

## Supporting Information

### Supporting Materials and Methods

#### *DNA extraction, amplification, sequencing*

Handling and preparation of samples, DNA isolation and PCR setup were conducted in two laboratories dedicated for ancient DNA: the Swedish Museum of Natural History (E-labelled specimens), and the Centre for Precolumbian Studies at the University of Warsaw (L-labelled specimens), following standard procedures to avoid contamination.

*Description of DNA analyses for E-labelled specimens:* 10-50mg of bone powder was obtained from each specimen using a multitool drill. DNA extractions were performed following a modified version of protocol C in Yang *et al.* (1998) as described in Brace *et al.* (2012). In brief, ~10-50mg of bone powder were incubated overnight in extraction buffer including 0.45M EDTA, 0.1M Urea (instead of SDS) and 0.15mg proteinase K. The extracts were concentrated using 30K MWCO Vivaspin filters (Sartorius) and purified with silica spin columns (QIAGEN). Because of the degraded nature of DNA in ancient material, we first screened all specimens for DNA by amplifying a 171bp fragment of the mitochondrial cytochrome *b* (cyt *b*) gene using primer pair A in Brace *et al.* (2012). Successfully amplified specimens were further amplified for 780bp of the cyt *b* gene in eight overlapping fragments using primer pairs A-G, I from Brace *et al.* (2012). Primers were tagged on their 5'end with custom-designed six-nucleotide sequences (indexes) to allow for parallel sequencing of multiple individuals (Binladen *et al.*, 2007). Each forward and reverse primer was tagged with 8 different indexes producing 8 x 8 unique tag combinations that enabled sequencing of 64 individuals in parallel. This strategy resulted in amplicons that were tagged in both ends. PCR reactions and conditions were the same as those described for Caverne Marie Jeanne samples in Brace *et al.* (2012). Negative controls were included in each extraction and amplification

batch to control for contamination. Each fragment was replicated at least once with independent amplification of each individual to monitor DNA damage.

Tagged amplicons from different individuals were pooled and purified using a MinElute column (QIAGEN) for library preparation. We followed the Rapid Library (RL) preparation protocol from 454 Life Sciences for sequencing on a GS Junior instrument. The following modifications were made to the library protocol: *i)* The DNA fragmentation by nebulization step was omitted since we were sequencing PCR products. *ii)* The fragment end-repair step was omitted and instead only phosphorylation of the pooled PCR products was performed in a mix of 2.5µl RL 10x buffer, 2.5µl RL ATP, 1µl PNK and 3µl elution buffer (EB; QIAGEN). This modification was introduced to eliminate the problem of switched indexes that we observed during initial experiments; out of the 64 unique tag combinations at least two combinations were used for amplification of negative controls (NC). Although NCs did not yield PCR products when checked on an agarose gel and thus were not included in the pool of PCR products, resulting sequences possessed NC tag combinations indicating that PCR products were switching tags at some point during library preparation, emulsion PCR or sequencing. We discovered that tag-switching occurred during the end-repair step and therefore proceeded by excluding this step from the protocol. *iii)* After adaptor ligation, purification was performed using a MinElute column (QIAGEN) instead of AMPure beads since our amplicons were comparatively short. *iv)* The quality of the libraries was assessed on agarose gels and high-sensitivity DNA chips on the Bioanalyzer 2100 (Agilent). Library quantification was performed on a TBS 380 Fluorometer (Turner Biosystems) as described on the RL preparation protocol. Each library was amplified in an emulsion PCR following the emPCR Amplification Method Manual – Lib-L from 454 Life Sciences. We used Live Amp Mix B for paired-end libraries with 0.6 DNA molecules per bead and 35 cycles instead of 50 because our amplicons were shorter than 400bp. Bead recovery and enrichment were

performed as described in the manual. Amplified libraries were sequenced on a GS Junior instrument following the Sequencing Method Manual from 454 Life Sciences with 200 nucleotide cycles. We performed a total of 5 sequencing runs (5 libraries of pooled PCR products).

Following data processing, we discovered cases of *i*) missing fragments for a few individuals, *ii*) fragments from which replicates did not work, *iii*) replicate-fragments of the same individual that contained mismatches. In order to fill in these gaps and resolve mismatches between replicates, we re-amplified the particular fragments/individuals and purified them using Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase. Sequencing was performed in both directions on an ABI3730xl (Applied Biosystems) instrument.

*Description of DNA analyses for L-labelled specimens:* Prior to DNA extraction each tooth was washed with bleach solution (6% w/v sodium hypochlorite), rinsed with water and UV-irradiated for 20 minutes on each side. Then it was crushed with a sterile pipette tip and up to 50mg of bone powder were used for DNA extraction following a protocol described in Baca *et al.* (2012). Bone powder was incubated overnight at 40°C with agitation in 1.5 ml of buffer containing 100mM EDTA, 20mM DTT, 5mM PTB, 0.5% *N*-Lauroylsarcosine, 0.4mg/ml Proteinase K (Applichem). Then a phenol/chloroform/isoamyl alcohol (25:24:1) extraction followed by chloroform extraction was performed followed by a isopropanol precipitation with 750 mM ammonium acetate. The obtained DNA pellet was dissolved in 60 µl TE buffer. For initial screening, a 163bp fragment of the mitochondrial *cyt b* was amplified using primer pair *cytb4* designed in this study (Table S4, Supporting Information). Successfully amplified samples were further amplified for 1050bp of the *cyt b* gene in two multiplex PCR reactions using eleven primer pairs designed in this study (Table S4, Supporting Information). PCR reactions were set up in 25µl reactions containing 1X AmpliTaq Gold MasterMix (LifeTech), 5µg BSA, 0.1-0.2µM of each primer and 1.5µl of DNA extract. Reaction conditions were

75 95°C for 12 min; 18 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 30 s; and 72°C for 5 min.

76 In each reaction, primer pairs amplifying non overlapping fragments were used. Multiplexed

77 PCR products from each individual were pooled and purified using solid phase reversible

78 immobilization (SPRI) beads. Sequencing libraries for each individual pool were prepared

79 according to the protocol proposed by Stiller *et al.* (2009). Briefly: i) multiplexed PCR

80 products were blunt end-repaired and phosphorylated in 30µl reactions that contained 15µl

81 purified PCR products, 1X Tango buffer, 1mM ATP, 15U T4 PNK, 3U T4 DNA Polymerase

82 and 100µM dNTPs for 7 min and 30 s at 12 °C and 7 min and 30 s at 25 °C, followed by

83 purification with SPRI, ii) truncated 454 FLX adapters A and B were ligated to the

84 multiplexed amplicons. Each A adapter contained a unique barcode sequence of ten

85 nucleotides. Barcode sequences differed from each other by at least three nucleotides. Up to

86 96 different A adaptors were used in each sequencing experiment. Ligation was performed in

87 30µl reactions that contained 15µl purified blunt-end products, 1X Ligase buffer, 5% PEG-

88 4000, 5U T4 DNA Ligase and 3mM of each adapter. Reactions were incubated for 20 min at

89 22°C and purified using SPRI. iii) Fill-in reactions were performed to eliminate single-

90 stranded overhangs in the ligated adapters including 15µl ligation products, 1X Thermopol

91 buffer, 8U Bst polymerase and 250µM dNTPs in a total of 30µl mix. Reactions were

92 incubated at 37 °C for 20 min and were SPRI purified. iv) Final amplification was performed

93 with primers MP-make454-A and MP-make454B to produce sequencing libraries with full

94 length universal 454 FLX Titanium adapters. Reactions included 10µl fill-in products, 1X

95 AmpliTaq Gold MasterMix (LifeTech) and 250nM primers. PCR conditions were 95°C for 12

96 min; 18 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 40 s; and 72°C for 5 min. PCR

97 products were checked on agarose gels. The amount of DNA molecules in each library was

98 estimated with real-time PCR using a 454 FLX Titanium Library Quantification kit (KAPA).

