Supplementary Material to

**Ancient DNA of narrow-headed vole reveal common features of the Late Pleistocene population dynamics in cold-adapted small mammals**

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# Supporting Methods and Results

## Ancient DNA extraction

DNA extraction and the pre-PCR library preparation steps were performed in the dedicated ancient DNA laboratory at the LPCG. All teeth were thoroughly cleaned with ultra-pure water in a 2 ml tube, crushed with a pipette tip and incubated overnight in 1 ml of extraction buffer (0.5 M EDTA pH = 8.0; 0.5% N-laurylsarcosine; 0.1 mg Proteinase K) at 38°C with agitation. After the incubation, one part of the extraction buffer was combined with 13 parts of binding buffer (5 M guanidine hydrochloride and 40% isopropanol) and eluted through a MinElute silica column (Qiagen, Hilden, Germany). The silica suspension was washed twice with 750 µl of PE buffer (80% ethanol, 10 mM Tris-HCl pH 7.5), dried and eluted twice with 30 µl of pre-warmed EB buffer (10 mM Tris-Cl, pH 8.5).

## Double-stranded library preparation

The double-indexed, double-stranded sequencing libraries were constructed following the protocol of Meyer and Kircher (2010) with minor modifications described by Baca et al. (2019) using 20 µl of DNA extract as input. The blunt-end repair was performed in a 30 µl reaction containing 1× buffer Tango, 15 U T4 polynucleotide kinase (Thermo), 3U T4 DNA polymerase, 100 µDNTPs, and 1 mM ATP. The reaction was incubated for 15 min at 25°C, followed by 5 min at 12°C and 20 min at 95°C to inactivate the enzymes. An adapter ligation step was performed by adding 10 µl of the adapter ligation mix directly to the blunt-end repair reaction resulting in a final reaction volume of 40 µl containing 1× T4 DNA ligase buffer, 5% PEG-4000, 5 U T4 DNA ligase (Thermo Scientific) and 1 µM of the P5 and P7 adapters. The reaction was incubated for 30 min at 22°C and purified using magnetic beads. Adapter fill-in was performed by adding 20 µl of purified ligation product to 15 µl of the reaction master mix, resulting in a 35 µl reaction containing 9.6 U of BST polymerase (New England Biolabs, Ipswich, MA, USA), 1× Thermopol buffer and 0.25 µM of the dNTPs. The reactions were incubated in a thermocycler for 20 min at 37°C, followed by heat inactivation at 80°C for 20 min. The DNA libraries were amplified in three replicates in 25 µl reaction volumes containing 10 µl of adapter-ligated DNA, 1× AmpliTaq Gold 360 Master Mix (Thermo Scientific) and 0.2 µM of the P5 and P7 indexing primers under the following conditions: 95°C for 12 min, 19 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1 min and 72°C for 10 min. Each indexing primer contained a 7-bp long index. The amplification replicates were combined and purified using magnetic beads. The libraries were visualised by 2% agarose gel electrophoresis and quantified with the Denoxiv spectrophotometer.

## Single-stranded library preparation

We prepared single-stranded libraries for five specimens with poorly preserved DNA in which the enriched double-stranded libraries yielded very few target DNA molecules. Twenty microliters of DNA extract were combined with 9 µl of water and 1 µl of USER enzyme (New England Biolabs) and incubated for 1 h at 37°C. Further steps strictly followed the protocol outlined by Gansauge et al. (2020). The appropriate number of PCR cycles was determined with qPCR using the Illumina Library Quantification kit before indexing (KAPA). Indexing PCR was performed in duplicate using AccuPrime™ *Pfx* DNA Polymerase (Thermo Scientific). Amplified libraries were combined, purified using magnetic beads and subjected to the target enrichment procedure (Section 1.4).

## Target enrichment of mtDNA

Target enrichment was performed to enrich the libraries with vole mtDNA. Hybridisation bait was produced using the modern DNA of the following vole species: common vole (*Microtus arvalis*), field vole (*M. agrestis*), root vole (*Alexandromys oeconomus*), narrow-headed vole (*Stenocranius gregalis*) and bank vole (*Clethrionomys glareolus*). Total genomic DNA was extracted from tissue fragments using the Syngen Tissue DNA Mini Kit The mitogenomes were amplified in four overlapping fragments using PrimeSTAR GXL DNA Polymerase (Takara Bio, Shiga, Japan).

Table S1. Primers used to generate the mitogenomes from various vole species

|  |  |  |
| --- | --- | --- |
| Primer ID | Sequence | Product length |
| MICMT01F | TGCAAGCATCCCATAAACAA | 3.8 kb |
| MICMT01R | ATGGGCCCGATAGCTTTATT |
| MICMT02F | CAAAATTCTCCGTGCTACCC | 4.4 kb |
| MICMT02R | TTGTGTGGTTGGGGTAAATG |
| MICMT03F | CGCCTCTTTCATTACCCCTA | 4.2 kb |
| MICMT03R | TCYCAGCCGATGAAGAGTTG |
| MICMT04F | ACCCHAACCTAAACCGATTC | 4.5 kb |
| MICMT04R | ATAAGGCCAGGACCAAACCT |

Each fragment was amplified separately. The amplification reaction was performed in 50 µl and comprised of 20–50 ng of genomic DNA, 1× PrimeSTAR GXL Buffer, dNTPs (200 µM each), 0.2 µM primers and 2.5 U PrimeSTAR GXL DNA Polymerase. The PCR conditions were 30 cycles at 98°C for 10 s, 55°C for 15 s and 68°C for 30 to 50 s depending on the target length.

The PCR products were mixed in equimolar ratios and sonicated to a length of ca. 200 bp using the Covaris S220 sonicator. The sonicated DNA from various species was pooled and converted into DNA bait following the protocol reported by Maricić et al. (2010). Target enrichment was performed in solution following the protocol of Horn (2012). Hybridisation was performed using the Oligo aCGH/ChiP-on-Chip Hybridisation Kit (Agilent Technologies, Palo Alto, CA, USA). Each reaction (50 µl) consisted of 12–15 µl pooled libraries (up to five libraries), 25 µl of 2× hybridisation buffer, 5 µl of blocking agent, 4 µl of blocking oligos (25 µM each) and 1–3 µl of DNA bait. The quantities of the pooled libraries and DNA bait were adjusted so that the library to bait ratio was 10 to 1. Hybridisation was carried out for 20–24 h at 65°C in a thermocycler. After the incubation, the hybridisation reaction was incubated for 20 min with 5 µl of streptavidin-coated beads (Dynabeads MyOne C1, Thermo Fisher) to immobilise the enriched libraries. The bead-immobilised libraries were washed five times with BWT buffer (see Horn 2012 for buffer composition), incubated for 2 min at 50°C with HWE buffer, washed once with BWT buffer, transferred to a new tube, washed once with TET and resuspended in 35 µl of TE buffer. To separate the enriched library from the bait, the mixture was incubated for 5 min at 95°C, the beads were collected on a magnet and the eluate was transferred to a new tube. The enriched library was amplified 15 cycles in three separate reactions of 20 µl each using Herculase II Fusion DNA polymerase (Agilent Technologies), which was purified using magnetic beads and subjected to the second round of hybridisation and amplification. Libraries from the ancient and modern specimens were never pooled in one hybridisation reaction. Multiple enriched library pools, either ancient or modern, were combined for sequencing ensuring that all P5 and P7 indices were unique in the pool, quantified using qPCR (Illumina Library Quantification kit, KAPA) and sequenced on the NextSeq550 at the CeNT UW NGS Core Facility using the 150 bp Mid Output kit and a 2 × 75 bp sequencing scheme. A custom Read1 primer was used for the single-stranded libraries, as described by Gansauge et al. (2020).

