

1 Ancient DNA reveals interstadials as a driver of the common vole population 2 dynamics during the last glacial period

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5 Running title: Evolutionary history of common vole

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48 **1 Abstract**

49 **Aim**

50 The common vole is a temperate rodent widespread across Europe. It was also one of the most
51 abundant small mammal species throughout the Late Pleistocene. Phylogeographic studies of
52 its extant populations suggested the Last Glacial Maximum (LGM, 26.5–19 ka ago) as one of
53 the main drivers of the species' population dynamics. However, analyses based solely on extant
54 genetic diversity may not recover the full complexity of past population history. The main aim
55 of this study was to investigate the evolutionary history and identify the main drivers of the
56 common vole population dynamics during the Late Pleistocene.

57 **Location**

58 Europe

59 **Taxon**

60 Common vole (*Microtus arvalis*)

61 **Methods**

62 We generated a dataset comprising 4.2 kb-long fragment of mitochondrial DNA from 148
63 ancient and 51 modern specimens sampled from multiple localities across Europe and covering
64 the last 60 thousand years (ka). We used Bayesian inference to reconstruct their phylogenetic
65 relationships and to estimate the age of specimens that were not directly dated.

66 **Results**

67 We estimate the time to the most recent common ancestor of all Last Glacial and extant common
68 vole lineages to 90 ka ago and the divergence of the main mtDNA lineages present in extant
69 populations to between 55 and 40 ka ago, earlier than previous estimates. We find multiple
70 lineage turnovers in Europe in the period of high climate variability at the end of Marine Isotope
71 Stage 3 (MIS 3; 57–29 ka ago) in addition to those found previously around the
72 Pleistocene/Holocene transition. Conversely, data from the Western Carpathians suggest
73 continuity throughout the LGM even at high latitudes.

74 **Main conclusions**

75 Our results suggest that the main factor affecting the common vole populations during the last
76 glacial period was the reduction of open habitats during the interstadial periods while the
77 climate deterioration during the LGM had little impact on species' population dynamics.

78

79 **Keywords:** ancient DNA, *Microtus arvalis*, habitat, Late Pleistocene, paleoclimate, small
80 mammals

81

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103

104 **3 Introduction**

105 The climatic and environmental changes during the last glacial period (ca. 115–11.7 ka
106 ago) had a great impact on the evolutionary histories of most species. It has been suggested that

107 species have responded to those changes according to their individual characteristics and there
108 is no basis for considering them as communities responding to climate and environmental
109 changes in the same manner (Baca et al., 2017; Lorenzen et al., 2011; Stewart, Lister, Barnes,
110 & Dalén, 2010). However, within the ecosystem, species depend on a whole range of
111 interactions at different trophic levels and across different ecological niches (Walther, 2010).
112 Thus, investigations of species with different adaptations can reveal the spectrum of responses
113 to the same climatic and environmental fluctuations and allow identification of the key factors
114 driving ecosystem responses (Cooper et al., 2015). Small mammals may be especially well
115 suited for such investigations as, in contrast to megafaunal species, they seem to be little
116 affected by activities of Palaeolithic hunter-gatherers and their population dynamics were
117 mainly driven by environmental changes.

118 The common vole *Microtus arvalis* (Pallas, 1779) is a temperate rodent species which
119 is present in most of continental Europe (Figure 1b). The species feeds primarily on leaves and
120 grasses and prefers well-drained grasslands, pastures, and alpine meadows from lowlands up to
121 ca. 3000 m above sea level. At present it mainly utilizes secondary habitats, such as agricultural
122 fields, where it is often considered a pest (Jacob, Manson, Barfknecht, & Fredricks, 2014). The
123 earliest remains of ancestral common voles (*Microtus arvalis*-group) in Europe date to ca. 0.6-
124 0.5 Mya (Berto, Nadachowski, Pereswiet-Soltan, Lemanik, & Kot, 2021; Kučera, Suvova, &
125 Horáček, 2009; Maul & Markova, 2007). The fossil record from the last glacial period (115–
126 11.7 ka ago), attests to its continuous presence on most of the continent (Chaline, 1972; Horáček
127 & Ložek, 1988; Jánossy, 1986; Nadachowski, 1989). In many localities on the European Plain,
128 from France to Poland, the common vole was the most abundant small mammal, alongside the
129 collared lemming (*Dicrostonyx torquatus*) and the European narrow-headed vole
130 (*Lasiopodomys anglicus*) (Royer et al., 2016; Socha, 2014). The mitochondrial DNA (mtDNA)
131 phylogeography of extant populations of the common vole has been intensively studied, with
132 the aim of reconstructing the post-glacial history of the species. The extant mtDNA diversity is
133 partitioned into six divergent lineages with parapatric distribution: Western-South (WS),
134 Western-North (WN), Italian (ITA), Balkan (B), Central (CEN), and Eastern (E) (Bužan,
135 Förster, Searle, & Kryštufek, 2010; Haynes, Jaarola, & Searle, 2003; Heckel, Burri, Fink,
136 Desmet, & Excoffier, 2005; Stojak, McDevitt, Herman, Searle, & Wójcik, 2015) (Figure 1b).
137 Most of the previous studies estimated the time to the most recent common ancestor (tMRCA)
138 of the extant common vole populations to between 65 and 50 ka ago and subsequent divergence
139 of main lineages to between 50 and 20 ka ago (García et al., 2020; Heckel et al., 2005; Stojak
140 et al., 2016). In contrast, Fink et al. (2004) and Tougaard et al. (2008) used fossil calibration to

141 suggest a much older diversification in the Middle Pleistocene. Analyses of nuclear DNA
142 revealed overall a very good correspondence with the spatial distributions of mtDNA lineages
143 (Fischer, Foll, Heckel, & Excoffier, 2014; Heckel et al., 2005) and detailed analyses of the
144 contact areas between lineages demonstrated admixture only in narrow hybrid zones (Beysard
145 & Heckel, 2014; Braaker & Heckel, 2009). However, the divergence time estimates of the
146 evolutionary lineages based on the nuclear data were generally much more recent than those
147 based on mtDNA and suggested that the diversification of common vole evolutionary lineages
148 took place during or after the LGM (Heckel et al., 2005; Lischer, Excoffier, & Heckel, 2014).

149 The present-day distribution of common vole mtDNA lineages (Figure 1b) was
150 interpreted as evidence for the survival of common vole populations during the LGM in both
151 traditional Southern glacial peninsular refugia, as well as at higher latitudes, especially in
152 Central France (WN), north of the Alpine region (CEN) and in the Carpathian area (E) (Heckel
153 et al., 2005; Stojak et al., 2015; Tougaard et al., 2008). Examination of nuclear DNA from
154 multiple European populations revealed a south-west cline of genetic diversity which was
155 interpreted as indicative of a westward expansion of the common vole at a time prior to the
156 LGM (Heckel et al., 2005), nevertheless the pre-LGM history of the species remains largely
157 unknown.