99 Libraries were pooled in equimolar concentrations and sequenced on 1/8 of a Pico-Titer plate

on the GS FLX Titanium platform in the Institute of Biochemistry and Biophysics, PAS. Amplification and sequencing of each fragment was replicated at least once and in cases of mismatches between replicates appropriate fragments were amplified in single-plex PCR (with the same conditions as described above but with 45 cycles instead of 18) and sequenced with the Sanger method on an ABI3730xl (as described below).

For modern L-labelled *Dicrostonyx* specimens, DNA was isolated from dried tissue using the Wizard Genomic DNA extraction kit according to manufacturer's recommendations. Amplification of 849bp of the mitochondrial *cyt b* was performed using primers CytBwspF, CytBwspR (Table S4, Supporting Information) in 20µl reactions containing 1X polymerase master mix (Biomix, Bioline), 0.1µM primers and 1µl of DNA extract. Reaction conditions were: 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds; and 72°C for 5 minutes. PCR products were purified with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase and sequenced on an ABI3730xl (Applied Biosystems) in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences PAS. Sequences were analysed and aligned in BioEdit version 7.2.5.

#### *Data analysis*

Data generated with the 454 GS junior technology were processed by the 454 Sequencing System Software (version 2.9) using the full processing option for Shotgun or Paired End Rapid Libraries. Passed filter reads were used in further analyses. We used SFF Tools' (454 Sequencing System Software version 2.9) command 'sfffile' to assign reads to each individual, based on tag identification in both ends allowing for one mismatch. Indexes were designed to differ from each other by at least two base pairs in order to avoid misidentification due to sequencing errors. Following individual identification, 'sff' files were converted to 'fastq' files using the program sff2fastq (<https://github.com/indraniel/sff2fastq>).

After this step we followed the bioinformatics pipeline described in Clarke *et al.* (2014). In brief, primer sequences were trimmed from both ends using TagCleaner (Schmieder *et al.* 2010). The complete cyt *b* sequence of a *Dicrostonyx torquatus* specimen available in GenBank (accession number AF119275) was used as a reference sequence. BWA's (Li & Durbin 2009); version 0.7.8) 'index' command was used to index the reference sequence. Reads were aligned to the reference using the BWA-MEM algorithm, which is recommended since it's faster and more accurate than BWA-SW. SAM format files were converted to BAM format, sorted according to coordinates and indexed with SAMtools version 0.1.19 (Li *et al.* 2009). Picard's (<http://picard.sourceforge.net>; version 1.92) 'MergeSamFiles' function was used to merge BAM files from the same individual/replicate into one BAM file. Consensus sequences were called using SAMtools' 'mpileup' command, bcftools and vcftools' 'vcf2fq' command. Consensus sequences were converted to fastq files and renamed according to individual/replicate using the perl script provided in Clarke *et al.* (2014). Replicate-consensus sequences from each individual were then aligned and visually inspected for mismatches on Geneious version 5.5.7. In cases of mismatches between replicates from the same individual, we re-amplified the particular fragments containing the mismatches to resolve these positions (see above).

Data generated with the 454 FLX technology were processed by the 454 Sequencing System Software (version 2.9). SFF Tools were used to assign reads to each individual and convert 'sff' files to 'fna' files. Primer sequences were trimmed in BioEdit (Hall, 1999). Reads were assembled and consensus sequences were called in SeqMan Pro (DNASTAR). For each individual, consensus sequences from two replicates were aligned and visually inspected in BioEdit.

Sequences generated from Sanger sequencing on the ABI3730xl were assembled and analysed on Geneious version 5.5.7.

## References

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**Table S1. List of sampling localities.**

| Locality                      | Country | Number of samples | Number of samples with complete <i>cyt b</i> sequences | Coordinates                    |
|-------------------------------|---------|-------------------|--------------------------------------------------------|--------------------------------|
| Marie-Jeanne cave             | Belgium | 24                | 9                                                      | 50°7'48" N, 4°28'33.6" E       |
| Trou Al'Wesse cave            | Belgium | 16                | 4                                                      | 50°25'15.6" N, 5°17'38.4" E    |
| Kitley cave                   | UK      | 1                 | 1                                                      | 50°20'36" N, 4°00'12" W        |
| Merlin cave                   | UK      | 6                 | 6                                                      | 51°50'6" N, 2°39'24" W         |
| Bois laiterie                 | Belgium | 2                 | 2                                                      | 50°21'47.88" N, 4°52'19.2" E   |
| Walou Cave                    | Belgium | 5                 | 0                                                      | 50°34'44" N, 5°40'24" E        |
| Kleine Scheuer am Hohlenstein | Germany | 4                 | 4                                                      | 48°32'57.57" N, 10°10'20.75" E |
| Neanderthal Cave              | Germany | 5                 | 0                                                      | 51°13'36" N, 6°56'49" E        |
| Biśnik cave                   | Poland  | 256               | 137                                                    | 50°25'35" N, 19°39'54" E       |
| Oblazowa cave                 | Poland  | 12                | 2                                                      | 49°25'48" N, 20°9'36" E        |
| Deszczowa cave                | Poland  | 6                 | 0                                                      | 50°35'15" N, 19°32'34" E       |
| Nietoperzowa cave             | Poland  | 2                 | 0                                                      | 50°11'38" N, 19°46'28" E       |
| Mamutowa cave                 | Poland  | 3                 | 0                                                      | 50°10'14" N, 19°48'20" E       |
| Jankovich cave                | Hungary | 15                | 0                                                      | 47°43'38"N, 18°33'21" E        |
| Pilisszanto rock shelter      | Hungary | 4                 | 0                                                      | 47°43' N, 18° 55' E            |
| Mezin site                    | Ukraine | 9                 | 0                                                      | 51°48'41"N, 33°4'12"E          |
| Nowgorod Sewerski             | Ukraine | 10                | 0                                                      | 52°00'24"N, 33°15'49"E         |
| Yudinovo                      | Russia  | 9                 | 4                                                      | 52°40'14.5" N, 33°15'44.64" E  |
| Eliseevichi                   | Russia  | 9                 | 0                                                      | 55°16'48.72" N, 32°04'02.64" E |
| Studenaya                     | Russia  | 18                | 15                                                     | 62°00' N, 58°44' E             |
| Betovo                        | Russia  | 42                | 38                                                     | 53°20'38.76" N, 34°1'1.9" E    |
| Lobva                         | Russia  | 22                | 8                                                      | 59°10'44.79" N, 60°29'54.12" E |
| Pymva Shor                    | Russia  | 8                 | 8                                                      | 67°10' N, 60°51' E             |
| Yangana Pe-                   | Russia  | 12                | 9                                                      |                                |
| Rasik                         | Russia  | 15                | 13                                                     | 59°02'24" N 57°19'48" E        |
| Makhnevskaya-2 cave           | Russia  | 30                | 16                                                     | 59°27'00" N, 57°41'20" E       |
| Sikiyaz Tamak 22              | Russia  | 1                 | 1                                                      | 55°11'24" N, 59°11'24" E       |
| Ushminskaya cave              | Russia  | 3                 | 0                                                      | 61°16'12" N, 60° 01'48" E      |
| Dyrovatyi Kamen cave          | Russia  | 3                 | 2                                                      | 57°23'24" N, 58°32'24" E       |
| Staroe Logovo                 | Russia  | 3                 | 1                                                      | 55°13'31" N, 58°07'33" E       |
| Kipievo site                  | Russia  | 1                 | 1                                                      | 65°39'48" N, 54°31'29" E       |
| Shapkina-18                   | Russia  | 1                 | 0                                                      | 67°10'46" N, 53°59'04" E       |
| Shapkina-1                    | Russia  | 1                 | 1                                                      | 67°40'32" N, 56°43'01" E       |
| Kur'yador                     | Russia  | 1                 | 0                                                      | 61°40'60" N, 54°53'34" E       |
| Sedyu-1                       | Russia  | 1                 | 1                                                      | 63°24'57" N, 53°59'46" E       |
| Pizhma-1                      | Russia  | 1                 | 1                                                      | 64°55'09" N, 51°39'49" E       |
| Serche'yu-3                   | Russia  | 1                 | 0                                                      | 67° 00'28" N, 55°33'13" E      |
| Laya-2 site                   | Russia  | 1                 | 0                                                      | 67°10'10" N, 55°46'41" E       |
| Bison's site                  | Russia  | 7                 | 1                                                      | 65°27' N, 124°07' E            |

