of the field vole species complex revealed by modern and ancient genomes. BioRxiv 4. Żeromska et al. Reconstruction of phylogeographic relationships and evolution of tundra vole, *Alexandromys oeconomus* (Rodentia, Cricetidae), based on ancientDNA (submitted). 5. Baca M et al. 2020 Diverse responses of common vole (*Microtus arvalis*) populations to Late Glacial and Early Holocene climate changes – Evidence from ancient DNA. Quat Sci Rev 233, 106239 6. Baca M et al. 2023 Ancient DNA reveals interstadial as a driver of common vole population dynamics during the last glacial period. J Biogeogr 50, 183–196 7. Lord E et al. 2022 Population dynamics and demographic history of Eurasian collared lemmings. BMC Ecol Evol 22, 126 8. M. Krajcarz M. 2023 New insights into Late Pleistocene cave hyena chronology and population history - the case of Perspektywiczna cave, Poland. Radiocarbon 6(5): 1038–1056 9. Grotzinger J et al. 2019 Large-scale mitogenomic analysis of the phylogeography of the Late Pleistocene cave bear. Sci Rep 9, 1–11 10. Krajcarz MT et al. 2020 Shelter in Smoleń III - A unique example of stratified Holocene clastic cave sediments in Central Europe, a lithostratigraphic stratotype and a record of regional paleoecology. PLoS One 15(2): e0228546 11. Lemanik A et al. 2020 The impact of major warming at 14.7 ka on environmental changes and activity of Final Palaeolithic hunters at a local scale (Crawa-Nowy Targ Basin, Western Carpathians, Poland). Archaeol Anthropol Sci 12, 66 12. Berto, C et al. Chronological, paleoclimatic, and paleoenvironmental data from Łabajowa Cave (Kraków-Częstochowa Upland, Poland): a comprehensive approach for investigating a complex Late Pleistocene sequence (submitted)