158 A detailed study of the Eastern mtDNA lineage suggested that it originated in the
159 Carpathian area and its current distribution is the result of an expansion which started after the
160 LGM with a possible bottleneck during the Younger Dryas (12.8–11.7 ka ago) (Stojak et al.,
161 2016). Recently, an ancient DNA investigation of the common vole remains from the Western
162 Carpathians has shown the presence of the Eastern lineage from the early Holocene onwards.
163 However, it also showed that from at least 18 ka ago the Central lineage was present in this
164 region suggesting a population replacement around the Pleistocene/Holocene transition (Baca
165 et al., 2020). This challenged the simple model of post-glacial recolonisation of the Eastern
166 lineage from the Carpathian refugium and suggested that the analyses based solely on extant
167 genetic diversity may not recover the full complexity of the Late Pleistocene population
168 dynamics. To refine the evolutionary history of common vole populations in Europe and to
169 compare it with the past histories of coeval cold-adapted species such as the collared lemming,
170 we generated and analysed a new mitochondrial dataset consisting of nearly 200 sequences
171 from Late Pleistocene and extant individuals.

172 **4 Material and Methods**

173 **4.1 Ancient specimens**

174 Isolated lower first molars or mandible fragments with molars classified as *M. arvalis*
175 or *Microtus sp.* based on morphology of the occlusal surface were collected from various
176 palaeontological sites across Europe (Table S1). Each tooth was photographed at the Institute
177 of Systematics and Evolution of Animals, PAS.

178 **4.2 Modern specimens**

179 DNA of modern specimens from various locations across Europe was extracted
180 previously (Table S2). A target 4.2 kb region of mtDNA spanning positions 12,000 to 16,247
181 according to the reference sequence (NC_038176; Folkertsma et al., 2018) was generated using
182 various approaches. It was either PCR amplified, sonicated, transformed into sequencing
183 libraries and sequenced or the genomic DNA was sonicated and transformed into sequencing
184 libraries. It was then either enriched for target fragment using in-solution hybridization and
185 sequenced or the target region was extracted from deep shotgun sequencing data (see Appendix
186 A1.1 and Table S2 for more details).

187 **4.3 Ancient DNA extraction, target enrichment and sequencing**

188 DNA extraction and pre-PCR library preparation steps were performed in the dedicated
189 ancient DNA laboratory at the Centre of New Technologies at the University of Warsaw. Each
190 tooth was thoroughly cleaned with ultra-pure water in a 2 ml tube and crushed with a pipette
191 tip. DNA was extracted following a silica spin column-based protocol optimized for retrieval
192 of short DNA molecules (Dabney et al., 2013). A negative control without biological material
193 was processed alongside each batch of 15 specimens. Double-stranded, double-indexed
194 sequencing libraries were produced from half of the DNA extract (20 µl) following a previously
195 established protocol (Meyer & Kircher, 2010) with minor modifications (Baca et al., 2019). For
196 some specimens that yielded low-quantity DNA additional double-indexed, single-stranded
197 sequencing libraries were prepared following the protocol proposed by Gansauge et al. (2020)
198 (see Appendix A1.3–A1.4 for more details).

199 Libraries were enriched for vole mitochondrial DNA using an in-solution hybridization
200 protocol described in Baca et al. (2019). Up to five libraries were pooled for hybridization
201 reaction. We performed two rounds of hybridization in 65°C for 22–24h each. After each round,
202 library pools were washed and amplified in triplicate for 10 to 15 cycles. Enriched library pools

203 were combined, quantified using qPCR and sequenced on Illumina NextSeq550 platform (MID
204 output, 2x75 bp kit; see Appendix A1.2–A1.5 and Tables S4–S5 for more details).

205 **4.4 Sequence processing**

206 Sequencing reads were demultiplexed using *bcl2fastq* v. 2.19 (Illumina). Overlapping
207 reads were collapsed, adaptor and quality trimmed using *AdapterRemoval* v. 2.2.2 (Schubert,
208 Lindgreen, & Orlando, 2016). Then, reads were mapped to the common vole mtDNA genome
209 using the *mem* algorithm in *bwa* v. 0.7.17 (Li & Durbin, 2010). Duplicates, short (<30 bp) and
210 low mapping quality reads ($\text{mapq} < 30$) were removed using *samtools* v. 1.9. Variants and
211 consensus sequences were called using *bcftools* v. 1.9 (Li et al., 2009). Read alignments and
212 vcf files were inspected manually using *Tablet* v. 1.17 (Milne et al., 2013). Positions with
213 coverage below three were masked with N. If a base was supported by less than 75% of reads,
214 an IUPAC symbol was inserted. *MapDamage* v. 2.08 (Jónsson, Ginolhac, Schubert, Johnson,
215 & Orlando, 2013) was used to assess the damage patterns and length distribution of DNA
216 molecules. See Appendix A1.6 for more details.

217 **4.5 Phylogenetic analyses and molecular dating of specimens**

218 We used a Bayesian approach, implemented in BEAST 1.10.4 (Suchard et al., 2018), to
219 estimate divergence times of common vole lineages and the age of specimens which were not
220 directly dated. For the phylogenetic inference we used only ancient and modern sequences with
221 at least 70% and 90% of the target mtDNA fragment (4.2 kb) recovered, respectively.
222 Sequences were aligned with MAFFT v. 7.407 (Katoh & Standley, 2013). The best substitution
223 model selected by jModelTest2 (Darriba, Taboada, Doallo, & Posada, 2012), TIM2+F+I+G4,
224 was not easily available in BEAST we therefore used the closest available one: GTR +I+G.

225 First, we used Bayesian evaluation of temporal signal (BETS) (Duchene et al., 2020) to
226 check whether there is sufficient temporal resolution within our dataset to calibrate the
227 molecular clock. We used all directly radiocarbon dated ($n = 20$) and modern ($n = 51$) specimens
228 and tested four alternative models. In two of them, we assigned real sampling times to the
229 sequences (heterochronous analysis) and used either strict clock or uncorrelated relaxed
230 lognormal clock. In the two other models, we used the same sampling time for all sequences
231 (isochronous analysis) and applied either strict clock or uncorrelated relaxed lognormal clock
232 (see Appendix A1.7 for more details). Then, we performed the leave-one-out analysis on the
233 directly radiocarbon dated specimens to check the accuracy of the age estimates produced using
234 the available calibration dataset. In this analysis we estimated the age of each directly
235 radiocarbon dated specimen using all the remaining radiocarbon dated and modern specimens

236 to calibrate the molecular clock. Next, we estimated the age of each ancient, not directly dated,
237 specimen (n = 128) in a separate BEAST run, again using all directly dated and modern
238 specimens to calibrate the molecular clock. Finally, we ran a joint analysis with all the
239 sequences. For the specimens that were not directly dated we set a lognormal prior with a mean
240 equal to the mean age estimated in the individual analysis and the range covering the 95% HPD
241 interval of the individual estimate (see Appendix A1.7, Tables S6–S9 for more details).