|                                                     |        |     |     |                                 |
|-----------------------------------------------------|--------|-----|-----|---------------------------------|
| Ostrov Bolshevik                                    | Russia | 7   | 7   | 79°29'47.95" N, 97°19'00.82" E  |
| Kyttyk peninsula                                    | Russia | 16  | 15  | 69°29'17" N, 167°45'6" E        |
| Batagaika                                           | Russia | 1   | 1   | 67°41'33.98" N, 134°39'14.15" E |
| Yana HRS site                                       | Russia | 2   | 2   | 70°43' N, 135°25' E             |
| Tordokh site                                        | Russia | 2   | 1   | 75°20'50" N, 146°47'50" E       |
| Duvanny Yar site                                    | Russia | 3   | 2   | 68°37' N, 159°11' E             |
| Bulunskiy district, Sakha Republic<br>(modern)      | Russia | 5   | 5   | 72°34'12"N, 124°43'48"E         |
| Taymyr Peninsula-Lukunskiy<br>sector (modern)       | Russia | 6   | 6   | 72° 29' 24"N, 105° 4' 48"E      |
| Taymyr Peninsula-Verchnya<br>Taymira river (modern) | Russia | 2   | 2   | 74° 6' 36"N, 98° 50' 24"E       |
| January Cave                                        | Canada | 2   | 2   | 50°10'60" N, 114°31'00" W       |
| Eagle Cave                                          | Canada | 5   | 2   | 49°38'10" N, 114°38'24" W       |
| Total                                               |        | 621 | 317 |                                 |

**Table S2. Details of collared lemming specimens analysed in this study. Radiocarbon dates ( $^{14}\text{C}$ ) and median, 95% upper and lower calibrated dates are given in years before present. Median ages, although not recommended for use on single age calibration, were used as tip-dates for internal calibration of the tree. Specimens that provided complete *cyt b* sequences are shown.**

| Specimen ID | Region   | Site              | $^{14}\text{C}$ date | $^{14}\text{C}$ error ( $\pm$ ) | 14C Lab Nr    | Median cal. date | lower 95% cal. date | upper 95% cal. date | Median age or age-range used for tip-dating | Haplotype | Accession No. |
|-------------|----------|-------------------|----------------------|---------------------------------|---------------|------------------|---------------------|---------------------|---------------------------------------------|-----------|---------------|
| E011        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H51       | KT867382      |
| E031        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H52       | KT867383      |
| E032        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H53       | KT867384      |
| E033        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H54       | KT867385      |
| E039        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H55       | KT867386      |
| E040        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H56       | KT867387      |
| E042        | Belgium  | Marie-Jeanne cave | 20310                | 150                             | OxA-30002     | 24412            | 24022               | 24942               | 24412                                       | H57       | KT867388      |
| E043        | Belgium  | Marie-Jeanne cave | 13760                | 70                              | OxA-30003     | 16630            | 16350               | 16923               | 16630                                       | H06       | JX867569      |
| E046        | Belgium  | Marie-Jeanne cave | -                    | -                               | -             | -                | -                   | -                   | -                                           | H58       | KT867389      |
| E073        | W.Russia | Yudinovo          | 14465                | 80                              | OxA-X-2575-46 | 17634            | 17408               | 17895               | 17634                                       | H59       | KT867390      |
| E081        | W.Russia | Studenaya         | -                    | -                               | failed        | -                | -                   | -                   | 26200-29385                                 | H60       | KT867391      |
| E082        | W.Russia | Studenaya         | -                    | -                               | failed        | -                | -                   | -                   | 26200-29385                                 | H61       | KT867379      |
| E083        | W.Russia | Studenaya         | -                    | -                               | failed        | -                | -                   | -                   | 26200-29385                                 | H62       | KT867392      |
| E084        | W.Russia | Studenaya         | -                    | -                               | failed        | -                | -                   | -                   | 26200-29385                                 | H63       | KT867393      |
| E085        | W.Russia | Yudinovo          | 14345                | 75                              | OxA-30001     | 17481            | 17202               | 17711               | 17481                                       | H59       | KT867390      |
| E088        | W.Russia | Yudinovo          | -                    | -                               | failed        | -                | -                   | -                   | 17202-17895                                 | H64       | KT867394      |
| E089        | W.Russia | Yudinovo          | -                    | -                               | failed        | -                | -                   | -                   | 17202-                                      | H59       | KT867390      |

|      |          |           |       |     |              |       |       |       |             |     |          |
|------|----------|-----------|-------|-----|--------------|-------|-------|-------|-------------|-----|----------|
|      |          |           |       |     |              |       |       |       | 17895       |     |          |
| E092 | W.Russia | Studenaya | -     | -   | failed       | -     | -     | -     | 26200-29385 | H65 | KT867395 |
| E093 | W.Russia | Studenaya | 23730 | 170 | OxA-X-2581-7 | 27819 | 27534 | 28179 | 26200-29385 | H66 | KT867396 |
| E094 | W.Russia | Studenaya | 24180 | 180 | OxA-X-2581-8 | 28218 | 27834 | 28625 | 26200-29385 | H67 | KT867397 |
| E096 | W.Russia | Studenaya | 24790 | 220 | OxA-30032    | 28842 | 28380 | 29385 | 28842       | H68 | KT867398 |
| E097 | W.Russia | Studenaya | 22470 | 170 | OxA-30033    | 26783 | 26304 | 27229 | 26783       | H69 | KT867399 |
| E098 | W.Russia | Studenaya | 22380 | 170 | OxA-30034    | 26676 | 26200 | 27143 | 26676       | H70 | KT867400 |
| E099 | W.Russia | Studenaya | -     | -   | -            | -     | -     | -     | 26200-29385 | H71 | KT867401 |
| E100 | W.Russia | Studenaya | -     | -   | -            | -     | -     | -     | 26200-29385 | H72 | KT867402 |
| E101 | W.Russia | Studenaya | -     | -   | -            | -     | -     | -     | 26200-29385 | H73 | KT867380 |
| E102 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H74 | KT867403 |
| E103 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H75 | KT867404 |
| E104 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H76 | KT867405 |
| E105 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H77 | KT867406 |
| E106 | W.Russia | Betovo    | 27240 | 290 | OxA-29989    | 31188 | 30813 | 31599 | 31188       | H78 | KT867407 |
| E107 | W.Russia | Betovo    | 23610 | 190 | OxA-29750    | 27733 | 27427 | 28072 | 27733       | H79 | KT867408 |
| E108 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H80 | KT867409 |
| E109 | W.Russia | Betovo    | 24320 | 210 | OxA-29751    | 28351 | 27889 | 28774 | 28351       | H81 | KT867410 |
| E110 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H82 | KT867411 |
| E111 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-31599 | H83 | KT867412 |
| E112 | W.Russia | Betovo    | 23150 | 220 | OxA-29681    | 27433 | 27057 | 27769 | 27433       | H84 | KT867413 |
| E113 | W.Russia | Betovo    | 24660 | 220 | OxA-29752    | 28705 | 28195 | 29245 | 28705       | H81 | KT867410 |
| E114 | W.Russia | Betovo    | -     | -   | failed       | -     | -     | -     | 26254-      | H82 | KT867411 |