Leave-one-out analysis on the directly radiocarbon dated samples

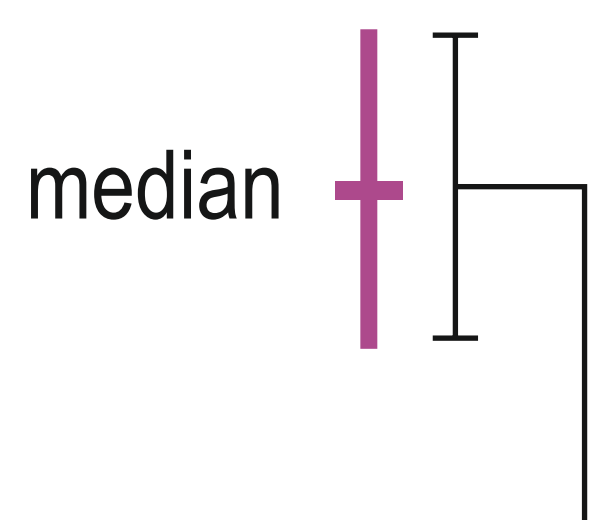


Molecular age estimates of small mammals are accurate



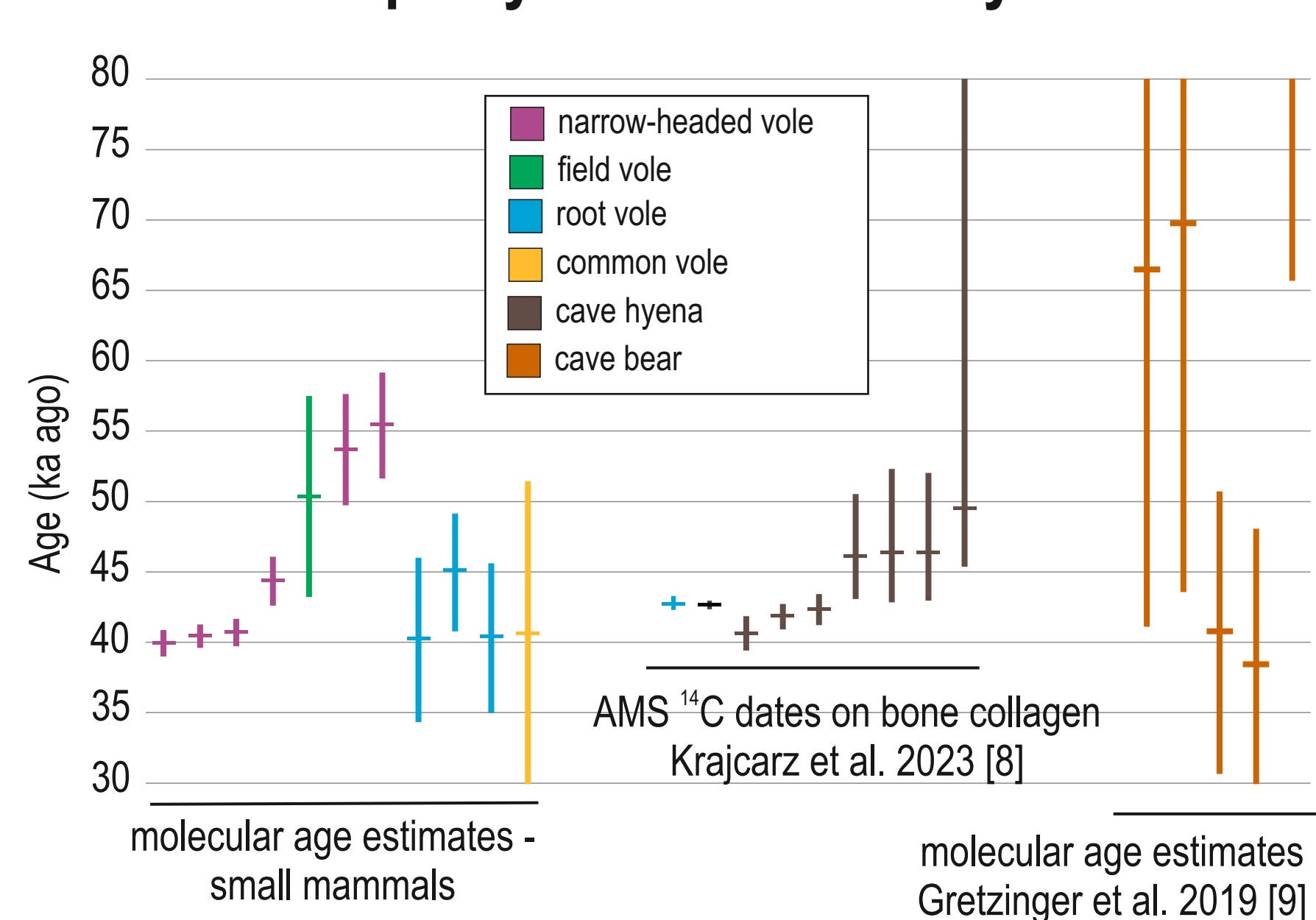
Common vole sample
used for DNA extraction

Date Notation



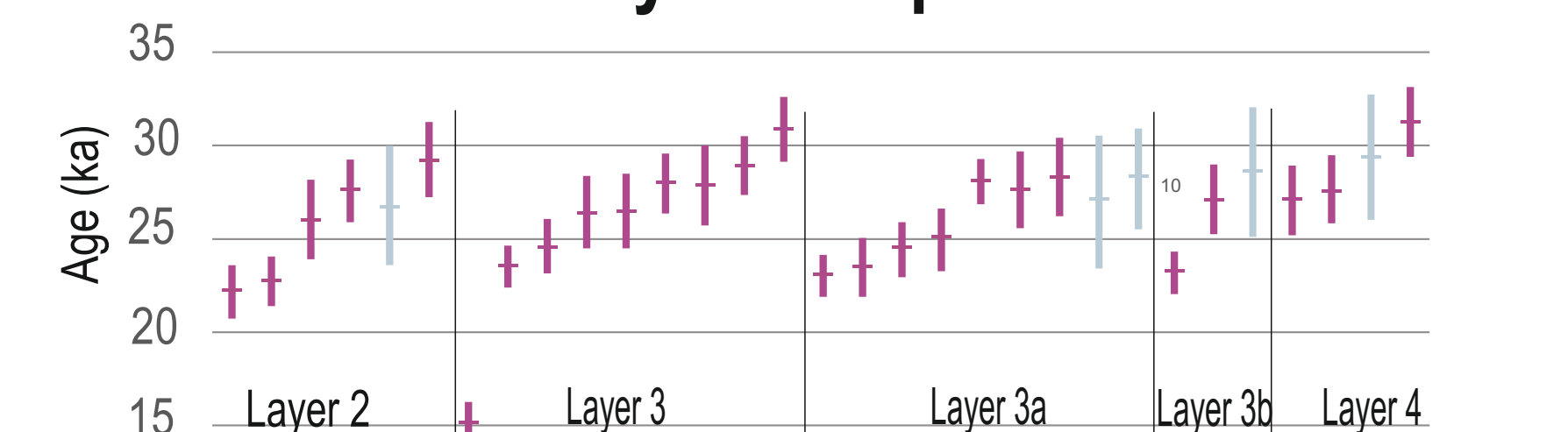
95% HPD interval – molecular estimates
95.4% range (2-sigma) – AMS ¹⁴C dates