242 The demographic reconstructions using the Bayesian Skyline and Bayesian SkyGrid
243 methods implemented in BEAST 1.10.4 were performed for the WN lineage specimens from
244 Spain (n=58). In the case of other localities either the number of available sequences was low
245 or the assumption of population continuity (i.e., lack of lineage turnovers) were violated (see
246 Appendix A1.8 for details).

247 **4.6 Radiocarbon dating**

248 Selected vole mandibles were pretreated for radiocarbon dating in the Department of
249 Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA,
250 Leipzig, Germany) following the protocol for <100 mg bone samples described in Fewlass et
251 al. (2019). The quality of the collagen extracts was assessed based on the collagen yield as a
252 percentage of the original bone weight (minimum requirement 1%). The elemental and isotopic
253 ratios of the extracts (~0.5 mg) were measured at the MPI-EVA on a Thermo Finnigan Flash
254 elemental analyser coupled to a Thermo Delta plus XP isotope ratio mass spectrometer (EA-
255 IRMS). Where sufficient collagen was extracted, collagen was graphitised using the automated
256 graphitisation equipment (AGE) (Wacker, Němec, & Bourquin, 2010) in the Lab of Ion Beam
257 Physics at ETH-Zurich (Switzerland) and dated on a MIni CARbon DAting System
258 (MICADAS) accelerator mass spectrometer (AMS) (Wacker, Bonani, et al., 2010). Where the
259 extracted collagen yield was insufficient for graphitization, it was combusted to CO₂ and
260 measured directly using the gas interface system coupled to the gas ion source of the MICADAS
261 (Wacker et al., 2013) following the protocol described in Fewlass et al. (2019) (see Appendix
262 A1.9 for more details).

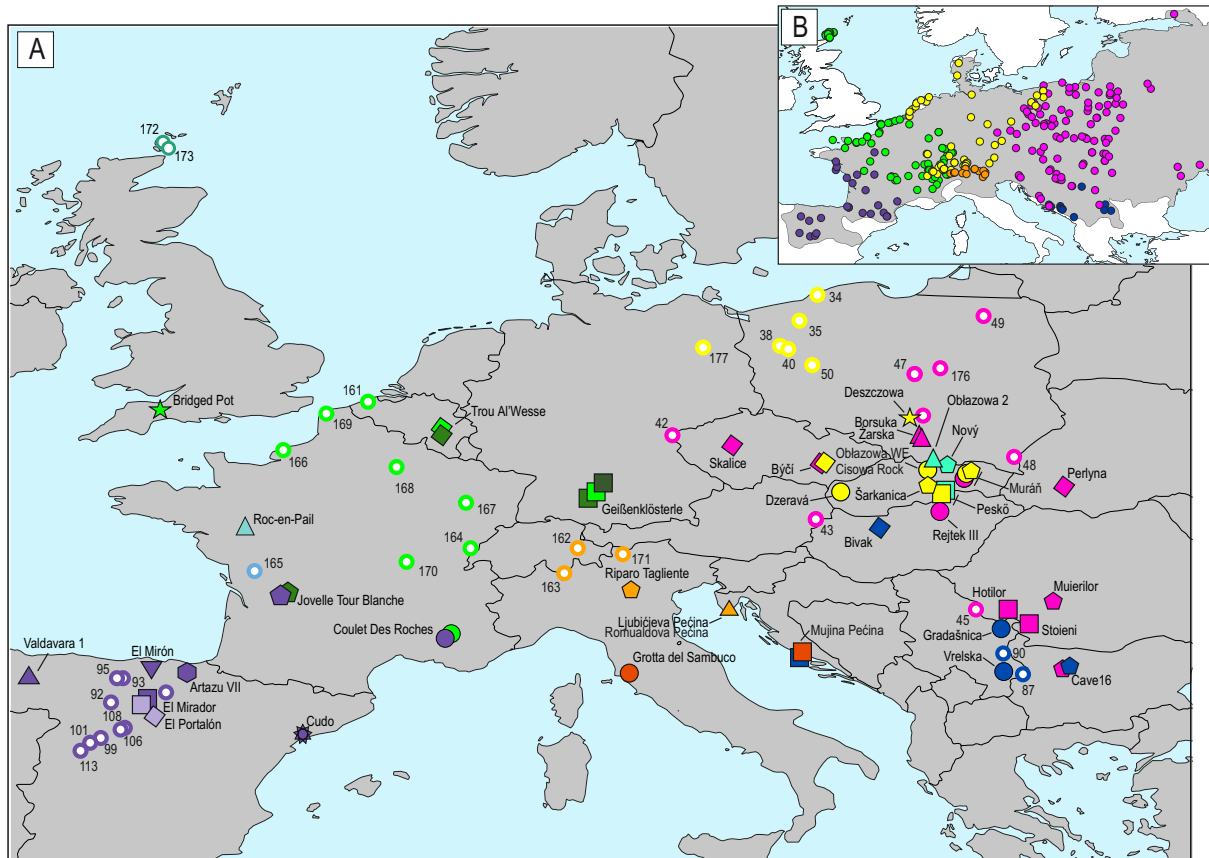
263 To improve stratigraphic information available for the sites from which the analysed
264 specimens originated we also obtained radiocarbon dates from five palaeontological sites
265 (Appendix A1.10, Table S10). Radiocarbon dates were calibrated in OxCal v4.4 (Bronk
266 Ramsey, 2009) using the IntCal20 (Reimer et al., 2020) calibration curve.