|      |          |                           |       |     |           |       |       |       |             |     |          |
|------|----------|---------------------------|-------|-----|-----------|-------|-------|-------|-------------|-----|----------|
|      |          |                           |       |     |           |       |       |       | 31599       |     |          |
| E115 | W.Russia | Betovo                    | 24080 | 200 | OxA-29753 | 28132 | 27755 | 28571 | 28132       | H79 | KT867408 |
| E116 | W.Russia | Betovo                    | -     | -   | failed    | -     | -     | -     | 26254-31599 | H85 | KT867414 |
| E117 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H84 | KT867413 |
| E118 | W.Russia | Betovo                    | 22460 | 190 | OxA-29682 | 26769 | 26254 | 27241 | 26769       | H82 | KT867411 |
| E119 | W.Russia | Betovo                    | 22680 | 150 | OxA-29754 | 27014 | 26576 | 27368 | 27014       | H86 | KT867415 |
| E120 | W.Russia | Betovo                    | 24030 | 190 | OxA-29755 | 28085 | 27731 | 28517 | 28085       | H87 | KT867416 |
| E121 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H88 | KT867417 |
| E122 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H81 | KT867410 |
| E123 | W.Russia | Betovo                    | 23970 | 200 | OxA-29756 | 28036 | 27687 | 28487 | 28036       | H77 | KT867406 |
| E125 | W.Russia | Betovo                    | 25630 | 250 | OxA-29757 | 29821 | 29175 | 30552 | 29821       | H89 | KT867418 |
| E126 | W.Russia | Betovo                    | 23000 | 190 | OxA-29683 | 27322 | 26927 | 27675 | 27322       | H74 | KT867403 |
| E127 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H90 | KT867419 |
| E128 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H74 | KT867403 |
| E132 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H82 | KT867411 |
| E133 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H91 | KT867420 |
| E134 | W.Russia | Betovo                    | -     | -   | -         | -     | -     | -     | 26254-31599 | H82 | KT867411 |
| E140 | UK       | Merlin cave               | -     | -   | failed    | -     | -     | -     | 14362-15301 | H06 | JX867569 |
| E141 | Canada   | January Cave (P80.1.7900) | -     | -   | -         | -     | -     | -     | 33688-39994 | H92 | KT867421 |
| E142 | Canada   | January Cave (P80.1.7886) | -     | -   | failed    | -     | -     | -     | 33688-39994 | H93 | KT867422 |
| E146 | W.Russia | Studenaya                 | -     | -   | failed    | -     | -     | -     | 26200-29385 | H94 | KT867423 |
| E147 | W.Russia | Studenaya                 | -     | -   | failed    | -     | -     | -     | 26200-29385 | H95 | KT867424 |

|      |          |                  |         |         |           |              |              |                 |             |      |          |
|------|----------|------------------|---------|---------|-----------|--------------|--------------|-----------------|-------------|------|----------|
| E157 | E.Russia | Bison's site     | -       | -       | -         | -            | -            | -               | 50000-70000 | H96  | KT867425 |
| E165 | W.Russia | Lobva            | -       | -       | -         | -            | -            | -               | 0-14401     | H97  | KT867426 |
| E166 | W.Russia | Lobva            | -       | -       | -         | -            | -            | -               | 0-14401     | H98  | KT867427 |
| E175 | W.Russia | Lobva            | -       | -       | -         | -            | -            | -               | 0-14401     | H99  | KT867428 |
| E176 | W.Russia | Lobva            | -       | -       | failed    | -            | -            | -               | 0-14401     | H100 | KT867429 |
| E177 | W.Russia | Lobva            | -       | -       | failed    | -            | -            | -               | 0-14401     | H101 | KT867430 |
| E180 | W.Russia | Lobva            | -       | -       | -         | -            | -            | -               | 0-14401     | H102 | KT867431 |
| E181 | W.Russia | Lobva            | -       | -       | -         | -            | -            | -               | 0-14401     | H103 | KT867432 |
| E183 | W.Russia | Lobva            | 12540   | 60      | OxA-29633 | 14834        | 14401        | 15130           | 14834       | H104 | KT867433 |
| E184 | W.Russia | Betovo           | 23760   | 170     | OxA-29758 | 27843        | 27558        | 28219           | 27843       | H83  | KT867412 |
| E185 | W.Russia | Betovo           | -       | -       | failed    | -            | -            | -               | 26254-31599 | H87  | KT867416 |
| E186 | W.Russia | Betovo           | -       | -       | failed    | -            | -            | -               | 26254-31599 | H105 | KT867434 |
| E187 | W.Russia | Betovo           | 23020   | 150     | OxA-29759 | 27345        | 27055        | 27615           | 27345       | H106 | KT867435 |
| E188 | W.Russia | Betovo           | 23210   | 180     | OxA-29760 | 27480        | 27181        | 27753           | 27480       | H107 | KT867436 |
| E189 | W.Russia | Betovo           | -       | -       | failed    | -            | -            | -               | 26254-31599 | H108 | KT867437 |
| E191 | W.Russia | Betovo           | 26440   | 230     | OxA-29761 | 30712        | 30214        | 31089           | 30712       | H109 | KT867438 |
| E192 | W.Russia | Betovo           | -       | -       | failed    | -            | -            | -               | 26254-31599 | H110 | KT867439 |
| E193 | W.Russia | Betovo           | 23100   | 170     | OxA-29762 | 27403        | 27097        | 27684           | 27403       | H111 | KT867440 |
| E195 | C.Russia | Ostrov Bolshevik | 1,30992 | 0,00352 | OxA-29748 | <b>50</b>    | <b>46</b>    | <b>54</b>       | 50          | H112 | KT867441 |
| E196 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H113 | KT867442 |
| E197 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H114 | KT867443 |
| E199 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H115 | KT867444 |
| E200 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H116 | KT867445 |
| E202 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H117 | KT867446 |
| E206 | C.Russia | Ostrov Bolshevik | -       | -       | -         | -            | -            | -               | (0) modern  | H118 | KT867447 |
| E208 | UK       | Merlin cave      | -       | -       | failed    | -            | -            | -               | 14362-15301 | H104 | KT867433 |
| E313 | E.Russia | Batagaika        | >50100  | -       | OxA-29747 | <b>50229</b> | <b>48290</b> | <b>Date out</b> |             | H119 | KT867448 |