## Sequence processing

The raw reads were demultiplexed using bcl2fastq v. 2.19 (Illumina). Overlapping reads were collapsed and adaptor and quality trimmed (-trimns, -trimqualities) using AdapterRemoval v. 2.2.2 (Schubert et al., 2016) with the following parameters: --collapse, --minalignmentlength 4, --trims, --trimqualities, --gzip, --basename ‘sample’. We indicated the sequence of the second adapter the single-stranded libraries as --adapter2 GGAAGAGCGTCGTGTAGGGAAAGAGTGT. The reads were mapped to a narrow-headed vole mtDNA reference using the BWA-MEM algorithm (Li, 2013). We used either *S. gregalis* or *S. anglicus* mtDNA as a reference respectively. In some cases we used competitive mapping approach to filter out human contaminations (Feuerborn et al., 2020). In this approach human and vole mtDNA sequences were combined into a single reference with two chromosomes.

Duplicated, short (<30 bp) and low mapping quality reads (mapq <30) were removed by *samtools* v.1.9 (Li et al., 2009) using the *samtools view-q 30*; *samtools sort* and *samtools rmdup* commands. Consensus sequence was called using in-house script utilizing bcftools mpileup and *ivar call* commands (Grubaugh et al., 2019). The BED file, which is used for masking low coverage positions, was generated using the *genomecov* command from BEDtools v. 2.27 (Quinlan & Hall, 2010) and filtered to retain only positions with coverage less than 3 using the *awk* script. Read alignments and vcf files were inspected manually using Tablet v. 1.17 software (Milne et al., 2013).

## Radiocarbon dating of vole bones

Vole bones were pre-treated for radiocarbon dating in the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA, Leipzig, Germany) (lab code: EVA) following the protocol for <100 mg bone samples described in (Fewlass et al., 2019). In brief, the samples were demineralised in 0.5 M HCl at 4°C. Demineralisation was stopped when CO2 effervescence had stopped, and the samples were soft (3–23 h). The samples were treated with 0.1 M NaOH for 15 min at room temperature to remove humic acids and then re-acidified in 0.5 M HCl. The samples were rinsed to a neutral pH with ultrapure Milli-Q water between each step. The samples were gelatinised in HCl at pH 3 on a heater block at 70°C until fully solubilised (3–7 h). The resulting gelatine was filtered to remove particles >80 µm (Ezee filters, Elkay labs, UK, cleaned by sonication in Milli-Q water for 20 min) and then ultrafiltered to concentrate the >30 kDa fraction (Sartorius VivaSpin Turbo 15 with 30 kDa molecular weight cut off) with pre-cleaned ultrafilters (Brock et al., 2007). Samples were freeze-dried for 48 h.

The quality of the collagen extracts was assessed based on collagen yield as a percentage of the original bone weight (minimum requirement 1%). The elemental and isotopic ratios of the extracts (~0.5 mg) were measured at the MPI-EVA on a Thermo Finnigan Flash elemental analyser coupled to a Thermo Delta plus XP isotope ratio mass spectrometer. Stable carbon isotope ratios were expressed relative to Vienna PeeDee Belemnite, and stable nitrogen isotope ratios were measured relative to air (atmospheric N2) by using the delta notation (δ) in parts per thousand (‰). Analysis of internal (methionine δ13C = −28.13 ± 0.13 ‰ and δ15N = −6.35 ± 0.03 ‰ (1SD); in-house collagen MRG δ13C = −19.77 ± 0.22 ‰ and δ15N = 5.00 ± 0.06 ‰) and international standards (IAEA-CH-6 sucrose, δ13C = −10.4 ± 0.25 ‰; IAEA-CH-7 polyethylene, δ13C = −32.15 ± 0.14 ‰; IAEA-N-1 ammonium sulphate, δ15N = 0.43 ± 0.04 ‰; IAEA-N-2 ammonium sulphate, δ15N = 20.41 ± 0.2 ‰) indicates an analytical error of 0.2‰ (1σ) for δ13C and δ15N. Collagen extracts were considered suitable for dating where the collagen yield was >1% and elemental values (C: 30%–45%, N: 11%–16%, C:N: 2.9–3.6) fell within established ranges of well-preserved collagen (DeNiro 1985; van Klinken 1999).

Collagen was weighed into tin cups and graphitised using the automated graphitisation equipment (Wacker et al., 2010a) in the Lab of Ion Beam Physics at ETH-Zurich (Switzerland) and dated on a Mini Carbon Dating System (MICADAS) accelerator mass spectrometer (AMS) (Synal et al., 2007) (AMS lab code: ETH). Background bones (>50,000 years) of equal size were prepared and dated alongside the vole samples to monitor lab-based contamination. Oxalic acid standards and background collagen samples measured in the same session were used to calculate the age of the samples with BATS software (Wacker et al., 2010a). An external error of 1‰ was propagated in the error calculation of the samples. Radiocarbon dates were calibrated in OxCal v4.4 (Bronk Ramsey, 2009) by using the IntCal20 (Reimer et al., 2020) calibration curve.