267

268 5 Results

269 We generated a dataset of 4.2 kb long mtDNA sequences from 199 ancient and modern
270 common vole specimens (148 ancient and 51 modern). Sequences of 82 ancient specimens are
271 reported for the first time and either 4.2 kb or 1kb fragments of mtDNA of the remaining 66
272 specimens were reported previously (Baca et al., 2020; Baca et al., 2021; Lemanik et al., 2020)
273 (Table S1). All 82 newly reported specimens yielded short inserts and elevated level of cytosine
274 deamination at the terminal nucleotides characteristic for ancient DNA (Table S1). Ancient
275 specimens which yielded mtDNA sequences came from 40 sites scattered across Europe and
276 covered the period of the last ca. 60 thousand years (Table S1, Figure 1a). MtDNA cytochrome
277 *b* sequences of most modern specimens were reported previously (García et al., 2020; Stojak et
278 al., 2016) and here only the longer mtDNA fragment was generated to increase the phylogenetic
279 resolution. Direct radiocarbon dating was undertaken for 12 vole mandibles and 10 yielded
280 collagen of sufficient quality for AMS dating (Table S3). The carbon to nitrogen ratio (C:N) of
281 one specimen from Geißenklösterle (MI935) was at the limit (3.6) of accepted values for well-
282 preserved collagen (2.9-3.6; Van Klinken, 1999) and yielded a relatively recent date with
283 respect to the latest Electron Spin Resonance dating of the cave sediments (Richard et al., 2019)
284 indicating that contamination with modern carbon, and therefore under-estimation of the true
285 age, is likely. We therefore discarded this radiocarbon date and estimated the age of this
286 specimen using the molecular approach. All other direct dates are regarded as reliable based on
287 their chemical indicators (collagen yield, stable isotopic and elemental values; Table S3). As a
288 result, we used sequences of 20 directly radiocarbon dated and 51 extant specimens to calibrate
289 the molecular clock. The analysis of temporal signal (BETS) showed that the dated specimens
290 included in our dataset are suitable for calibration of the molecular clock. The heterochronous
291 model, with a strict clock and correct sampling times assigned to specimens, was strongly
292 supported over all other models ($2\ln BF > 9$) (Table S4). The leave-one-out analysis revealed
293 that the dated dataset enables relatively accurate estimation of specimen ages, although in the
294 case of three specimens (MI074, MI1337 and MI1355) the 95% highest posterior density
295 intervals (95% HPD) of estimated ages did not overlap within 2-sigma ranges of the
296 radiocarbon dates (Figure S1; Appendix A). In addition, the estimated ages of most specimens
297 agreed with their stratigraphic position, providing evidence for accuracy of this approach (Table
298 S1).

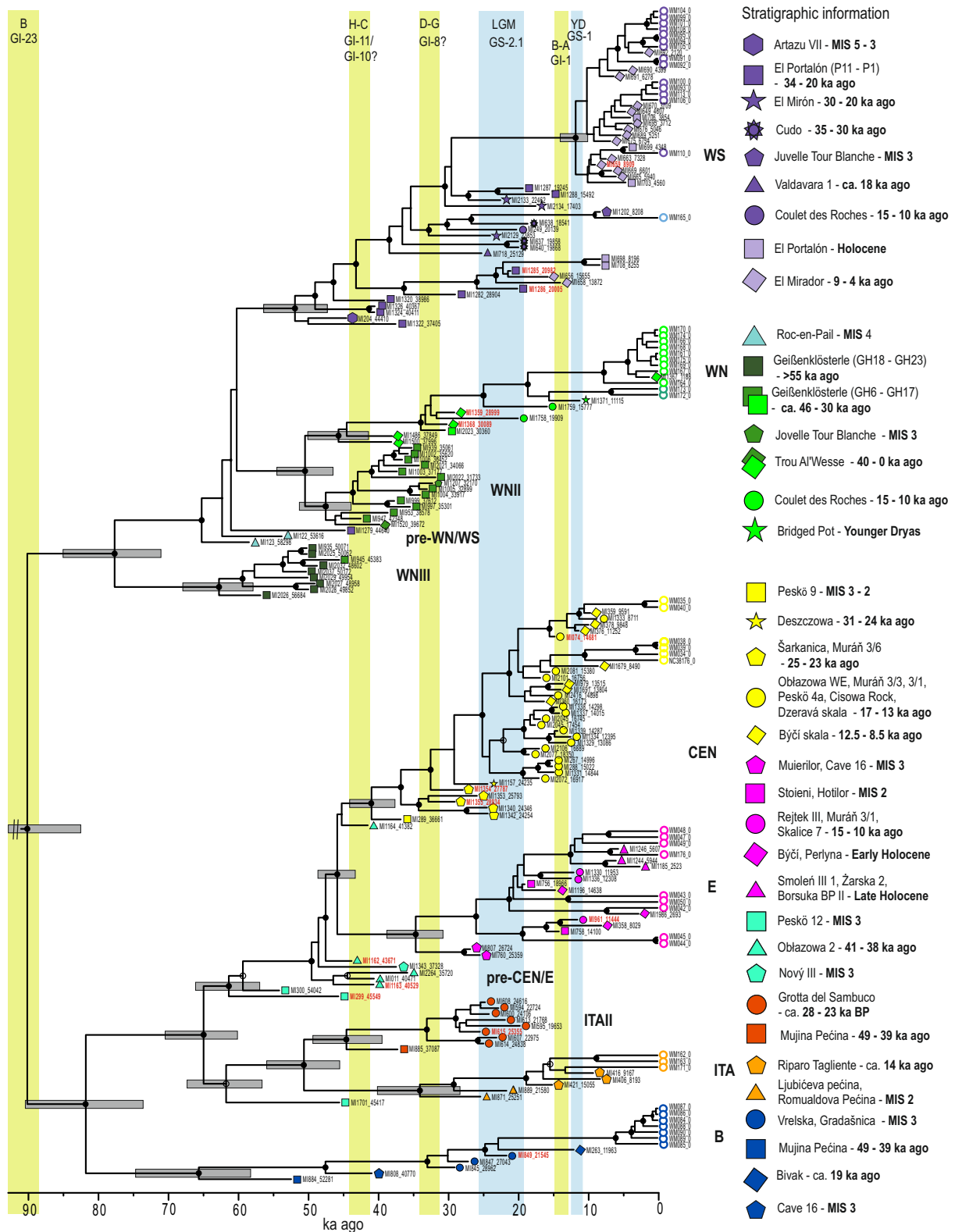
299
300



301
302 Figure 1. Sampling localities of modern and ancient common voles across Europe (a). Filled symbols
303 represent paleontological sites, while unfilled circles denote localities of modern specimens. Numbers
304 near modern localities correspond to specimen numbers (WM...) in Figure 2 and Table S2. Symbols
305 and names are consistent with those in Figure 2 and are coloured according to the vole mtDNA lineage
306 found at the site. If multiple mtDNA lineages were found at the site, two or more symbols are presented.
307 Distribution of the main mtDNA lineages in extant populations (b). The grey area depicts the current
308 range of common vole in Europe. The coloured circles represent sampling localities of the common vole
309 and mtDNA lineages compiled from previous studies: pink – Eastern (E); yellow – Central (CEN);
310 orange – Italian (ITA); green – Western-North (WN); violet – Western-South (WS); navy blue – Balkan
311 (B).