Perspektywiczna cave – layer 7c



Molecular age estimates of small mammals show higher precision than those of large mammals

Pietraszyn 11 - open air site

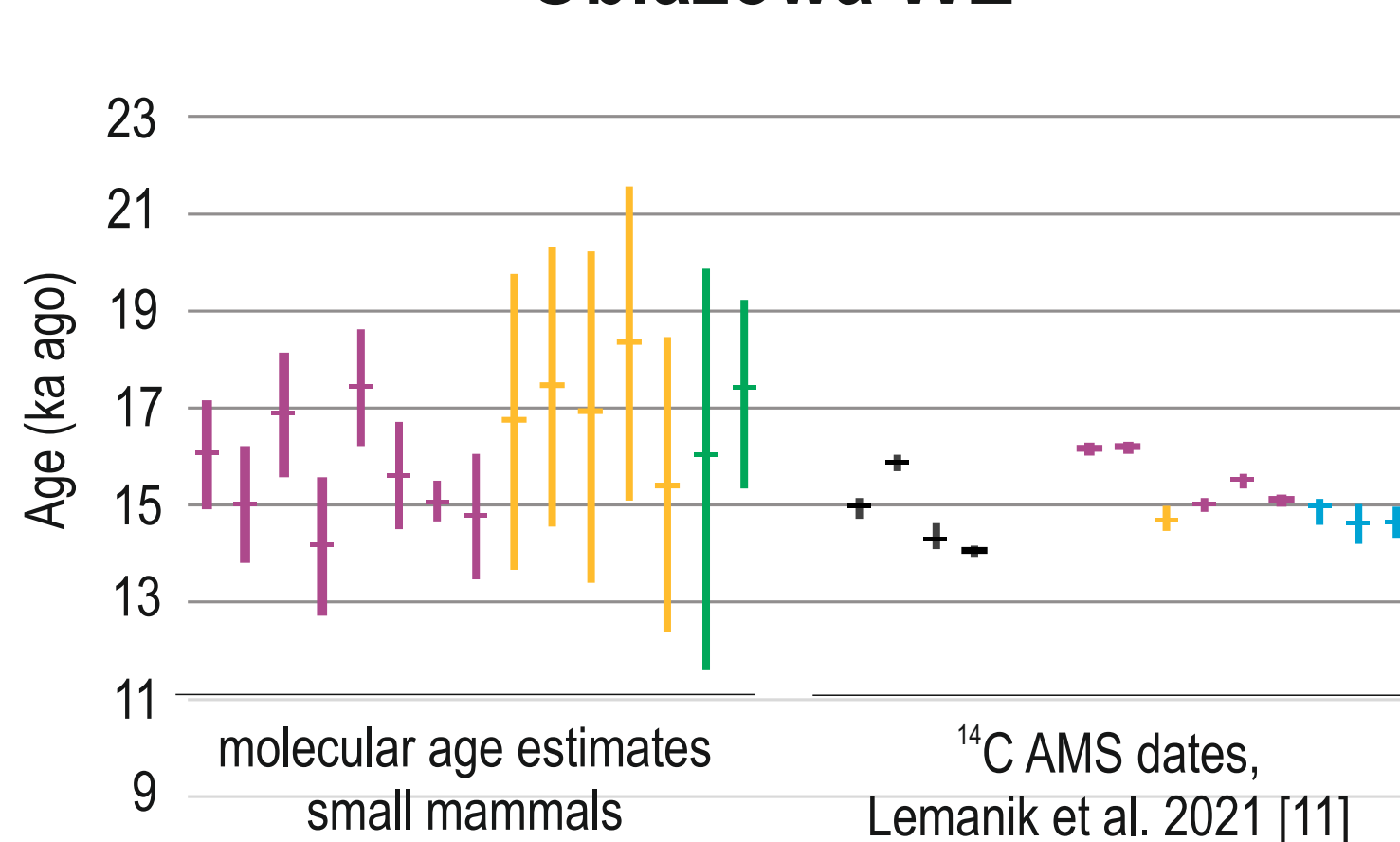


Ancient mtDNA can often be recovered from sites where poor collagen preservation hinders radiocarbon dating