## Phylogenetic analyses - testing the mitogenomic dataset for temporal signal and age estimation accuracy

The sequences used in this study were aligned in MAFFT (Katoh & Standley, 2013). The alignment was visually inspected, the variations in length around nucleotide homopolymers were trimmed and singleton indels were deleted. Then we use PartitionFinder v.2 (Lanfear et al., 2016) to select the most appropriate partitioning scheme and DNA substitution models (electronic supplementary material, table S2).

|  |  |  |  |
| --- | --- | --- | --- |
| Table S2. Best partitioning scheme of mitochondrial DNA and DNA substitution models identified by PartitionFinder2. | | | |
| No | Model | No. Sites | Partitions |
| 1 | GTR+I+G+X | 5095 | 9844–10140\3, 6982–7665\3, 1–66, 1018–1086, 2648–2717, 3678–3746, 3747–3817, 3818–3885, 4922–4988, 4990–5056, 5059–5128, 5162–5229, 5230–5295, 6843–6908, 6913–6980, 7669–7732, 9358–9425, 9775–9842, 11512–11579, 11580–11638, 11639–11707, 14041–14109, 15259–15388, 13520–14040\3, 11708–13519\3, 69–1017, 1087–2647; |
| 2 | TRN+I+G+X | 940 | 67–68, 2718–2722, 4921–4921, 4989–4989, 5057–5058, 5129–5161, 5296–5297, 6909–6912, 6981–6981, 7666–7668, 9774–9774, 9843–9843, 14110–14114, 15258–15258, 15389–16092, 13521–14040\3; |
| 3 | K80+I+G | 1476 | 5298–6842\3, 8577–9357\3, 2723–3677\3, 14115–15257\3; |
| 4 | TRN+I+G+X | 2481 | 3887–4920\3, 9427–9773\3, 7734–7936\3, 7937–8574\3, 11709–13519\3, 9845–10140\3, 8575–9357\3, 10141–11511\3, 2724–3677\3; |
| 5 | GTR+G+X | 2695 | 7938–8574\3, 14117–15257\3, 11710–13519\3, 8576–9357\3, 2725–3677\3, 10142–11511\3, 9428–9773\3, 3888–4920\3; |
| 6 | HKY+G+X | 1198 | 9426–9773\3, 7733–7936\3, 7939–8574\3, 3886–4920\3, 10143–11511\3; |
| 7 | HKY+X | 1124 | 5299–6842\3, 14116–15257\3, 6983–7665\3; |
| 8 | TRN+G+X | 1083 | 9846–10140\3, 6984–7665\3, 5300–6842\3, 13522–14040\3, 7735–7936\3; |

The BEAST 1.10.4 software (Suchard et al., 2018) was used to reconstruct phylogenetic relationships, estimate the divergence times of the common vole lineages and age the not directly dated specimens. In all analyses, we used eight data partitions as suggested by PartitionFinder2 with unlinked substitution and clock models.

The Bayesian evaluation of temporal signal (BETS, Duchene et al., 2020) was used to determine whether the temporal signal within our dataset was sufficient to calibrate the molecular clock. In this analysis we used 17 sequences, 6 from extant and 11 from directly radiocarbon-dated narrow-headed voles. The medians of the calibrated radiocarbon ages were set as the sequence sampling times. The support for the four models was compared. In the first two models, real sampling times were assigned to the sequences (heterochronous analysis), and then either a strict clock or an uncorrelated relaxed log-normal clock (UCLN) was used. In the two other models, the same sampling time (i.e. isochronous analysis) was used for all sequences, and either a strict clock or the UCLN clock was applied. A constant population size tree prior to all analyses and a CTMC rate reference prior for the heterochronous datasets were applied (Ferreira & Suchard, 2008). Each analysis was run 50 million steps sampled every 5,000 steps and with the first 5 million steps discarded as burn-in. Convergence and stationarity were inspected in Tracer 1.7 (effective sample size; ESS >200 for all parameters). The log marginal likelihood (MLE) of each model was estimated using the generalised stepping-stone (GSS) sampling approach (Baele et al., 2016). The MLE calculation consisted of 50 path steps, each run for one million iterations. Two replicates of each BEAST analysis were performed. The results are presented in table S3. We were not able to obtain marginal likelihood estimates for model IV, with one sampling time across all sequences and the UCLN clock model. The Marginal Likelihood estimator failed in each of several replicates, yielding unrealistic logML values. Among the other three models, model I with real sampling times assigned to sequences and a strict clock was decisively supported over Model III (2lnBF=1900) and Model II (2lnBF=213) indicating that our dated dataset is suitable for calibrating the molecular clock and a strict clock model fits the data better than the UCLN clock model.

Table S3. Results of Bayesian evaluation of the temporal signal

|  |  |  |  |
| --- | --- | --- | --- |
| ID | Description | logML | mean logML |
| **Model I** | **Tips; strict clock, CTMC rate prior** | **−36747,71** | −**36747,70** |
| **−36747,69** |
| Model II | Tips; UCLN clock, CTMC rate prior | −36853,82 | −36854,27 |
| −36854,71 |
| Model III | No tips; strict clock | −37700,81 | −37698,08 |
| −37695,35 |
| Model IV | No tips; UCLN clock | −1.7E91 | failed |
| −1.3E93 |

To additionally test the dated dataset, we performed a date randomization test (Duchêne et al., 2015). We compared the clock rate of each partition estimated as in Model I of BETS with 20 replicates in which tip dates were shuffled across sequences. We did not see that the 95% HPD interval of the clock rate estimated with the real tip-dates overlapped with the clock rate resulting from the reshuffled replicates, indicating that the dataset is appropriate to calibrate the molecular clock (figure S1).

Next, we tested which tree prior fit best to our dated dataset. A constant population size and Skygrid tree priors were compared using GSS MLE. The analyses were conducted the same as for the BETS analysis, and ‘positive’ support was found for the constant population size tree prior (table S4).

Table S4. Testing models for the ‘dated’ dataset using GSS

|  |  |  |  |
| --- | --- | --- | --- |
| Analysis details | logML (replicates) | Mean logML | 2lnBF |
| Dated dataset, strict clock, constant pop. Size tree prior, CTMC rate prior | −36747,71 | **−36747,704** | **3,57** |
| −36747,70 |
| Dated dataset, strict clock, Skygrid tree prior, CTMC rate prior | −36748,46 | −36749,488 |
| −36750,52 |



Figure S1. Results of date-randomisation test on 'dated' dataset. For each of the eight partitions clock rate estimated using the real dataset is compared against 20 replicates in which tip-dates were shuffled across sequences.

Next, we performed a leave-one-out analysis on the directly dated specimens to determine the precision of the age estimates produced using the available calibration dataset. In this analysis, the age of each directly radiocarbon-dated specimen was estimated using all of the remaining directly dated and modern specimens to calibrate the molecular clock. We set a gamma prior (shape = 2; scale = 50,000) on the age of the directly dated specimen whose age was to be estimated using the molecular dating approach and increased the operator weight of the age estimation to 5. We set a constant population size tree prior and used a CTMC rate reference prior. Each analysis was run in duplicate for 50 million generations sampled every 5,000 steps and with the first 5 million generations discarded as burn-in. Convergence and stationarity were inspected in Tracer 1.7 (ESS >200 for all parameters).