| of range |          |                        |         |        |           |     |     |     |              |      |          |
|----------|----------|------------------------|---------|--------|-----------|-----|-----|-----|--------------|------|----------|
| E314     | Canada   | Eagle Cave (P83.1.762) | -       | -      | failed    | -   | -   | -   | 25121-100000 | H120 | KT867449 |
| E315     | Canada   | Eagle Cave (P95.7.419) | -       | -      | failed    | -   | -   | -   | 25121-100000 | H121 | KT867450 |
| E316     | E.Russia | Kyttyk peninsula       | -       | -      | failed    | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E317     | E.Russia | Kyttyk peninsula       | 335     | 24     | OxA-28677 | 386 | 310 | 471 | 386          | M05  | AJ131442 |
| E318     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | H122 | KT867451 |
| E319     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E320     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | H123 | KT867452 |
| E321     | E.Russia | Kyttyk peninsula       | 260     | 24     | OxA-28678 | 301 | 151 | 428 | 301          | M05  | AJ131442 |
| E322     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E324     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E325     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | H122 | KT867451 |
| E326     | E.Russia | Kyttyk peninsula       | 842     | 27     | OxA-29255 | 748 | 690 | 794 | 748          | H123 | KT867452 |
| E327     | E.Russia | Kyttyk peninsula       | -       | -      | failed    | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E328     | E.Russia | Kyttyk peninsula       | 1,55063 | 0,0043 | OxA-29256 | 50  | 46  | 54  | 50           | M05  | AJ131442 |
| E329     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E330     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | M05  | AJ131442 |
| E331     | E.Russia | Kyttyk peninsula       | -       | -      | -         | -   | -   | -   | 0-1000       | H122 | KT867451 |
| E332     | W.Russia | Pymva Shor             | -       | -      | -         | -   | -   | -   | 10780-12511  | H124 | KT867453 |
| E333     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 25707-26807  | H125 | KT867454 |
| E334     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 15408-15946  | H102 | KT867431 |
| E335     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 15408-15946  | H126 | KT867455 |
| E336     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 15408-15946  | H104 | KT867433 |
| E337     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 15408-15946  | H127 | KT867456 |
| E338     | W.Russia | Pymva Shor             | -       | -      | failed    | -   | -   | -   | 15408-15946  | H128 | KT867457 |

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|      |          |                  |       |     |               |       |       |       |              |      |          |
|------|----------|------------------|-------|-----|---------------|-------|-------|-------|--------------|------|----------|
| L200 | Poland   | Biśnik Cave      | 16040 | 230 | OxA-X-2507-16 | 19360 | 18855 | 19940 | 19360        | H59  | KT867390 |
| L202 | Poland   | Biśnik Cave      | 14705 | 65  | OxA-27591     | 17897 | 17684 | 18083 | 17897        | H165 | KT867493 |
| L209 | Poland   | Biśnik Cave      | 15800 | 70  | OxA-27592     | 19051 | 18875 | 19255 | 19051        | H59  | KT867390 |
| L210 | Poland   | Biśnik Cave      | 15600 | 80  | OxA-27633     | 18847 | 18677 | 19025 | 18847        | H143 | KT867472 |
| L211 | Poland   | Biśnik Cave      | 19470 | 130 | OxA-27906     | 23452 | 23052 | 23821 | 23452        | H157 | KT867485 |
| L215 | Poland   | Biśnik Cave      | 13430 | 75  | OxA-27905     | 16159 | 15901 | 16411 | 16159        | H10  | JX867573 |
| L216 | Poland   | Biśnik Cave      | 14390 | 70  | OxA-27589     | 17539 | 17295 | 17795 | 17539        | H148 | KT867477 |
| L222 | Poland   | Biśnik Cave      | 14480 | 90  | OxA-27907     | 17652 | 17407 | 17920 | 17652        | H166 | KT867494 |
| L223 | Poland   | Biśnik Cave      | 15025 | 75  | OxA-27631     | 18256 | 18011 | 18475 | 18256        | H59  | KT867390 |
| L224 | Poland   | Biśnik Cave      | 20610 | 140 | OxA-27632     | 24822 | 24407 | 25251 | 24822        | H167 | KT867495 |
| L225 | Poland   | Biśnik Cave      | 15220 | 90  | OxA-27908     | 18487 | 18265 | 18711 | 18487        | H147 | KT867476 |
| L236 | Poland   | Biśnik Cave      | 13580 | 65  | OxA-27514     | 16362 | 16146 | 16626 | 16362        | H59  | KT867390 |
| L237 | Poland   | Biśnik Cave      | 15120 | 70  | OxA-27634     | 18380 | 18151 | 18581 | 18380        | H147 | KT867476 |
| L241 | Poland   | Biśnik Cave      | 15485 | 80  | OxA-X-2504-49 | 18747 | 18577 | 18905 | 18747        | H59  | KT867390 |
| L242 | Poland   | Biśnik Cave      | 13075 | 65  | OxA-2759      | 15674 | 15369 | 15925 | 15674        | H141 | KT867470 |
| L243 | Poland   | Biśnik Cave      | 17790 | 90  | OxA-27593     | 21550 | 21247 | 21841 | 21550        | H10  | JX867573 |
| L248 | Poland   | Oblazowa Cave    | 21770 | 180 | OxA-X-2543-31 | 26017 | 25706 | 26422 | 26017        | H87  | KT867416 |
| L257 | Poland   | Oblazowa Cave    | 25930 | 380 | OxA-X-2543-17 | 30139 | 29314 | 30917 | 30139        | H168 | KT867496 |
| L260 | E.Russia | Yana RHS         | -     | -   | -             | -     | -     | -     | 30881-33440  | H169 | KT867497 |
| L261 | E.Russia | Yana RHS         | -     | -   | -             | -     | -     | -     | 30881-33440  | H169 | KT867497 |
| L269 | W.Russia | Sikiyaz-Tamak 22 | -     | -   | -             | -     | -     | -     | -            | H170 | KT867498 |
| L270 | W.Russia | Rasik            | -     | -   | -             | -     | -     | -     | 34871-100000 | H171 | KT867499 |
| L271 | W.Russia | Rasik            | -     | -   | -             | -     | -     | -     | 34871-100000 | H172 | KT867500 |
| L273 | W.Russia | Rasik            | -     | -   | -             | -     | -     | -     | 34871-100000 | H173 | KT867501 |
| L275 | W.Russia | Rasik            | -     | -   | -             | -     | -     | -     | 14239-16281  | H102 | KT867431 |
| L276 | W.Russia | Rasik            | -     | -   | -             | -     | -     | -     | 14239-16281  | H174 | KT867502 |