Location of the mentioned paleontological sites

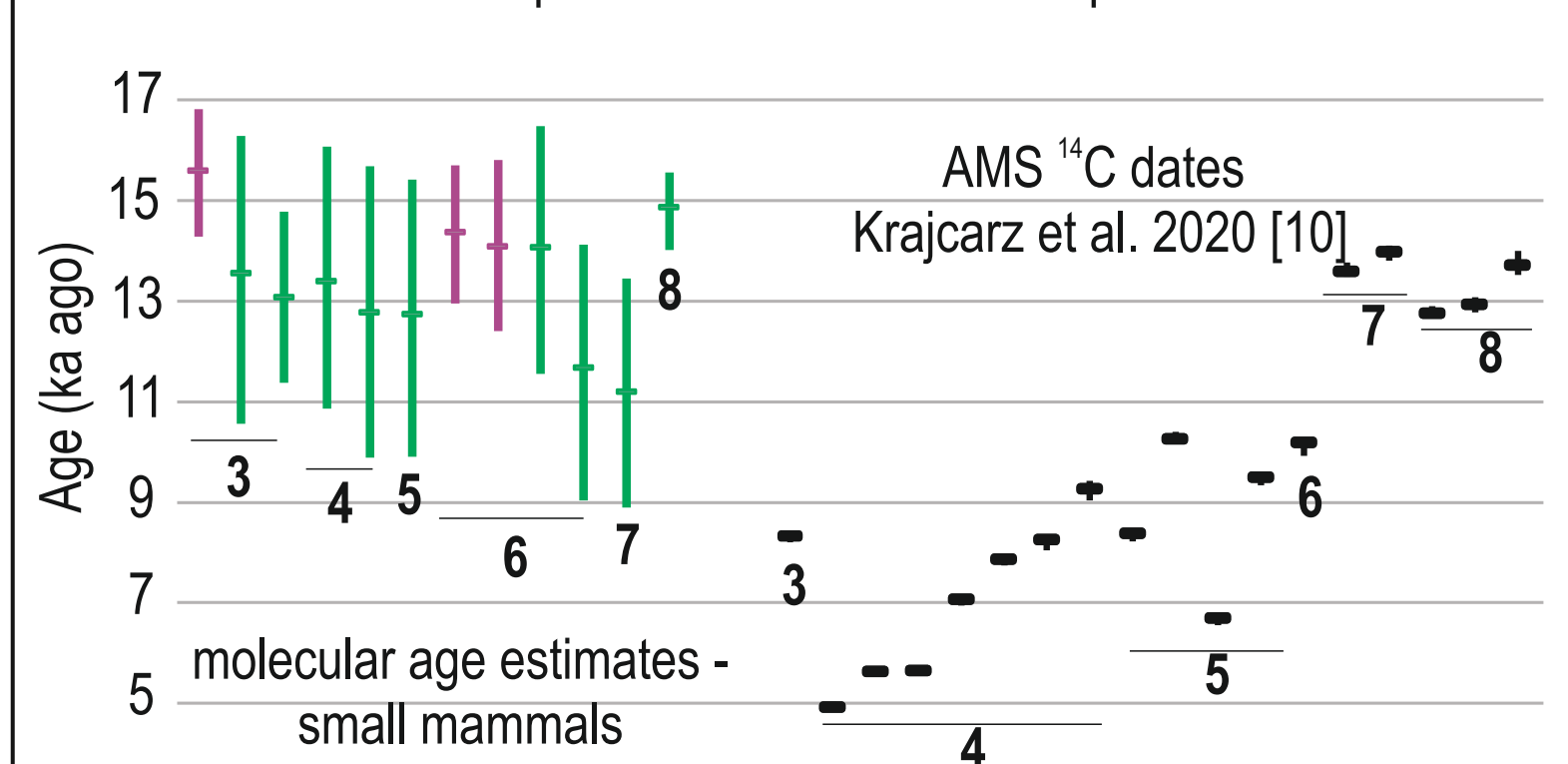
Oblazowa WE



Age estimates from independent datasets allow identification of potential species-specific biases