312 5.1 Diversification of common vole mtDNA lineages

313 The maximum clade credibility tree obtained in BEAST 1.10.4 (Figure 2), recovered,
314 with high support, all six mtDNA lineages characterised previously in analyses of the mtDNA
315 cytochrome *b* of modern individuals (Bužan et al., 2010; Haynes et al., 2003; Heckel et al.,
316 2005). In addition, we identified three lineages which were only present in the Late Pleistocene
317 specimens. These lineages were named by their geographic provenance and phylogenetic
318 position: WNII and WNIII in Western Europe (Germany and France) and ITAII in Italy and
319 Croatia (Figure 1a and 2).



320

321 Figure 2. Maximum clade credibility tree of common vole mtDNA obtained in BEAST 1.10.4 and
 322 calibrated with radiocarbon dated specimens. Black dots indicate nodes with posterior probability above
 323 0.95 and grey bars show the 95% HPD intervals of node ages. The tips are annotated with sample names,
 324 colour symbols (consistent with those in Figure 1) and, medians of age estimates (black font) or medians
 325 of calibrated radiocarbon dates (red font). The empty circles represent modern specimens. The legend

326 on the right side of the figure provides information on stratigraphy and dating of the source sites and
327 layers. The green and blue strips indicate the main interstadials and stadials: B – Brørup; H–C –
328 Hengelo–Charbon; D–G Denekamp–Grand Bois; LGM – Last Glacial Maximum, B–A – Bølling–
329 Allerød; YD – Younger Dryas.

330
331 The tMRCA of all European common voles was estimated to 90 ka ago (95% HPD: 98–
332 83 ka ago). Divergence time estimates of the subsequent lineages ranged between 82 ka ago
333 (95% HPD: 91–75 ka ago) for the split between B and ITA/C/E, through 77 ka ago (95% HPD:
334 84–70 ka ago) for the split between WNIII and WNII/WN/WS to ca. 50–45 ka ago for the splits
335 between a number of lineages: ITA and ITAII; CEN and E; WNII and WN (Figure 2).

336 **5.2 Temporal population structure and dynamics of the common vole**

337 *5.2.1 Western and Southwestern Europe*

338 The oldest specimens from Western Europe were classified as belonging to the WNIII
339 lineage which was the sister to the WS and WN/WNII lineages. The WNIII lineage contains
340 mainly individuals from the lowermost layers of Geißenklösterle (GH23-18). The age of these
341 individuals was estimated to between 56 and 45 ka (Figure 1a, 2, Table S1). Three haplotypes
342 of similar age as the latter (57–44 ka ago) from Western France (Roc-en-Pail; MI122, MI123)
343 and Northern Spain (El Portalon P9; MI1279) are located at the base of the WS, WNII and WN
344 lineages (pre-WN/WS; Figure 1a, Table S1). Around 42 ka ago the WS lineage appeared in
345 Northern Spain and the WNII lineage appeared in France, Belgium and Germany. The latter,
346 composed of specimens from the younger layers of Geißenklösterle, Trou Al’Wesse and
347 Jovelle, disappeared around 32 ka ago. The age of the oldest specimens from the WN lineage,
348 which come from the Trou Al’Wesse, was estimated to 37 ka ago. This lineage was found
349 among Late Pleistocene specimens from Western Germany, Belgium, France, and the UK.

350 The record from Spain suggests population continuity throughout the last 40 ka,
351 although nearly all modern and Holocene specimens coalesce around 11.5 ka ago (Figure 2),
352 which suggests significant reduction of population size around the Pleistocene/Holocene
353 transition. This was further confirmed by the Bayesian demographic analysis, which suggest
354 about five-fold reduction of female effective population size around the Pleistocene/Holocene
355 transition with the minimum around the Early Holocene (11.7–9 ka ago), followed by a slight
356 recovery around the Middle Holocene (9–6 ka ago) (Appendix A1.8, Figures S2 and S3). The
357 WS lineage was also detected in southern France at Jovelle and Coulet des Roches during MIS
358 2 (29–14 ka ago).

359 5.2.2 *Central and Southeastern Europe*

360 The oldest specimens in Central and Southeastern Europe come from Obłazowa 2, Nový
361 and layer 12 of Peskö, all from the Western Carpathians. Their ages were estimated to between
362 55 and 35 ka ago and they hold a basal position with respect to Central and Eastern lineages
363 (pre-CEN/E). The two oldest specimens from Peskö (MI299, MI300) were noticeably more
364 divergent than the others. Starting from 35 ka ago we found the CEN lineage in the Western
365 Carpathians as well as to their north, and the most recent specimens belonging to this lineage
366 were dated to the Early Holocene. The earliest specimens assigned to the Eastern lineage were
367 estimated to ca. 27–25 ka ago and were found in Central Romania (Muierilor Cave; MI760)
368 and Northwestern Bulgaria (Cave 16; MI807). The more recent specimens from this lineage
369 seem to represent a north and northwestern expansion of this population, with the first
370 appearance in the Ukrainian Carpathians in Perlyna at around 14 ka ago and in the Western
371 Carpathians around 12 ka ago (Rejtek III, Muráň 3/1).

372 The Balkan lineage occupied the same area as its current distribution starting from at
373 least 50 ka ago (Mujina pećina). A single specimen from Bivak Cave in the Northern Hungary
374 suggests that prior to the Holocene, its range extended further to the northeast.

375 The ITA and ITAII lineages were detected on the Italian and Balkan Peninsulas. ITAII
376 specimens were found in central Italy (Grotta del Sambuco) and in middle Dalmatia (Mujina
377 pećina) with ages estimated to between 36.5 and 18.5 ka ago. These were generally older than
378 specimens from the ITA lineage, dated to 22.6–0 ka ago and found in northern Italy (Riparo
379 Tagliente) and Istria (Ljubićeva pećina). The single individual from layer 12 of Peskö cave
380 (Western Carpathians; MI1701), directly radiocarbon dated to 45 ka cal BP, was located at the
381 base of the ITA lineages.

382 **6 Discussion**

383 The mitochondrial diversity of the common vole has been studied in detail to reconstruct
384 the evolutionary history of species and elucidate its reactions to climate change. However,
385 inferences from modern mitochondrial diversity are limited, since the signal of the population
386 history is usually erased by the most recent reduction of effective population size (Mourier et
387 al., 2012). Ancient DNA enables direct observation of changes in genetic diversity in response
388 to climate or environmental changes by sampling populations prior and after such changes.
389 Another great advantage of ancient DNA is that directly dated specimens may be used to
390 estimate substitution rates and to calibrate the molecular clock providing robust estimates of
391 divergence times without the need of external calibration. In this study, we make use of these

392 advantages and reconstructed the evolutionary history of the common vole at much greater
393 temporal depth providing new insights into its paleoecology.