Figure S2. Results of the leave-one-out analysis on directly dated specimens. Three age estimates are presented for each specimen. The first one is a radiocarbon age (green dot denotes median calibrated age, and whiskers denote 94.5% probability range), whereas the next two are the two replicates of the molecular age estimates from BEAST (red dot denotes median age, and whiskers denote 95% HPD interval).

Leave-one-out analysis revealed that our dataset enables an accurate estimation of the ages of the specimens. In all cases but one the 95% HPD intervals of estimated ages overlapped with the probability range of the calibrated radiocarbon date. It did not overlap only in the case of the oldest specimen radiocarbon dated to 42,640 cal BP. It’s hard to determine what was the reason for this. The date was close to the limit of the radiocarbon dating method and given the small sample size, we cannot exclude the possibility of rejuvenation.

## Estimation of the age of not directly dated specimens and reconstruction of the mitogenomic phylogeny

The previous analyses showed that our mitogenomic dataset is suitable to calibrate the molecular clock and to accurately estimate the age of the not dated specimens. Knowing this, we estimated the age of each not directly dates specimen (n=128) using the mtDNA sequence of this specimen together with all directly dated and modern specimens. We used the same prior distributions for parameters as in the leave-one-out analysis. We set a gamma prior (shape = 2; scale = 50,000) on the age of the not directly dated specimen and increased the operator weight of the age estimate to 5. We set a constant population size tree prior and used a CTMC rate reference prior. Each analysis was ran with 50 million generations sampled every 5,000 steps and with the first 5 million generations discarded as burn-in. Convergence and stationarity were inspected in Tracer 1.7 (ESS >200 for all parameters).

Then we run a joint analysis using the full dataset of all the 145 ancient and modern sequences. In this analysis we set a lognormal prior on the age of each not directly dated specimen so that the mean equals to the mean age estimate from the individual analysis of this specimen and the range corresponds to the 95% HPD interval of the individual analysis. The analysis was run in duplicate for 500 million generations sampled every 25,000 generations. The first 50 million generations were discarded as a burn-in. We used GSS MLE to determine whether the strict or UCLN clock model and whether the constant population size or Skygrid tree prior fits better to this dataset. We found that analysis with a strict clock and constant population size tree prior was strongly supported over the others (table S5).

Table S5. Testing models for the full dataset using GSS

|  |  |  |
| --- | --- | --- |
| Analysis details | logML (replicates) | Mean logML |
| Full dataset, strict clock, constant pop. size tree prior, CTMC rate prior | −62247,46 | **−62242,768** |
| −62238,07 |
| Full dataset, strict clock, Skygrid tree prior, CTMC rate prior | −62270,17 | −62261,493 |
| −62252,81 |
| Full dataset, UCLN clock, constant pop. size tree prior, CTMC rate prior | −62322,02 | −62322,023 |
| −62324.25 |

We combined the trees from the two replicates runs using *logcombiner* from the BEAST 1.10.4 suitesummarised it and generated the Maximum Clade Credibility (MCC) tree using *treeannotator*.

In the full analysis, most of the parameters reached high ESS values in each of the replicates but the ESS of the age parameter of seven specimens was lower than 200 in at least one replicate. In all cases the age values obtained in the full analysis were very similar to those obtained in the individual analysis of each sample and in all cases the ESS values obtained in the individual analysis were high (Table S6). Given the above, we considered the estimated ages of these samples as reliable.

Table S6. The ESS, mean and 95% HPD interval for age parameter of samples with low ESS in the full analysis

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Full analysis | | | | | | | | Individual analysis | | | |
| Rep1 | | | | Rep2 | | | |
| ESS | mean age | 95% HPD | ESS | | mean age | 95% HPD | ESS | | mean | 95% HPD |
| MI1210 | 111 | 26379.21 | [24270.32, 28472.70] | 112 | | 26464.16 | [24406.62, 28540.22] | 8019 | | 26981.68 | [22343.70, 31338.92] |
| MI1232 | 99 | 25005.25 | [21801.59, 28179.56] | 108 | | 24642.14 | [21606.41, 27688.45] | 1316 | | 23991.80 | [15743.01, 32775.41] |
| MI1233 | 102 | 24689.58 | [21496.09, 27904.90] | 113 | | 24323.17 | [21354.87, 27432.51] | 1443 | | 26284.76 | [17960.53, 35771.98] |
| MI1512 | 247 | 34974.07 | [33024.96, 36972.15] | 176 | | 35067.27 | [33103.24, 37023.04] | 3494 | | 35469.39 | [30494.08, 40609.58] |
| MI2735 | 71 | 25639.02 | [22638.94, 28952.35] | 72 | | 25198.10 | [22273.28, 28223.74] | 816 | | 24546.74 | [16061.07, 33392.72] |
| MI2737 | 45 | 25728.54 | [22857.72, 28947.85] | 40 | | 25168.83 | [22330.92, 27968.11] | 1318 | | 26028.78 | [17891.11, 34880.76] |
| MI2738 | 44 | 25730.96 | [22923.16, 29002.85] | 40 | | 25165.88 | [22432.78, 28052.03] | 1228 | | 26395.65 | [18204.98, 35637.48] |

## Phylogeny reconstruction using the mtDNA cytochrome b fragment

To investigate the known mtDNA diversity of modern Asiatic *S. gregalis* we reconstructed a phylogeny using an 889-bp-long fragment of mtDNA cytochrome b. This dataset consisted of 311 sequences, 139 from ancient and 172 from extant specimens (table S11). We used PartitionFinder2 to determine the best partitioning scheme and assign the DNA substitution model to partitions (table S7).

Table S7. Best partitioning scheme of 889 bp cyt b fragment and DNA substitution models assigned by PartitionFinder2.

|  |  |  |  |
| --- | --- | --- | --- |
| Subset | Model | No. Sites | Partitions |
| 1 | K80+I+G | 297 | cp1 |
| 2 | HKY+I+X | 296 | cp2 |
| 3 | GTR+G+X | 296 | cp3 |

We ran the analysis in duplicate for 100 million generations sampled every 10,000 generations and with first 10 million generations discarded as a burn-in. We assigned the dates estimated in the full analysis to the not directly dated specimens. We set the CTMC rate prior. We used GSS MLE to determine whether a constant population size or Skygrid tree prior fits better to this dataset and obtained strong support for the Skygrid tree prior. We combined the trees from the two replicates runs using *logcombiner* from the BEAST 1.10.4 suite,summarised it and generated the MCC tree using *treeannotator*.

The tree reconstructed using the mtDNA cytochrome b was similar to that obtained using the mtDNA genome sequences (figure S3. Three main lineages were observed corresponding to European *S. anglicus* and Asiatic *S. gregalis* and *S. raddei*. Within the *S. gregalis* lineage, four main lineages (A–D) were observed with the specimens from layers 14 and 12.3 from the Denisova cave placed as a sister group.