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|      |          |                                         |       |     |               |       |       |       |            |      |                 |
|------|----------|-----------------------------------------|-------|-----|---------------|-------|-------|-------|------------|------|-----------------|
| L317 | W.Russia | Shaplkina-1                             | -     | -   | -             | -     | -     | -     | -          | H104 | KT867433        |
| L319 | W.Russia | Sed'Yu-1                                | -     | -   | -             | -     | -     | -     | -          | H190 | KT867518        |
| L320 | W.Russia | Pizhma-1                                | -     | -   | -             | -     | -     | -     | -          | H192 | KT867520        |
| LP12 | Poland   | Biśnik Cave                             | 16900 | 90  | OxA-28798     | 20383 | 20107 | 20625 | 20383      | H10  | <i>JX867573</i> |
| LP17 | Poland   | Biśnik Cave                             | 19010 | 120 | OxA-28799     | 22885 | 22535 | 23267 | 22885      | H202 | KT867530        |
| LP19 | Poland   | Biśnik Cave                             | 17210 | 90  | OxA-28800     | 20757 | 20511 | 21025 | 20757      | H145 | KT867474        |
| LP20 | Poland   | Biśnik Cave                             | 18320 | 320 | OxA-X-2543-30 | 22157 | 21390 | 22921 | 22157      | H10  | <i>JX867573</i> |
| L372 | E.Russia | Tordokh Site                            | -     | -   | -             | -     | -     | -     | -          | H194 | KT867522        |
| L373 | E.Russia | Duvanny Yar site                        | -     | -   | -             | -     | -     | -     | -          | H195 | KT867523        |
| L374 | E.Russia | Duvanny Yar site                        | -     | -   | -             | -     | -     | -     | -          | H196 | KT867524        |
| LW1  | E.Russia | Bulunskiy district                      | -     | -   | -             | -     | -     | -     | 0 (modern) | H205 | KT867533        |
| LW2  | E.Russia | Bulunskiy district                      | -     | -   | -             | -     | -     | -     | 0 (modern) | H206 | KT867534        |
| LW3  | E.Russia | Bulunskiy district                      | -     | -   | -             | -     | -     | -     | 0 (modern) | H207 | KT867535        |
| LW4  | E.Russia | Bulunskiy district                      | -     | -   | -             | -     | -     | -     | 0 (modern) | H208 | KT867536        |
| LW5  | E.Russia | Bulunskiy district                      | -     | -   | -             | -     | -     | -     | 0 (modern) | H208 | KT867536        |
| LW6  | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | M09  | <i>AJ131440</i> |
| LW7  | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | M09  | <i>AJ131440</i> |
| LW9  | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | H209 | KT867537        |
| LW10 | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | M09  | <i>AJ131440</i> |
| LW13 | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | M09  | <i>AJ131440</i> |
| LW14 | C.Russia | Taymyr Peninsula-Lukunskiy sector       | -     | -   | -             | -     | -     | -     | 0 (modern) | M09  | <i>AJ131440</i> |
| LW15 | C.Russia | Taymyr Peninsula-Verchnya Taymira river | -     | -   | -             | -     | -     | -     | 0 (modern) | H203 | KT867531        |
| LW16 | C.Russia | Taymyr Peninsula-Verchnya Taymira river | -     | -   | -             | -     | -     | -     | 0 (modern) | H204 | KT867532        |

*Median calibrated date and 95% upper and lower calibrated date in italics indicates that the distribution of the calibrated date extended outside the range of the calibration curve. Accession No. in italics indicates previously published haplotype.*

**Table S3. Details of previously published modern and ancient DNA collared lemming sequences. Radiocarbon dates ( $^{14}\text{C}$ ) and median, 95% upper, lower calibrated dates are given in years before present.**

| Specimen ID | Region  | Site              | $^{14}\text{C}$ date | $^{14}\text{C}$ error ( $\pm$ ) | $^{14}\text{C}$ Lab Nr | Median cal. date | lower 95% cal. date | upper 95% cal. date      | Median age or age-range used for tip-dating | Haplotype | Accession No. |
|-------------|---------|-------------------|----------------------|---------------------------------|------------------------|------------------|---------------------|--------------------------|---------------------------------------------|-----------|---------------|
| E012        | Belgium | Marie-Jeanne cave | -                    | -                               | failed                 | -                | -                   | -                        | -                                           | H37       | JX867600      |
| E014        | Belgium | Marie-Jeanne cave | -                    | -                               | failed                 | -                | -                   | -                        | -                                           | H33       | JX867596      |
| E015        | Belgium | Marie-Jeanne cave | 47600                | 3300                            | OxA24115               | <b>49601</b>     | <b>42622</b>        | <i>Date out of range</i> | 49601                                       | H39       | JX867602      |
| E017        | Belgium | Marie-Jeanne cave | > 43900              | -                               | OxA24116               | -                | -                   | -                        | -                                           | H50       | JX867613      |
| E018        | Belgium | Marie-Jeanne cave | 43000                | 1900                            | OxA24117               | <b>46667</b>     | <b>44124</b>        | <i>Date out of range</i> | 46667                                       | H41       | JX867604      |
| E019        | Belgium | Marie-Jeanne cave | 43600                | 2100                            | OxA24118               | <b>47101</b>     | <b>44458</b>        | <i>Date out of range</i> | 47101                                       | H40       | JX867603      |
| E020        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H43       | JX867606      |
| E023        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H36       | JX867599      |
| E024        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H45       | JX867608      |
| E026        | Belgium | Marie-Jeanne cave | 40500                | 1400                            | OxA24119               | <b>44356</b>     | <b>42195</b>        | <b>47430</b>             | 44356                                       | H35       | JX867598      |
| E027        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H44       | JX867607      |
| E029        | Belgium | Marie-Jeanne cave | 44400                | 2300                            | OxA24120               | <b>47561</b>     | <b>44919</b>        | <i>Date out of range</i> | 47561                                       | H42       | JX867605      |
| E036        | Belgium | Marie-Jeanne cave | > 43700              | -                               | OxA24121               | -                | -                   | -                        | -                                           | H34       | JX867597      |
| E045        | Belgium | Marie-Jeanne cave | 12275                | 55                              | OxA24122               | 14199            | 14008               | 14546                    | 14199                                       | H06       | JX867569      |
| E047        | Belgium | Marie-Jeanne cave | 20930                | 140                             | OxA24123               | 25267            | 24807               | 25648                    | 25267                                       | H20       | JX867583      |
| E049        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H49       | JX867612      |
| E050        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H47       | JX867610      |
| E051        | Belgium | Marie-Jeanne cave | -                    | -                               | -                      | -                | -                   | -                        | -                                           | H48       | JX867611      |
| E052        | Belgium | Marie-Jeanne cave | >43000               |                                 | OxA24124               | -                | -                   | -                        | -                                           | H46       | JX867609      |

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|       |          |                    |   |   |   |   |   |   |            |     |          |
|-------|----------|--------------------|---|---|---|---|---|---|------------|-----|----------|
| D.t01 | E.Russia | Olenekskiy Bay     | - | - | - | - | - | - | 0 (modern) | M01 | AJ238423 |
| D.t02 | E.Russia | W.Kolyma           | - | - | - | - | - | - | 0 (modern) | M02 | AJ131441 |
| D.t03 | E.Russia | E.Kolyma           | - | - | - | - | - | - | 0 (modern) | M03 | AJ238425 |
| D.t04 | E.Russia | E. Kolyma          | - | - | - | - | - | - | 0 (modern) | M04 | AJ238424 |
| D.t05 | E.Russia | E. Kolyma          | - | - | - | - | - | - | 0 (modern) | M05 | AJ131442 |
| D.t06 | W.Russia | Pechora            | - | - | - | - | - | - | 0 (modern) | M06 | AF119275 |
| D.t07 | W.Russia | NE Kanin Peninsula | - | - | - | - | - | - | 0 (modern) | M07 | AJ238421 |
| D.t08 | W.Russia | Yamal Peninsula    | - | - | - | - | - | - | 0 (modern) | M08 | AJ131439 |
| D.t09 | C.Russia | Taymyr Peninsula   | - | - | - | - | - | - | 0 (modern) | M09 | AJ131440 |
| D.t10 | C.Russia | Taymyr Peninsula   | - | - | - | - | - | - | 0 (modern) | M10 | AJ238422 |
| D.g01 | E.Russia | Wrangel Island     | - | - | - | - | - | - | 0 (modern) | M11 | AJ131443 |
| D.g02 | Alaska   |                    | - | - | - | - | - | - | 0 (modern) | M12 | AJ131444 |
| D.g03 | Alaska   |                    | - | - | - | - | - | - | 0 (modern) | M13 | AJ238426 |
| D.g04 | Alaska   |                    | - | - | - | - | - | - | 0 (modern) | M14 | AJ238427 |
| D.g05 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M15 | AJ238428 |
| D.g06 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M16 | AJ238429 |
| D.g07 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M17 | AJ238430 |
| D.g08 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M18 | AJ238431 |
| D.g09 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M19 | AJ238432 |
| D.g10 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M20 | AJ238433 |
| D.g11 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M21 | AJ238434 |
| D.h01 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M22 | AJ238437 |
| D.h02 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M23 | AJ238436 |
| D.h03 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M24 | AJ238438 |
| D.r01 | Canada   |                    | - | - | - | - | - | - | 0 (modern) | M25 | AJ238435 |

