

# Middle Pleistocene genome calibrates a revised evolutionary history of extinct cave bears

Axel Barlow<sup>\*1,2</sup>, Johanna L. A. Paijmans<sup>3</sup>, Federica Alberti<sup>1</sup>, Boris Gasparyan<sup>4</sup>, Guy Bar-Oz<sup>5</sup>, Ron Pinhasi<sup>6</sup>, Irina Foronova<sup>7</sup>, Andrey Y. Puzachenko<sup>8</sup>, Martina Pacher<sup>9</sup>, Love Dalén<sup>10,11</sup>, Gennady Baryshnikov<sup>12</sup>, Michael Hofreiter<sup>1</sup>.

<sup>1</sup>School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, United Kingdom.

<sup>2</sup>Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24–25, 14476 Potsdam, Germany.

<sup>3</sup>School of Archaeology and Ancient History, University of Leicester, University Road, Leicester LE1 7RH, UK.

<sup>4</sup>Institute of Archaeology and Ethnography, National Academy of Sciences of the Republic of Armenia, 0025, RA, Yerevan, 15 Charents st., Armenia.

<sup>5</sup>The Zinman Institute of Archaeology, University of Haifa, 199 Aba-Hushi Avenue, Haifa, Israel 3498838

<sup>6</sup>Department of Evolutionary Anthropology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria.

<sup>7</sup>V.S. Sobolev Institute of Geology and Mineralogy, Siberian Branch of the Russian Academy of Sciences, 3, Ac. Koptuga ave., Novosibirsk, Russia, 630090.

<sup>8</sup>Institute of Geography, Russian Academy of Sciences, Staromonetnyy Pereulok, 29, Moscow, Russia, 119017.

<sup>9</sup>Institute of Paleontology, University of Vienna, Geozentrum, UZA II, Althanstraße 14, A - 1090 Vienna, Austria.

<sup>10</sup>Centre for Palaeogenetics, Stockholm University, SE-106 91 Stockholm, Sweden.

<sup>11</sup>Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Svante Ahrenius väg 3, 114 18 Stockholm, Sweden.

<sup>12</sup>Zoological Institute, Russian Academy of Sciences, Universitetskaya Naberezhnaya 1, 199034 St. Petersburg, Russia.

\*Lead Contact: axel.barlow.ab@gmail.com

## SUMMARY

Palaeogenomes provide the potential to study evolutionary processes in real time, but this potential is limited by our ability to recover genetic data over extended timescales [1]. As a consequence, most studies so far have focused on samples of Late Pleistocene or Holocene age, which covers only a small part of the history of many clades and species. Here, we report the recovery of a low coverage palaeogenome from the petrous bone of a ~360,000 year old cave bear from Kudaro 1 cave in the Caucasus Mountains. Analysis of this genome alongside those of several Late Pleistocene cave bears reveals widespread mito-nuclear discordance in this group. Using the time interval between Middle and Late Pleistocene cave bear genomes, we directly estimate ursid nuclear and mitochondrial substitution rates to calibrate their respective phylogenies. This reveals post-divergence mitochondrial transfer as the dominant factor explaining their mito-nuclear discordance. Interestingly, these transfer events were not accompanied by large-scale nuclear introgression. However, we do detect additional instances of nuclear admixture among other cave bear lineages,

and between cave bears and brown bears, which are not associated with mitochondrial exchange. Genomic data obtained from the Middle Pleistocene cave bear petrous bone has thus facilitated a revised evolutionary history of this extinct megafaunal group. Moreover, it suggests that petrous bones may provide a means of extending both the magnitude and time depth of palaeogenome retrieval over substantial portions of the evolutionary histories of many mammalian clades.

## KEYWORDS

Palaeogenomics, ancient DNA, cave bear, *Ursus*, Middle Pleistocene, evolution

## RESULTS AND DISCUSSION

Analyses of palaeogenomes have provided unparalleled insights into the evolution of numerous vertebrate lineages. Assembling these datasets represents a considerable technical challenge, however, due to postmortem degradation and the loss of endogenous DNA molecules over time [1]. This is especially true for warm temperate and tropical environments, where DNA degradation proceeds more rapidly than in colder boreal or arctic environments [1]. As a consequence, comparatively few ancient DNA studies have successfully retrieved genetic data from temperate zone samples of Middle Pleistocene age (Chibanian age, 129–774 ka [2]). Notable successes include high coverage genome datasets from samples dating around the Middle to Late Pleistocene boundary from Germany [3,4] and from the Altai Mountains [5]. Much older DNA sequences have been retrieved from samples dating to ~430 ka from Spain, but with a lower magnitude of data recovery, comprising of mitochondrial genome sequences [6,7] and 1–2 megabases of nuclear DNA [8]. Nonetheless, these achievements suggest that the retrieval of genome-scale datasets of this age is possible, provided samples of sufficient quality can be found.