Figure S3. Maximum Clade Credibility tree of the narrow-headed voles generated using the 889 bp fragment of mtDNA cytochrome b. Dots at nodes indicate posterior probability higher than 0.95. The branches leading to S. raddei and S. anglicus were collapsed for better visibility. The letters D1–D5 mark the nodes discussed in the text and in table S10.

Within lineage A, we detected six sublineages (A1–A6) with a single specimen from layer 11.3 of Denisova cave placed as a sister group. The geographic distribution of the lineage is presented in figure S4. The divergence times of the three main lineages estimated using the mtDNA cytochrome b fragment were younger than those estimated using the mitogenomic dataset. The divergence of *S. anglicus* was estimated to be 201 (95% HPD: 238–170) ka ago and the divergence of *S. gregalis* and *S. raddei* was estimated to be 178 (95% HPD: 210–148) ka ago. However, the other divergence times were very similar to those obtained from the mitogenomic dataset (table S10); therefore we considered the divergence of the main mtDNA lineages present in modern *L. gregalis* (lineages A–D) estimated using the mtDNA cytochrome *b* dataset as reliable and used for comparison with divergence times obtained from the mitogenomic datasets.

Table S12. Comparison of the divergence times obtained from the mitogenomic and cytochrome b datasets. Node numbers correspond to those on the figure S3.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Node | mitogenomic dataset | | cytichrome b dataset | |
| Age (ka) | 95% HPD | Age (ka) | 95% HPD |
| D1 (*anglicus*) | 237 | 255, 218 | 201 | 238, 170 |
| D2 (gregalis−raddei) | 219 | 240, 200 | 178 | 210, 148 |
| D3 (*Denisova–AD*) | 125 | 136, 114 | 123 | 134, 116 |
| D4 (*Denisova–A*) | 59 | 65, 53 | 58 | 64, 53 |
| D5 (*A1–A3*) | 41 | 44, 37 | 36 | 41, 31 |



Figure S4. Distribution of mtDNA lineages identified in Asiatic narrow-headed voles. Circles denote sampling localities of modern specimens while other symbols denote ancient localities. If more than one lineage was observed in the ancient locality multiple symbols are presented. Colors correspond to figure S3. Green areas denote present day range of S. gregalis and S. raddei. Only localities with exact GPS coordinates available are shown (see table S11).

# Description of archaeological/palaeontological sites

Descriptions of most of the sites discussed or mentioned in the text can be found in Supporting Information of previous papers (Baca et al., 2020, <https://doi.org/10.1016/>

j.quascirev.2020.106239; Baca et al. 2021, [ece38289-sup-0001-Supinfo.docx](https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fece3.8289&file=ece38289-sup-0001-Supinfo.docx) and [ece38289-sup-0002-TableS1-S7.xlsx](https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1002%2Fece3.8289&file=ece38289-sup-0002-TableS1-S7.xlsx); Baca et al., 2022, SI TBA). Here, only descriptions of new sites from which the studied material originated are presented.

## Austria

Doris Nagel and Thomas Filek

**Teufelslucke**

48°40` N, 15°51’ E

The cave is situated on the west flank of the Königsberg (Röschitz) at an altitude of 314 m. Maximum extension of this cave is 40 m and the height mostly around 1.0 to 1.5 m. Over Palaeozoic rocks of the Bohemian Massif marine sediments from the Lower Miocene (Eggenburgium) were deposited. Some of them are quite soft and eroded easily (Gauderndorf sands) thus forming the cave. The cave has been known since 1874 but the first scientific excavations took place in 1930 lead by A. Stifft-Gottlieb (Krahuletz Museum) and J. Bayer (Natural History Museum Vienna). The main publication was delayed due to WW II and the description of three layers is reconstructed based on the manuscript of Kernerknecht (Ehrenberg 1966). They distinguish a basal layer of quartz sand without fossils, a fossiliferous main layer formed by different coloured quartz and calcareous sands sometimes interlayered by clay, and a layer of manganese oxide particles. The uppermost part contains weathered sandy limestone mixed with humus. The faunal list contains almost 60 bird taxa and 45 mammal taxa. Biochronologically the material fits into MIS 3 and MIS 2 (e.g. *Ursus spelaeus*, *Crocuta* *c*. *spelaea*, *Mammuthus* *primigenius*, *Dicrostonyx*, *Lemmus*, *Allactaga*). In 1982, the first attempt was made to date the material resulted in ca. 23 ka BP (ca. 27.3 cal ka BP). New Radiocarbon dates are in progress.

**Teufelsrast**

48°27` N, 15°24’ E

Teufelsrast is one of the “Kremstalhöhlen” (caves in the gorge of the little river Krems, Lower Austria) at an altitude of 597 m. The cave is only 7 m long, 2.5 m wide and approx. 0.5 m high. It is embedded in the Bohemian Massif. Between Palaeozoic schist and amphibolites small layers of marble are enclosed. Along fissures in this marble some caves weathered out. Excavations took place in 1983 by Neugebauer-Maresch (1993) where they discovered a connection to another small cave, the Teufelsrast-Felsdach. They sampled micromammals in the sandy sediments which D. Jánossy (Budapest) was able to identify: twelve bats, 19 rodents, two lagomorphs and three carnivores. Additionally, twelve bird taxa were found. The material has only been dated biochronologically. *Lemmus*, *Dicrostonyx* and *Chionomys* *nivalis* are probably from MIS 2 while *Allactaga* and *Lagurus* need drier conditions and judging from the latest *Allactaga* datings should be placed into the upper MIS 3. *Sciurus*, *Eliomys* and *Apodemus* seem to be mostly extant and typical for the woodland habitat today.