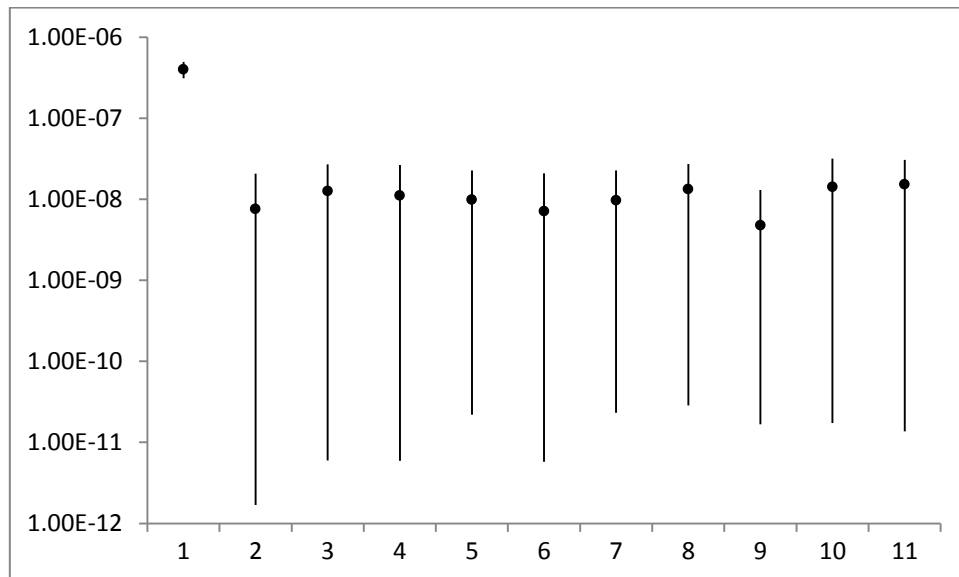
*Median calibrated date and 95% upper and lower calibrated date in italics indicates that the distribution of the calibrated date extended outside the range of the calibration curve.*

**Table S4. Primer pairs designed in this study to amplify the *cyt b* gene from *Dicrostonyx* samples.**

| Primer name                                 | sequence                    | conc.  | Length (bp) | Multiplex reaction |
|---------------------------------------------|-----------------------------|--------|-------------|--------------------|
| CytB1F                                      | TCGTTGTTATTCAACTATAGAAACACC | 0.1 μM | 165         | 1                  |
| CytB1R                                      | GGATTGATAATTAGGCATAGG       | 0.1 μM |             |                    |
| CytB2F                                      | GCTCCATCCAACATTTTCATCA      | 0.1 μM | 160         | 2                  |
| CytB2R                                      | CGTAATTTACATCTCGGCAAA       | 0.1 μM |             |                    |
| CytB3F                                      | ACAGCAACAGCATTCTCGTC        | 0.1 μM | 160         | 1                  |
| CytB3R                                      | TTTCAATTATGTTATAGGAGCCGTA   | 0.1 μM |             |                    |
| CytB4F                                      | TTCATCTGCTTATTCTTACACGTAG   | 0.1 μM | 163         | 2                  |
| CytB4R                                      | CTGTGGCTCCTCAGAAGGAT        | 0.1 μM |             |                    |
| CytB5F                                      | CAGCATTCATAGGATATGTTCTCC    | 0.1 μM | 169         | 1                  |
| CytB5R                                      | TGGAATGCAAAGAATCGTGT        | 0.1 μM |             |                    |
| CytB6F                                      | ATGAATCTGAGGGGGCTTCT        | 0.1 μM | 168         | 2                  |
| CytB6R                                      | ATTTTGTCTGCGTCGGAGTT        | 0.1 μM |             |                    |
| CytB7F                                      | TTTCTTCACGAAACAGGCTCT       | 0.1 μM | 166         | 1                  |
| CytB7R                                      | CTCCGAGAATATCTGGGAAAAA      | 0.1 μM |             |                    |
| CytB8F                                      | TTAGGAGCCCTCCTTCTATT        | 0.2 μM | 170         | 2                  |
| CytB8R                                      | GGATCGTAGGATGGCGTAGA        | 0.2 μM |             |                    |
| CytB9F                                      | CACCACACATTAAGCCAGGA        | 0.1 μM | 160         | 1                  |
| CytB9R                                      | CGGAATGTTAGGCCTCGTT         | 0.1 μM |             |                    |
| CytB10F                                     | AGTACTAGCCCTCATAACCTTCC     | 0.2 μM | 176         | 2                  |
| CytB10R                                     | TTTGCCGATGATGATGAATG        | 0.2 μM |             |                    |
| CytB11F                                     | CTCATCCTCACATGAATTGG        | 0.1 μM | 180         | 1                  |
| CytB11R                                     | TTAATCTAGGTCCAGGATGTTGTT    | 0.1 μM |             |                    |
| Primers for amplification of modern samples |                             |        |             |                    |
| CytBwspF                                    | GGCTCCCTACTTGGCCTAT         | 0.1 μM | 805         |                    |
| CytBWspR                                    | ATGTTAGGCCTCGTTGTTTG        | 0.1 μM |             |                    |