394 **6.1 The effects of climatic changes on diversification of common vole lineages**

395 The estimated tMRCA of mtDNA for the European common vole (90 ka ago; 95%
396 HPD: 98–83 ka ago) is substantially older than some previous estimates (García et al., 2020;
397 Heckel et al., 2005; Stojak et al., 2015). It was similar to the recent estimates for the initial
398 diversification of mtDNA lineages of the cold-adapted collared lemmings (100 ka ago, 95%
399 HPD: 109–92 ka ago; E. Lord, personal communication). It was suggested that this may be an
400 effect of a bottleneck during the Eemian Interglacial (MIS5e). It may also be related to the
401 Brørup Interstadial (MIS 5c; ~GS-23; ca. 104–88 ka). Vegetation during the Brørup Interstadial
402 was characterised by temperate deciduous forests in Western Europe and boreal forests more
403 to the north and east (Guiter et al., 2003; Helmens, 2014), providing unfavourable habitats for
404 the cold-adapted collared lemming as well as for the common vole, both of which rely on
405 various types of open habitat.

406 The initial divergence of the common vole lineages may have been the result of survival
407 in two distinct refugial areas in the Alpine and Carpathian regions causing a partition in the
408 mitochondrial diversity of the species in with two geographical areas. Western Europe,
409 including the territories of present-day Spain, France, Switzerland, and parts of Germany, was
410 occupied by the WNIII, WNII, WN and WS lineages. Meanwhile, Central and South-eastern
411 Europe, including territories to the East and to the South of Germany, was occupied by the
412 CEN, ITAII, ITA, B and E lineages. This geographic partitioning was maintained for at least
413 45 ka (Figure 3) and probably contributed to patterns of restricted gene flow and partial
414 reproductive isolation of the present-day populations of Western-North and Central lineages
415 (Beysard & Heckel, 2014).

416 The divergence of the main common vole mtDNA lineages estimated in this study was
417 also older than previously suggested. All mtDNA lineages present in extant European
418 populations (WS, WN, ITA, B, CEN, E) diverged prior to 40 ka ago, and the earliest specimens
419 belonging to each of those lineages in our dataset pre-date 25 ka ago. This suggests, contrary
420 to previous hypotheses (García et al., 2020; Heckel et al., 2005; Stojak et al., 2015), that climate
421 deterioration during the LGM did not play the major role in the initial divergence of the main
422 extant mtDNA lineages. However, the population decline and increased isolation during the
423 period of the most inhospitable climatic conditions may have reinforced the previously existing
424 divergence.

425 Most of the observed divergence events occurred between ca. 60 and 45 ka ago. They
426 may be related to a long interstadial period identified in the palynological records across
427 Europe: the Moershoofd Interstadial Complex in the Netherlands, the Pile Interstadial Complex
428 at La Grande Pile pollen sequence (eastern France) and the Oerel and Glinde Interstadials at
429 the Oerel pollen sequence (Northern Germany) dated to ca. 58–48 ka uncal BP and is usually
430 correlated with Greenland Interstadials (GI) 16 and 14 (ca. 58–56.5 and 54–49.5 ka ago)
431 (Helmens et al., 2014).

432 **6.2 Phylogeography and demographic history of the common vole**

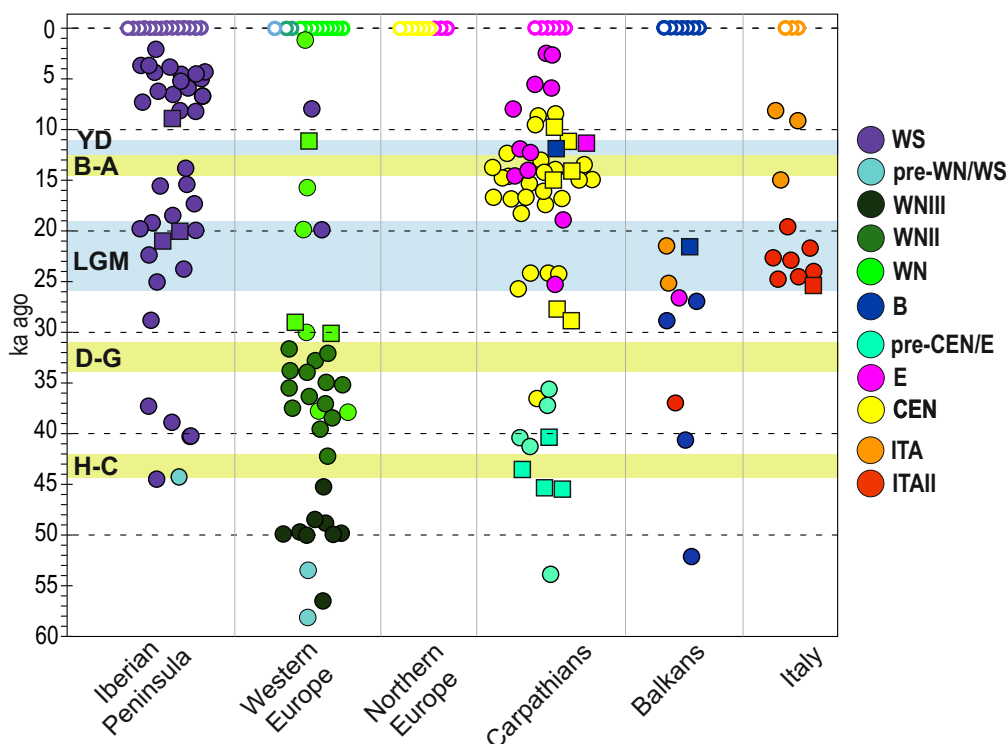
433 *6.2.1 Western Europe and Iberian Peninsula*