## Czechia

Ivan Horáček

**Malenice 2**

490 74' N, 130 53' E

A stratigraphic section excavated in 2000 by I. Horáček, S. Čermák and J. Wagner at entrance of small system of narrow underground passages in a rocky cliff of crystalline limestones above Volyňka river at northern outskirts of the village Malenice, close to the largest local cave (ca 50 m passages) Jiříčkova sluj, SW Bohemia. The cliff represents one of few small spots of limestones appearing within Proterozoic granites and metamorphic rocks of the Moldanubicum series composing area of southern and southwestern Bohemia, the region lacking relevant fossil records except for classical sites of the Late Pleistocene assemblages in Sudslavice (Woldřich 1880,1881,1883) and Zechovice (Želízko, 1922 etc.) situated in vicinity of the present locality. Basal layers 4 and 5 of a compact loess colluvium in the section (of total thickness about 1.6 m) yielded remains of at least 35 individuals (MNI) of the following taxa: 2 *Lagopus* sp., 2 *Spermophilus* sp., 1 *Clethrionomys glareolus*, 3 *Microtus arvalis*, 18 *Stenocranius anglicus*, 9 *Dicrostonyx torquatus*.

|  |  |
| --- | --- |
|  |  |

**Zkamenělý zámek (section D)**

49040' N, 160 56' E

A stratigraphical section (of total thickness 3.6 m) excavated in 1990 by I. Horáček, D. Storch and J. Mlíkovský in a sedimentary talus at rocky bridge (supposedly a collapsed cave entrance) in a cliff above Špraněk river near Javoříčko, N Moravia. Entrance of proper cave Zkamenělý zámek (with a mass assemblage of glacial microfauna - comp.- e.g. Horáček and Sánchez Marco, 1984) is situated some 20 m below the section. The section revealed 12 layers mostly relative rich in both mollusc and vertebrate remains: layers 1–7 was of the Holocene age, layers 7–8 with rich clastic component represented probably a horizon influenced by destruction in time of late LGM. The layer 9 was the uppermost member of loess layers 9–11 which in total thickness >2m overlayered sandy deposits at the base of section. They all provided rich fauna with dominant representation of *Dicrostonyx torquatus* and *Stenocranius anglicus*.

## France

Aurélien Royer and Marie Soressi

**Les Cottés cave**

46°41’N, 0°50’E

Les Cottés, Saint-Pierre-de-Maillé, is a limestone cave located in the center west of France on the south-western margin of the Paris Basin. The deposit in front of the cave were excavated from 2006 to 2018 by M. Soressi and her team (Soressi et al. 2010; Rendu et al. 2019). Late Middle and Early Upper Paleolithic finds are preserved in a diamicton of mostly centimetre-sized limestone clasts with a sandy clay matrix. The sequence contains five major stratigraphical units: one Mousterian, one Châtelperronian, one Proto-Aurignacian and two Early/Middle Aurignacian (Roussel and Soressi, 2009) with some very low density or sterile layers in between each of these units. Radiometric dates on bones and on sediment indicate that the site was occupied from at least 45,000 years ago up until around 35,000 years ago (Talamo et al. 2012; Jacobs et al. 2015). Small vertebrate remains were recovered in most of all these units, including the low-density unit US–07 that is estimated to date between 45 and 40,000 years (Jacobs et al., 2015). A total of thirteen different taxa of small fauna has been identified with sizable variations throughout the sequence (Royer, unpublished data). Several remains of *Stenocranius anglicus*, *Microtus arvalis* and lemmings were identified. Samples of *S. anglicus* come mainly from the Châtelperonnian US–06 unit for which climatic estimation of mean annual temperature based on small mammal associations (Royer et al., 2020) is around 0°C.

**Grotte des Gorges**

47°09’N, 5°32’E

La Grotte des Gorges is located close to Amange village (Jura) and was excavated between 2008 and 2017 by S. David. Two layers (1a and 1b) yielded archaeological material (David et al., 2014, 2017), from the end of Marine Isotope Stage (MIS) 3 with accelerator mass spectrometry (AMS) dates ranging from 33,030±750 BP (37,828±966 cal BP) to 29,390±170 (33,985±213 cal BP). The site yields a diverse association of mammalian species demonstrating a transition from arctic toward the steppe environment. The upper part of the sequence (layer 1a) delivered faunal remains dominated by typical taxa of cold‐climate phases such as reindeer (*Rangifer tarandus*), mammoth (*Mammuthus primigenius*), rhinoceros (Rhinocerotidae), collared lemmings (*Dicrostonyx torquatus*), tundra lemming *(Lemmus lemmus*), and wood lemming (*Myopus schisticolor*), which are associated with *Stenocranius anglicus* individuals (Arbez et al., 2021). The second phase of the sequence (layer 1b) is dominated by bison (*Bison priscus*), a typical taxon of the steppe environment.

**Les Pradelles**

45°44’N, 0°25’E

The Les Pradelles site, also known as Marillac, is located in the village of Marillac-le-Franc in Charente, region delivering many Neanderthal sites (Mann and Maureille, 2007). The first mention of the site dates from the end of the 19th century, and was excavated later by David, Vandermeersch and Maureille and Mann. The last excavation was performed between 2001 and 2013 and delivered many Neanderthal and faunal remains, and Quina lithics (Maureille et al., 2010a, b). The stratigraphy is constituted by 12 lithological units. Using Optically Stimulated Luminescence methods, main sedimentary deposits with faunal and Neanderthal remains have been assigned to the end of MIS 4 and beginning of MIS 3, up to ~50 ka BP (Frouin et al., 2017). The small mammal remains are present in most of these stratigraphic levels. Eleven rodent species have been identified, including a remarkable presence of *Dicrostonyx torquatus, Alexandromys oeconomus, Cricetulus migratorius* and *Stenocranius anglicus*, with their relative proportion varying throughout the sequence (Royer et al., 2013). A sizable change of small mammal association occurred in particularly between levels 4a and 4b, which is associated with changes in oxygen isotope compositions measured from rodent teeth. These changes are interpreted as a possible result from an arid event.

**Taillis des Coteaux**

46°31’N, 0°51’E

Discovered in 1998, the archaeological site, the Taillis-des-Coteaux, is located in southwestern France, near the village of Antigny, Vienne (Primault et al., 2007). It provides an exceptional sequence delivering several sedimentary levels from the Upper Palaeolithic, dated from 35 to 17 cal ka BP (Primault et al., 2007, 2018), from which has been recovered an abundant and diversified sample of micromammal remains (Jeannet, 2011; Royer et al., 2013) including *Alexandromys oeconomus*, *Dicrostonyx* *torquatus* and *Stenocranius gregalis*. The oxygen isotope composition of phosphate from tooth enamel of rodents offers opportunity to estimate summer mean temperature ranging from 16.0±2 to 19.1±1°C throughout the sedimentary sequence. Sample comes from the Bank Area sector, from the levels IIIB, which is dated to 17,460±110 BP (21,095±163 cal BP, Ly–6407).

## Germany

Lutz Maul

**Weinberghöhle**

48°46' N, 10°03' E

The cave system near village of Mauern (Weinberghöhlen) is situated on the western slope of the Wellheim valley on the elevation of 430 m a.s.l. The caves were excavated in 1937–1938 and in 1947–1949 and infilled with Late Pleistocene sediments. An overview of available sedimentological, archeological and faunal data was published by Koenigswald et al. (1974). The faunal remains represent 85 taxa including 51 mammals. The fauna of layers H to C belong to the last glaciation, mainly Middle Weichselian. However, the more detailed sub-division cannot be traced back based only on the fauna. Two 14C dates were determined from charcoal samples. The first one (Mauern I), from the entrance of cave 2, yielded the date 29,410±470 BP (33,877±557 cal BP, GrN–5000) and the second (Mauern IV) 28,265±325 BP (32,445±486 cal BP, GrN–6059), however, obtained not directly in the fossiliferous layer.