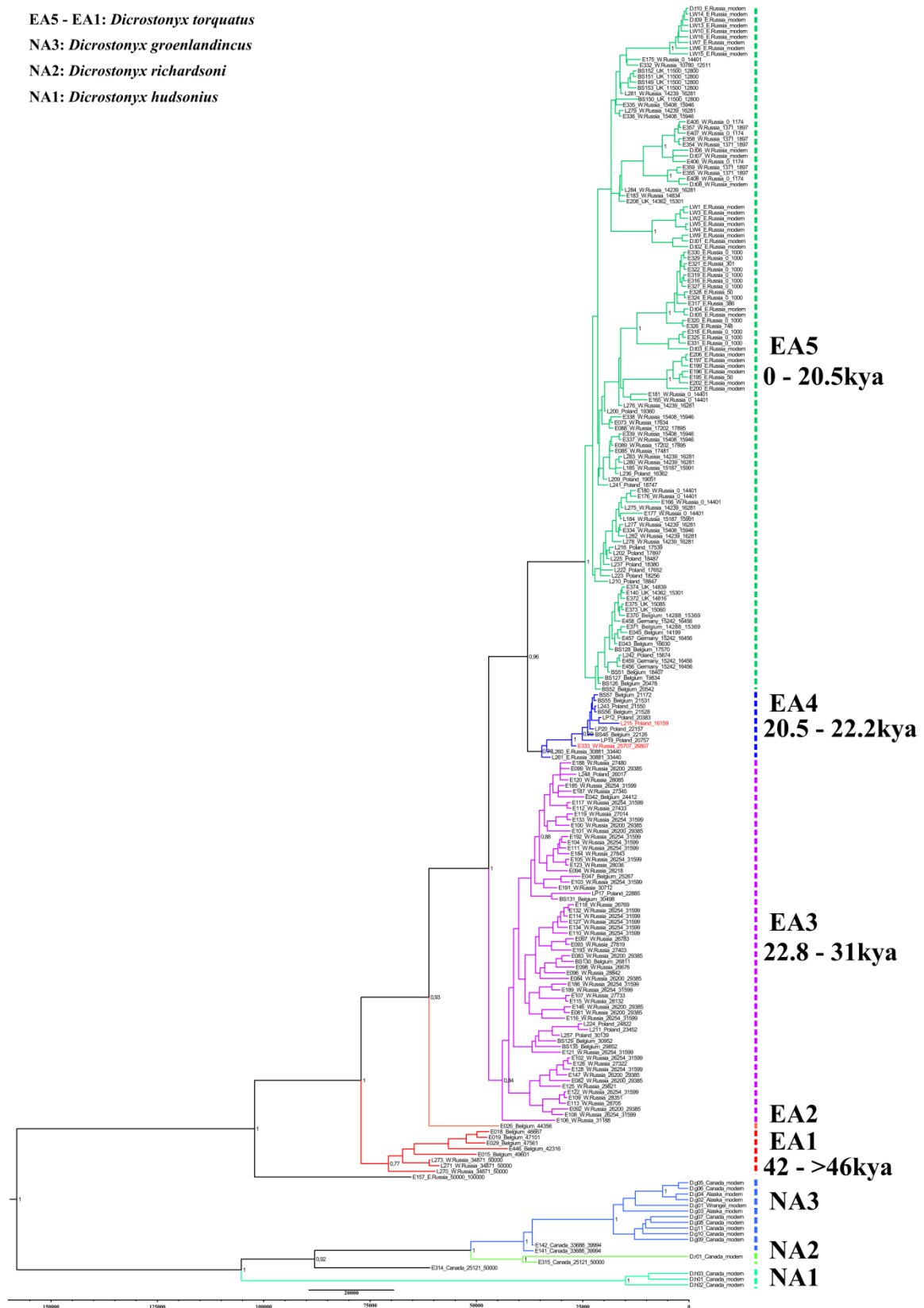
## Figures

**Figure S1.** Date randomization test performed on original data set (1) and ten replicates with dates randomly assigned to sequences (2-11). Filled circles indicate mean estimated substitution rate and lines appropriate 95% HPD intervals. The mean substitution rate estimated from the original dataset falls outside the 95% HPD intervals of substitution rates estimated from the date-randomized datasets, indicating that our original dataset has sufficient temporal signal.

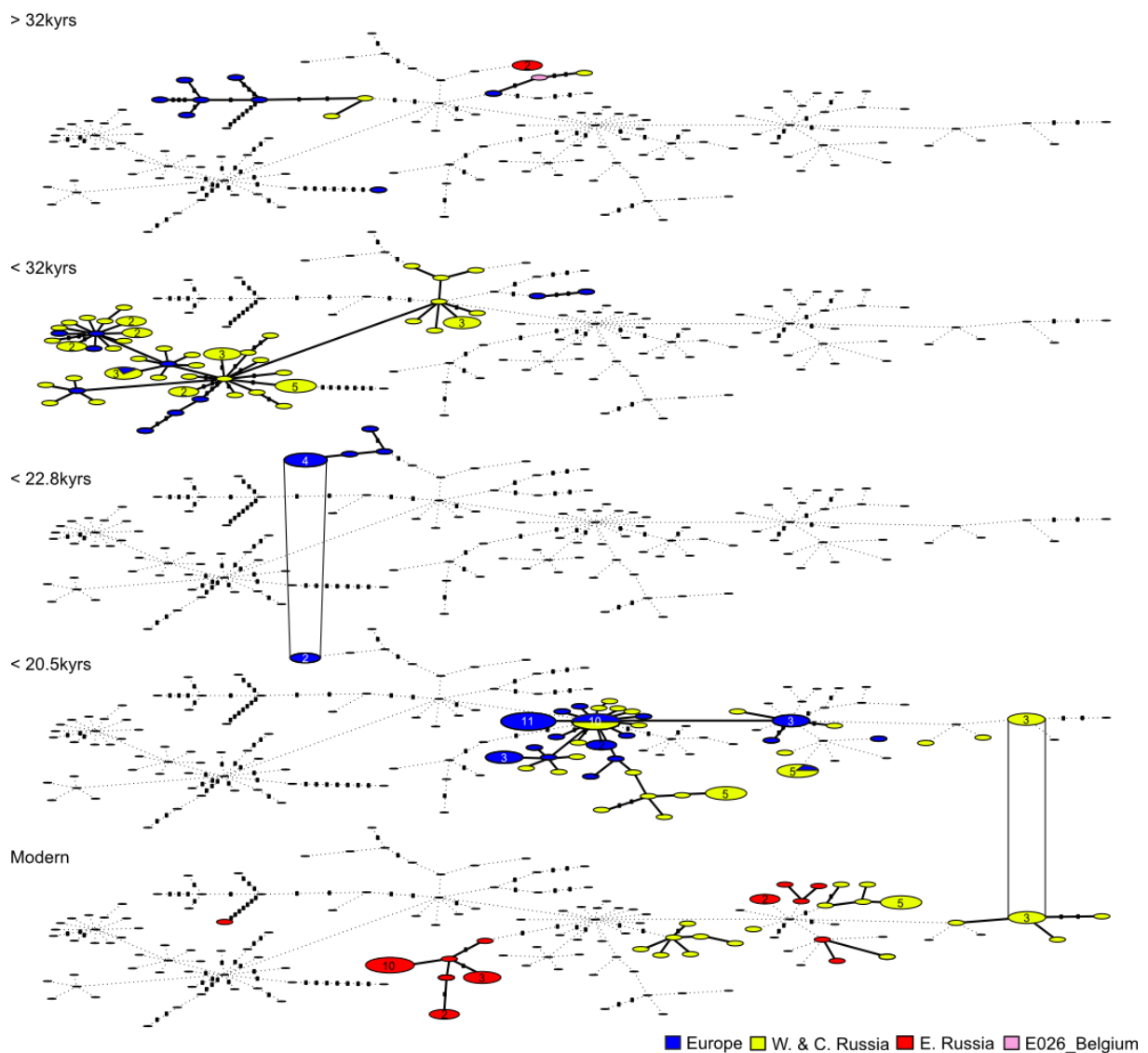


**Figure S2.** Bayesian phylogeny of mtDNA (cyt *b*) sequences that includes samples with finite radiocarbon dates and samples with assigned prior information on their age based on stratigraphic information and associated dates. Bayesian posterior probabilities of major nodes above 0.8 are shown. The name, locality and median calibrated date or prior range for tip-dating of each sequence are given as tip-labels. Sequences mentioned in the text are coloured in red. The temporal range of each mtDNA lineage is shown next to the vertical dotted lines indicating the lineages. The timescale on the x-axis is in calendar years before present (ky BP).

**NA1: *Dicrostonyx hudsonius***



**Figure S3.** Three-dimensional statistical parsimony network of *D. torquatus* mtDNA (cyt *b*) sequences. Layers correspond to the different temporal intervals. Haplotypes from different regions are indicated by different colors. Small black dots represent missing haplotypes. Small black circles represent haplotypes that are missing from a layer but are present in other layers. The size of each circle indicates haplotype frequency with the number in each circle indicating the number of samples carrying that particular haplotype when it is higher than one.



**Figure S4.** (A) Palynological record from Lago Grande di Monticchio in Italy (Allen *et al.* 1999), (B)  $\delta^{18}\text{O}$  records from the NorthGRIP ice core (GICC05) (Svensson *et al.* 2008), and (C) distribution of calibrated date ranges of radiocarbon-dated specimens for each of the distinct mitochondrial lineages. Time on the x-axis is given in years before present. Light blue boxes indicate the time ranges of mitochondrial lineage replacements.