434 In Western Europe, we documented two consecutive mtDNA lineage turnovers at the
435 end of MIS 3 (WNIII/WNII and WNII/WN). The first one took place around 45 ka ago (Figure
436 3). In our dataset there are few specimens of similar age from other parts of Europe; however,
437 the divergent position of three specimens from the Western Carpathians (MI299, MI300, and
438 MI1701; Figure 2) dated to 53 and 45 ka ago suggests that synchronous lineage turnovers may
439 also have taken place in other parts of Europe. The second one occurred around 32 ka ago and
440 appears to have been restricted to Western Europe (Figure 3). The oxygen isotope record from
441 Greenland ice cores shows that the period between 45 and 29 ka ago was characterised by
442 multiple short-term climatic oscillations (Rasmussen et al., 2014). Palynological records
443 revealed two main interstadials which stand out during this period and have been identified in
444 most of sediment sequences across Europe (Helmens, 2014). The older one, named Hengelo
445 from its type locality in the Netherlands or Charbon in the Grande Pile sequence from eastern
446 France, took place around 43–41 ka cal BP (38–36 ka uncal BP) (Helmens, 2014;
447 Vandenberghe & van der Plicht, 2016), whilst the younger one (Denekamp – Grand Bois)
448 occurred around 34–33 ka cal BP (31–29 ka uncal BP) (Guiter et al., 2003; Helmens, 2014),
449 approximately at the same time as the recorded mtDNA lineage turnovers. The exact correlation
450 of palynological data with Greenland ice-core records is problematic due to potential offsets
451 and the wide error ranges involved. Hengelo–Charbon is usually associated with GI-11 (ca.
452 43.3–42.2 ka ago) or GI-10 (ca. 41.5–40.8 ka ago), although at times to the earlier GI-12 (ca.
453 46.8–44.2 ka ago) as the longest and most pronounced interstadial around the end of MIS 3,
454 and Denekamp–Grand Bois with GI-8 (38.2–36.6 ka ago) (Helmens, 2014). Both interstadials
455 were characterised by the emergence of *Betula* and *Pinus* forests in Western Europe, and of
456 *Betula*, *Larix* and, *Pinus* forests in Central Europe, although it is assumed that the landscape
457 remained relatively open as the duration of these interstadials was too short for the development

458 of full forest cover (Guiter et al., 2003; Helmens, 2014). It was shown, however, that even
 459 partitioned landscapes limit dispersal and promote local extinctions of common vole
 460 populations (Delattre, Giraudoux, Baudry, Quere, & Fichet-Calvet, 1996), thus fragmentation
 461 of primarily open, stadial habitats into patchy and mosaic interstadial landscapes might have
 462 led to large scale decreases of population density and local or regional extinctions. A similar
 463 explanation for common vole population dynamics was previously suggested by Tougard et al.
 464 (2008). Martínková et al. (2013) showed partial replacement of mtDNA within the WN lineage
 465 in Late Holocene common vole populations from northern France and Belgium, and also
 466 suggested that the main factor driving this process was likely landscape reorganization.

467 The record from Northern Spain, occupied by the WS lineage, suggests population
 468 continuity throughout the last ca. 45 ka. The demographic reconstruction showed a drastic
 469 reduction of the effective population size around the Pleistocene/Holocene transition (Figures
 470 S2 and S3). This is in agreement with previous findings based on mtDNA cytochrome *b* and a
 471 smaller sample size (Baca et al., 2020). Palynological records from the region from which both
 472 modern and ancient specimens originated suggest that during the Bølling–Allerød interstadial
 473 (14.7–12.8 ka ago) a high proportion of open landscapes persisted until the expansion of
 474 deciduous woodlands started in the Early Holocene (Carrión et al., 2010).

475



476

477 Figure 3. Temporal distribution of the mtDNA lineages divided by the geographical regions. Western
 478 Europe here includes France, southern Germany, Belgium, and the United Kingdom; the Northern

479 Europe includes northern Germany and Poland (there were no ancient specimens from this region), the
480 Carpathians include southern Poland, Czechia, Slovakia, Hungary, Ukraine and Romania and the
481 Balkans include Bulgaria, Serbia and Croatia; Circles denote medians of age estimated using molecular
482 approach while squares denote medians of calibrated radiocarbon ages. The green and blue strips
483 indicate the main interstadials and stadials as in Figure 2.

484

485 6.2.2 *Central and South-eastern Europe*

486 The survival of common vole populations throughout the LGM at high latitudes,
487 including the Carpathians, has been previously suggested based on multiple lines of evidence.
488 The fossil record suggests the continuous presence of the common vole in the Pannonian Basin
489 (Pazonyi, 2004) and even north of the Carpathians (Sommer & Nadachowski, 2006), although
490 these findings were based only on the stratigraphic position of the specimens, rather than direct
491 dates. The Carpathians was also suggested as a northern refugium based on the distribution of
492 the Eastern mtDNA haplogroup in modern populations (Stojak et al., 2016, 2015) and
493 ecological niche modelling (Stojak, Borowik, Górny, McDevitt, & Wójcik, 2019). Our data
494 support a northern survival of the common vole throughout the LGM. In the Western
495 Carpathians, the northernmost part of this mountain range, we found all individuals with ages
496 estimated, or directly dated, to between 36 and 10 ka ago to belong to the CEN mtDNA lineage.
497 Two specimens from Šarkanica yielded pre-LGM, direct radiocarbon dates (28.9 and 27.8 ka
498 cal BP; Table S3) while the age of a further four specimens was estimated to between 25.9 and
499 24.2 ka ago (Figure 2, Table S1). Recently, Lemanik et al. (2020) found a signal of rapid growth
500 of the effective female population size (N_{ef}) of the common vole population from the Western
501 Carpathians starting ca. 21 ka cal BP that continued until ca. 15 ka cal BP. Together, these
502 results are consistent with population continuity through the LGM, although accompanied by a
503 significant reduction of population size.

504 Most of Central, Eastern, and Southeastern Europe is at present occupied by the Eastern
505 lineage. It was suggested, based on both modern and ancient DNA, that the expansion of this
506 lineage started around the Pleistocene/Holocene transition (Baca et al., 2020; Stojak et al.,
507 2016). Our new data allow us to refine the history of this lineage. Prior to the maximal extension
508 of the Scandinavian Ice Sheet (23–19 ka ago), we detected the Eastern lineage only in
509 Southwestern Romania and Bulgaria. The presence of younger specimens bearing this mtDNA
510 lineage in the same area, dated to 18.8 and 14 ka ago, suggests that this lineage may have
511 survived the LGM in this part of the Carpathians. The expansion of the Eastern lineage from

512 the South-eastern Carpathian area is also consistent with the finding of the highest genetic
513 diversity in the extant common vole populations in this area (Stojak et al., 2016). The
514 colonization of vast territories of Central and Eastern Europe, and replacement of Central and
515 Balkan mtDNA lineages, took place somewhat later during the Bølling–Allerød or the Younger
516 Dryas. In contrast to previous suggestions (Baca et al., 2020), the similar ages of Central and
517 Eastern lineage individuals from the Muráň 3 and Býččí sites estimated here, suggest that both
518 lineages may have coexisted in the area for some time (Figure 2, Table S1), prior to the final
519 extirpation of the Central lineage in the Early Holocene. Although, the limited resolution of
520 molecular age estimation and lack of possibility to track admixture with mtDNA does not allow
521 for fine scale reconstruction of this process.