## Poland

Adam Nadachowski, Magdalena Krajcarz and Anna Lemanik

**Komarowa cave**

50°43' N, 19°17'E

Komarowa cave is situated in the Sokole Mts., on the northern slope of Mt. Puchacz, Kraków-Częstochowa Upland. The studies in the cave started 1997 and finished in 2001. The trenches have been established inside the cave chamber (13 layers and sublayers from K do B) and on the terrace (10 layers and sublayers from Gtx to A). The cave chamber are filled with loams, limestone rubble, sand and silts and the section is about 3,5 m thick, while at the terrace the bottom was not reached at the depth of 6.7 m (Żarski, 2009). The lowermost layers at the terrace (Gtx and Ftx) are probably of Eemian (MIS 5e) or even earlier age followed by the series of sediments from MIS 5c–a (layers Kt, Jt, Gt, Ft). In the cave chamber the deepest layers (K, J, H, G4) are also probably of the MIS 5 age. An upper fragment of the profile inside the cave (layers F, E, D and C) was deposited during MIS 3, while at the terrace layer B is of mainly Late Glacial age (five OSL and TL dates and one AMS 14C date are available). The range of the dates covers a period between 16,700±2,000 cal BP (Lub-3790, TL date) and 9,900±1,300 cal BP (Lub-3788, TL date) (Nadachowski et al., 2009). The deposits yielded over 12,500 determined vertebrate remains (among total number of over 70 000) comprise at least 180 species including birds (over 100 species), insectivores (10 species), bats (20 species), carnivores (14 species), ungulates (12 species), rodents (20 species) and lagomorphs (3 species) (Ochman, 2003; Tomek and Bocheński, 2005; Rzebik-Kowalska, 2006; Wojtal, 2007; Nadachowski et al., 2009). Wojtal (2007) described in detail the taphonomy of the bone remains.

**Perspektywiczna cave**

50°26' N, 19°46'E

Perspektywiczna Cave is located in Udorka Valley in the middle part of Kraków-Częstochowa Upland, southern Poland (Sudoł et al. 2016a, Krajcarz et al. 2016; Gretzinger et al. 2019; Krajcarz et al. 2020). The cave includes two chambers connected by a gallery. Two entrances to the cave occur in a cliff of Upper Jurassic limestone, at an elevation of 340 and 345 m a.s.l. One entrance faces W; the other faces NW. The upper chamber is filled with Upper Pleistocene (MIS 3) and Holocene sediments. The lower one contains Upper Pleistocene and Holocene sediments with abundant animal bones and archaeological material. During the excavations of the lower chamber, nineteen archeological layers (1, A1, 2, A2, A3, 3, A4, 4, 5, 6, 7a, 7b, 7c, 8, 9a, 9b, 10, 11, and 12) were identified within a 4-m-thick sequence. The sediments of the lower chamber are dated to: the Late Glacial of the last glaciation (layers 9a, 9b and 7c, the latter is a colluvium composed of material dated to MIS 3 and re-deposited from the upper chamber); the Lower Holocene (layer 8); and the Middle to Upper Holocene (layers from 7b to 1, including the colluvial series of re-deposited LGM to Middle Holocene material, constituting layers 5, 6, 7a and 7b) (Sudoł et al. 2016a; Sudoł et al. 2016b; Krajcarz et al. 2018). Chronology of the lowest strata (layers 10, 11 and 12) is unknown.

## Russia

Alexander K. Agadzhanian and Tatiana Strukova

**Cheremukhovo 1**

60°24' N, 60°03'E,

Cheremukhovo 1, eastern slopes of the Northern Urals, was excavated in 1998–2000. Its entrance facing northwest is situated at the level of 5.5 m above the river flood plain, being 8 × 8.5 m in dimensions. The cave is 32 m long in total. The cave roof collapsed 11 m behind the roof dropping line along the distance of 12 m. Cave walls are pierced by holes and have numerous niches and grottos situated near the floor and higher, up to the level of 1.5–2.5 m above the floor. Four pits were made in the cave (Borodin et al., 2000). Pit 1 is located at the mouth of the cave. The deposits are represented by eleven lithological layers. Alluvial-speleogenic (layers 6–10) and speleogenic formations (layers 1–5) took part in the structure of the section. A series of dates (AMS, 14C) was obtained for the lower part of the profile. The sedimentation began in the second half of MIS 3 and continued during MIS 2 and the Holocene. For detailed description of the lithology, dating results, paleontological materials (Vertebrata remains) and palynological spectra, see Strukova et al. (2006). Pit 4 is located in a niche, which is a recess in the southeast wall of the cave. It is at a height of 1 m from the cave floor and 6 m from the drip line. The height of the niche at the entrance is 90 cm, width 5 m; the length of the niche in depth is 3 m. In the western part of the excavation is a narrow slot (80 cm deep), which probably has an exit to the surface (Bachura and Strukova, 2002). The deposits are represented by two lithological layers. Rodent remains are dominated by species forming the core of the Late Pleistocene faunas of northern Eurasia: *Dicrostonyx henseli*, *Lemmus sibiricus* and *Stenocranius gregalis*. The fauna was dated to MIS 2 (the radiocarbon date was obtained from Cricetidae bone remains: 18,784±379 BP (22,740±856 cal BP; IEMAE-1259).

**Denisova cave**

51°23' N, 84°40’ E

The site is located in the Northwestern Altai Mountains, on the right bank of Anuy River valley. Denisova cave consists of three large chambers named Main Chamber, East Chamber and South Chamber, each containing numerous layers, the Pleistocene sequences are complex and dated from MIS 9 to MIS 2 (Jacobs et al., 2019). Denisova cave is one of the most important Palaeolithic sites in Asia, known from discovery of previously unknown hominin lineage – Denisovans (Krause et al., 2010). Fossil remains of archaic hominins were discovered in the Palaeolithic layers, which yielded mitochondrial and nuclear DNA (Prüfer et al., 2014). The human DNA has also been extracted from the Pleistocene sediments (Slon et al., 2017). It was found that along with the Denisovans, the cave was inhabited by Neanderthals (e. g. Viola et al., 2012; Slon et al. 2018).