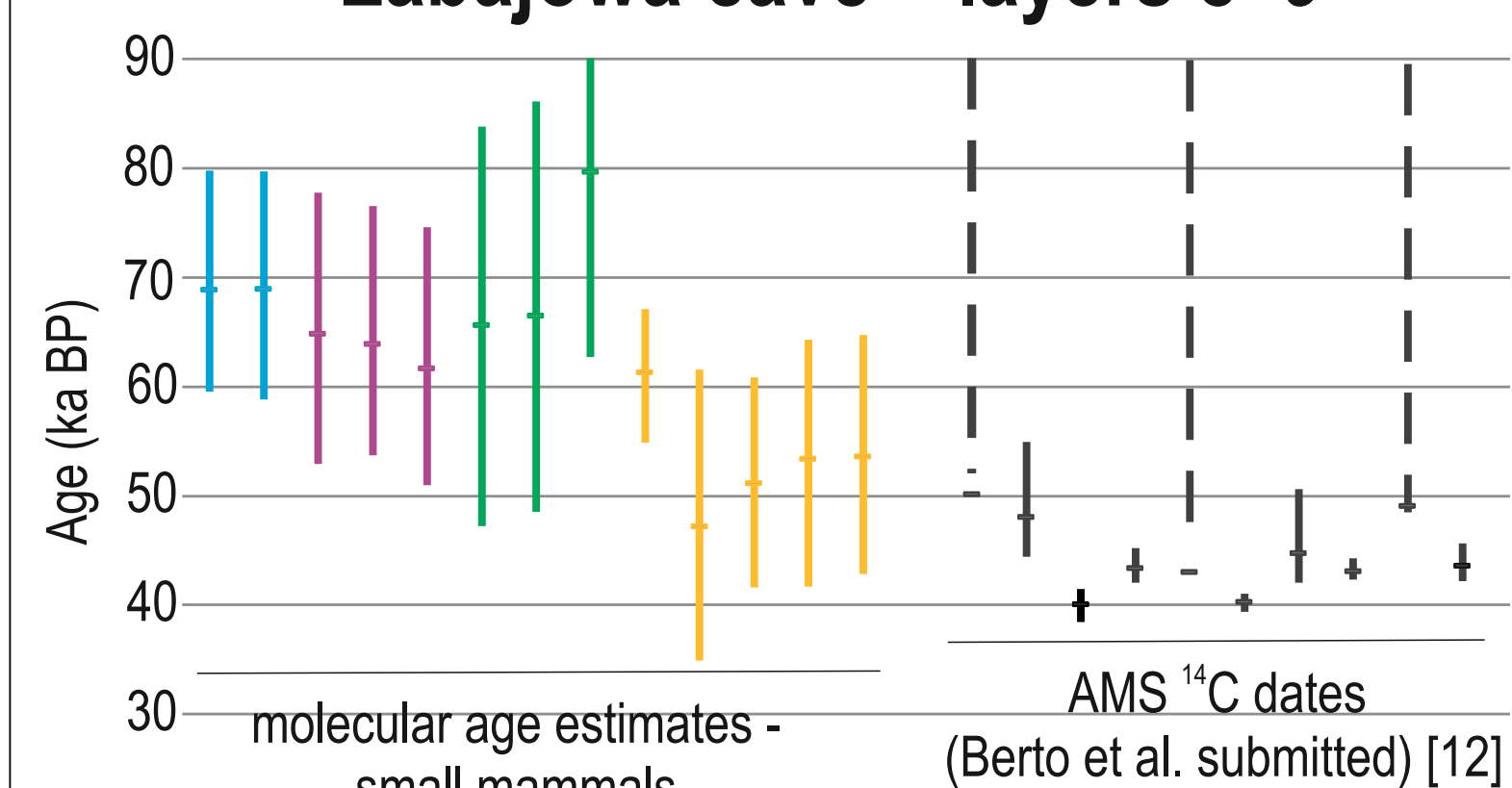
Shelter in Smoleń III

Unique stratified Holocene sequence



Problems with dating of Holocene samples

Łabajowa cave – layers 8–9



Molecular age estimates may improve dating of layers beyond the radiocarbon limit