522 The record from the Italian peninsula suggests a replacement of the ITAII lineage with
523 the ITA lineage which survived in Northern Italy and Switzerland until the present day.
524 Although the available data come from sites with a limited temporal span (Appendix A), our
525 dated phylogeny suggests that the extirpation of the ITAII lineage took place at some point after
526 the LGM and the expansion of the ITA lineage occurred no later than the Bølling–Allerød
527 warming. This lineage turnover may be reflected in the significant decrease in common vole
528 remains for a short period after the LGM which was observed in the southern Italian peninsula
529 (Berto, López-García, & Luzi, 2019). This is more consistent with repeated southward
530 expansions of subsequent common vole populations rather than with their continuous presence
531 in the region.

532 6.2.3 *Comparison with other European Late Pleistocene species*

533 The common vole, along with the collared lemming and narrow-headed voles, are
534 amongst the most numerous small mammals found in the assemblages from the last glacial
535 period in Europe and are assumed to have coexisted during much of this period. The explanation
536 for this paradox, in which a temperate species coexisted with cold-adapted ones, is the high
537 tolerance of common vole to low temperatures (Tougaard et al., 2008). Our study corroborates
538 the continuous presence of the species at middle and high latitudes of Europe throughout the
539 last at least 60 ka (Figure 3), although the evolutionary history of the common vole differed in
540 some respects from other species inhabiting Europe during the Late Pleistocene.

541 The long-term regional continuity of the main lineages of common vole with limited evidence
542 for migrations suggested by our mtDNA analysis is in contrast with the Late Pleistocene
543 evolutionary histories of megafaunal species such as cave bears and mammoths, both of which
544 showed evidence for long-distance migrations and large-scale population replacements

545 (Fellows Yates et al., 2017; Gretzinger et al., 2019). The collared lemming also showed a
546 distinct pattern of mtDNA diversity consistent with multiple continent-wide population
547 replacements (Palkopoulou et al., 2016). This was likely related to differences in mobility of
548 the two species, with collared lemmings being more capable of long-distance dispersals than
549 the common vole (Ehrich et al., 2001). On the other hand, available data suggest that despite
550 this apparently different, species-specific history, common vole populations were affected by
551 the same climatic and environmental changes as the cold adapted taxa. The extirpation of the
552 WNIII lineage in Western Europe, and potentially of other common vole populations across
553 Europe, took place around the same time as the continent-wide disappearance of collared
554 lemming populations represented by their mtDNA lineages 1 and 2 (Palkopoulou et al., 2016).
555 Similarly, the disappearance of the WNII lineage of the common voles around 32 ka ago is at
556 approximately the same time as the population replacement of cave bears recorded in the Ach
557 Valley, Germany (Münzel et al., 2011), and of woolly mammoth populations across the whole
558 of Europe (Fellows Yates et al., 2017; Palkopoulou et al., 2013). Around 32 ka ago a new
559 population of collared lemming (mtDNA lineage 3) appeared in Europe after a potential short-
560 term extirpation (Palkopoulou et al., 2016). It was suggested that the main driver affecting
561 mammalian species throughout the last glaciation were the abrupt warmings occurring at the
562 onset of the Greenland Interstadials (Cooper et al., 2015). Nevertheless, whether it was the
563 abruptness of the climatic changes, or the subsequent emergence of interstadial environments
564 is unclear, although the high environmental instability in the period between 45 and 29 ka ago
565 appears to have influenced a whole spectrum of differently adapted species including the
566 common vole.

567 The other period that seems to have had a large impact on common vole populations
568 was the Pleistocene/Holocene transition. The climatic warming and the emergence of forests
569 during the Bølling–Allerød interstadial (14.7–12.8 ka ago) and in the Early Holocene (11.7–9
570 ka ago) are considered a main causes of extirpation of many cold-adapted species from Europe
571 including the collared lemming and narrow-headed vole (Berto, Szymanek, et al., 2021; Royer
572 et al., 2016). Our data confirmed previous findings indicating a substantial population decline
573 of the common vole in Northern Spain, and mtDNA lineage replacement in the Western
574 Carpathians (Baca et al., 2020). In addition, we identified a potential lineage turnover on the
575 Italian peninsula. This, together with a probable extirpation of the common vole from the
576 British Isles in the Early Holocene (Baca et al., 2020), suggests a continent-wide impact of
577 interstadial environments on common vole populations.

578 **7 Conclusions**

579 Our study shows that the initial diversification of Last Glacial and extant common vole
580 took place around 90 ka ago, during the Brørup interstadial (MIS 5c, GI-23). The divergence
581 of mtDNA lineages present in extant common vole populations, as well as the first appearance
582 of specimens belonging to those lineages, occurred earlier than previously estimated, mostly
583 during the MIS 3 (57–29 ka ago). At high latitudes of Europe, we detected lineage turnovers
584 whose dating suggest that they were likely caused by the fragmentation of primary habitats
585 through reforestation during the interstadials occurring between 45 and 29 ka ago. In
586 comparison, the climate deterioration during the LGM appears to have had a milder effect on
587 common vole populations. More recent demographic changes and lineage turnovers like those
588 recorded in Spain, the Western Carpathians, and Italy took place after the LGM or around the
589 Pleistocene/Holocene transition and were also likely related to climatic warming. Altogether,
590 this suggests that during the last glacial period, the evolutionary history of the common vole
591 was distinct from typical cold-adapted species associated with steppe-tundra environments,
592 although they responded to similar climatic and environmental changes. However, in contrast
593 to the collared lemming and narrow-headed vole, the common vole did not become extinct in
594 Europe at the end of the Pleistocene, possibly due to higher ecological plasticity, and eventually
595 expanded during the mid-Holocene, taking advantage of secondary habitats such as agricultural
596 fields. Overall, this suggests that habitat availability, rather than climatic variables, is the
597 primary factor affecting common vole populations.

598 **8 Competing interests**

599 We have no competing interests.

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838 **10 Data Availability**

839 The consensus mtDNA sequences generated in this study have been deposited in
840 GenBank under accession numbers OL588336 - OL588524. The alignment used for the
841 reconstruction of phylogeny have been deposited in Dryad (doi:10.5061/dryad.4j0zpc8d9).
842 Mitochondrial alignments generated in this study have been deposited in the European
843 Nucleotide Archive under project number PRJEB53474.

844

845 **11 Author Contributions**

846 MB, DP and AN designed research; MB, DP, AL, HF and XW performed research; MB
847 analysed the data and prepared figures; SBC, NC, GCB, ED, JTG, GH, IH, MVK, LL, JMLG,
848 EL, ZM, JML, XM, AP, VP, TH, SER, BR, AR, JRS. JS, ST and JMW contributed samples;
849 MB and AN wrote the paper with the input from all co-authors.