The reconstruction of Pleistocene environments was inferred from the analyses of remains of 27 species of large mammals, more than 100 species of small vertebrates: 40 species of small mammals, 66 taxa of birds and some fish, reptiles and amphibians as well as pollen records of 30 species of trees, 36 shrubs and grasses and 6 species of spore-bearing plants (Derevianko et al., 2003; Bolikhovskaya and Shunkov, 2014). The rodent remains analyzed in this study come from the East Chamber of layers 14, 12.3, 11.3 and 11.1, rich in fauna of small mammals (Agadzhanian et al., 2021).

**Dyrovaty Kamen`**

57°39' N, 58°53’ E

The entrance to the cave, 8 m wide and 5 m high, was located 33 m above the Chusovaya river in a steep cliff 60 m high, western slopes of the Middle Urals. The area of the cave is approximately 160 m2. The upper layers of deposits were removed during archaeological excavations, which were carried out from the 1930s onwards and periodically resumed in the 1990s. The number of stone, bronze, bone and iron points from this site amounted to more than 10,000 (Serikov, 2000). All bone remains of small mammals were recovered from the brown loam. Based on a sample of micromammal bone remains, the layer was dated back to 13,757±250 BP (16,666±704 cal BP, IEMAE-1140). Later, a series of AMS dates of isolated jaws of rodents was obtained. Two mandibles of *Dicrostonyx torquatus* were dated back to 12,820±60 BP (15,307±108 cal BP, CAMS-35894) and 13,620±60 BP (16,449±103 cal BP, CAMS-35895); two mandibles of *Lagurus lagurus* – 12,610±60 BP (15,020±144 cal BP, CAMS-35896) and 12,620±60 BP (15,035±134 cal BP, CAMS-35897); two mandibles of *Circetulus migratorius* – 12,810±60 BP (15,291±108 cal BP, CAMS-35898) and 12,960±60 BP (15,500±108 cal BPCAMS-35899) (Stafford et al., 1999).

**Mironovskaya cave**

57°28 N, 61°43' E

Mironovskaya cave, eastern slope of the Middle Urals, was excavated in 2007. The entrance to the cave is at a height of 23 m above the river. The height of the entrance is 1.6 m, width - 3.5 m. The cave is well visible to the depth of 4 m. The mouth part of the cave is filled with sandy loams and sands with minor amounts of fine gravel. Seven layers were distinguished within the exposed profile. Sedimentation occurred during the Holocene. The stratigraphy of the cave and the complexes of small mammals are described by Rupysheva and Strukova (2010). Horizon 25 (layer 7) is dated to 9,830±130 BP (11,276±235 cal BP, Ki-15492) and horizon 20 (layer 6) to 5,340±80 BP (6,120±96 cal BP, Ki-15494).

**Pershinskaya cave 1**

57°26' N, 61°26' E

Pershinskaya 1 cave, placed on eastern slopes of the Middle Urals was excavated in 1995–1997. The entrance arch, 6 metres wide and 1.5 metres high, was oriented north-west; after 3–4 metres, the cave passage transformed into a fissure sloping down from left to right. Six layers were distinguished within the exposed profile. The stratigraphy of the cave and the complexes of small mammals are described by Erokhin et al. (1997) and Strukova (2000). Sedimentation occurred during MIS 2 and the Holocene. A series of radiocarbon dates were obtained for the lower part of the layer 6: the upper part was dated to 13,165±55 BP (15,796±93 cal BP, OxA–20263) based on a sample from *Coelodonta antiquitatis* radius shaft (Stuart and Lister, 2012); the lower part was dated to 12,980±240 BP (15,535±380 cal BP, SOAN-4542) based on Cricetidae bone remains. Layer 3 was dated to 7,380±150 BP (8,193±142 cal BP, SOAN-3824) by radiocarbon dating.

## Slovakia

Ivan Horáček

**Bojnice II - Prepošťská cave**

48° 47' N, 18° 35 E

An important archeological site with rich record of late Mousterian industry (Neruda and Kaminská 2013). The cave situated near Bojnice castle, W Slovakia, represent a spacious cavity eroded in a body of Eemian travertine cascade deposited by a local thermal spring. It was infilled by a complicated series of soft travertine deposit, soil colluvia and loess. It was many times visited and excavated by various amateur acheologists already in first half of 20th century. The first professional re-examination of the site was undertaken during 1950-1964 with aid of several sections by F. Prošek and V. Ložek (1951), later systematic excavations were conducted by J. Bárta (1965–1967). Numerous reports on the sites were summarized by Neruda and Kaminská (2013), bird remains from excavations by J. Bárta were recently surveyed by Šedivá et al. (2020). A rich mammalian microfauna obtained from revision sections by Prošek and Ložek was analyzed by Horáček and Sánchez Marco (1984). In total it includes remains of at least 694 individuals (MNI) of 16 spp. with predominating *Microtus arvalis* (357) and *Stenocranius anglicus* (250), the other glacial elements are quite rare (4 *Dicrostonyx*, 1 *Chionomys nivalis*, no *Lemmus, Lagurus*, but 3 *Allactaga major*). In agreement to lithological, archeological and malacological inferences concerning the site they proposed a biostratigraphic dating to beginning stage of the Vistulian.

## Ukraine

Leonid Rekovets

**Novhorod-Severskyi**

51°59'N, 30°16'E

Novhorod-Severskyi site is situated in Desna River valley, Tchernihiv region, Northern Ukraine. The first news of finding fossil bones in this place dates back to 1875. The excavations continued periodically until the middle of the 20th century, and the fauna of small mammals was published in monograph in 1985 (Rekovets, 1985). The remains of terrestrial vertebrates (large and small mammals, birds, lower vertebrates) were deposited in layers of loess-like clay of Quaternary age. The sediments were deposited on the surface of hard sandy Paleogene structures forming a kind of small caves. In the caves there was a site of ancient people, many artifacts were found, including gigantolites and bones of large mammals. With the help of birds of prey and other factors, the remains of small mammals and lower vertebrates were also gathered here. The faunal locality is *in situ* as evidenced by the taphonomy of the remains (all anatomical groups, fragments of skulls, entire mandibles with teeth and the postcranial bones were preserved). The remains of Rodentia (*Dicrostonyx toquatus, Lagurus lagurus major, Stenocranius anglicus/kriogenicus*), Lagomorpha (*Ochotona pusilla/spalaea*), Carnivora (*Canis lupus*), Aves (Passeriformes) dominated. Morphologically, the species were adapted to specific tundra-steppe (periglacial) ecological conditions. This served as the basis for description of many of them as separate extinct taxa (mainly on subspecies level) (Rekovets and Nowakowski, 2010). Only the mammoth tooth was dated from the Novhorod-Severskyi site: 19.800±350 BP (23,840±446 cal BP, OxA-698-698) (Nadachowski et al., 2018). However, it seems that fauna represents several colder phases of both MIS 2 and/or MIS 3.

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