### *Middle Pleistocene cave bear genome*

One recent and notable advance in palaeogenome sequencing has been the discovery of the mammalian petrous bone as a source of high purity ancient DNA [9]. One group where this approach has been successfully applied are extinct cave bears [10,11], which form the sister lineage to the clade consisting of the extant brown (*Ursus arctos*) and polar (*Ursus maritimus*) bears. To investigate if petrous bones may provide a way of extending both time depth and magnitude of Middle Pleistocene genome data retrieval, we investigated the petrous bone of a Middle Pleistocene cave bear from Kudaro 1 cave located in South Ossetia in the Southern Caucasus. The sedimentary layer (5c) from which this sample was recovered has been dated using radiothermoluminescence at  $360,000 \pm 90,000$  years [12], and multiple additional sources of evidence support a Middle Pleistocene age for the specimen (see STAR Methods). The Kudaro 1 sample is assigned to the taxon *Ursus kudarensis praekudarensis*, which is thought to be ancestral to the Late Pleistocene Caucasian cave bear *U. k. kudarensis* based on morphological evidence [13,14].

We extracted DNA from the *praekudarensis* petrous bone and sequenced it using Illumina technology. From a total of ~2.6 billion sequenced molecules, we were able to map 2.1 Gb of sequence with high confidence to the reference genome assembly of the polar bear (Table S1). This represents a low coverage genome dataset where the majority of sequenced positions are covered by a single sequencing read (Figure S1). The estimated proportion of endogenous DNA molecules in the *praekudarensis* extract is 3.6% (Table S1), which is remarkable given the age of the specimen,

and exceeds the endogenous proportions of some previously studied temperate-zone Middle Pleistocene extracts [8] by several orders of magnitude.

### ***Cave bear nuclear and mitochondrial relationships are highly incongruent***

Phylogenetic relationships among cave bear taxa have been largely guided by analysis of their mitochondrial DNA [15,16]. Our previous study on nuclear genomes did reveal one instance of mito-nuclear discordance among three Late Pleistocene taxa [10], but the limited sampling of this study precluded a broader assessment of cave bear nuclear relationships. To investigate this further, we analysed the Middle Pleistocene *praekudarensis* genome dataset alongside novel genome datasets of Late Pleistocene cave bears generated from their petrous bones, representing the taxa *rossicus* (Kizel Cave, Ural Mountains, Russia), *kanivetz* (Medvezhiya Cave, Ural Mountains, Russia), and *kudarensis* (Hovk 1 Cave, Armenia). We also included published datasets from the taxa *spelaeus* (Eiros Cave, Spain) [10], *eremus* (Windischkopf Cave, Austria) [10], *ingressus* (Gamssulzen Cave, Austria) [11], and a second *kudarensis* individual from Hovk 1 Cave [10] in addition to modern Georgian and Late Pleistocene Austrian brown bears [10], two modern polar bears [17], and a modern Asiatic black bear [18] as outgroup (see Table S1 & S2).

We investigated relationships among the cave bear nuclear genomes using Principal Components Analysis (PCA). This involved sampling a single mapped nucleotide from each individual at each position of the reference genome, which provided data from a total of 487,747 variable transversion sites after strict filtering (STAR Methods). PCA suggested three major groups (Figure 1A) comprising: the Caucasus cave bears *praekudarensis* and *kudarensis*, which cluster together as predicted by morphology; a second, geographically widespread and broadly European group including *spelaeus*, *ingressus*, *eremus*, and *kanivetz* from the Urals; and finally, the Urals cave bear *rossicus*, which is distinct from all other cave bears and may represent a Urals-specific group. PCA further suggests a hierarchy of relationships within these groups, with each successive PC separating different taxa from one another (Figure 1B).

The three major groups identified by the PCA deviate from expectations based on mitochondrial DNA, which instead supports two major clades comprising the Caucasus cave bears and all European and Urals cave bears, respectively, with *rossicus* nested within the latter (Figure 1C). We further investigated these contrasting nuclear relationships using phylogenetic analysis, including representative brown bears, polar bears and the Asiatic black bear outgroup. Palaeogenomic datasets are generally associated with high rates of error, which can distort estimates of phylogenetic branch lengths when only a single read is sampled [11]. We therefore applied a recently developed method, Consensify [11], which calls the majority base from a random sample of three mapped nucleotides. This method provided a single high quality allele from each individual for a total of 4,318,414 genomic sites, of which 39,122 were variable. Maximum likelihood phylogenetic analysis of this dataset (Figure 1D) supported the expected relationships between polar bears, brown bears, and the cave bear clade, as well as the position of the Caucasus cave bears within the latter. Among the sampled cave bears from Europe and the Urals, however, there is not a single sister-group relationship that agrees between the mitochondrial and nuclear phylogenies.

The relationships among cave bears inferred using nuclear DNA closely match estimates based on morphological characters [19,20], in contrast to mitochondrial relationships, which are frequently

139 incongruent with morphology. Mitochondrial DNA, which has provided the basis for our  
140 understanding of cave bear relationships for several decades, thus emerges as a phylogenetic outlier  
141 in contradiction with generally congruent evidence from morphology and nuclear genomes.  
142 Mitochondrial evolution in cave bears therefore appears to have been shaped to a large extent by  
143 incomplete lineage sorting and/or gene flow among cave bear lineages, as previously documented in  
144 brown bears and polar bears [17].

145  
146 The revised cave bear nuclear genome phylogeny provides several new insights into their evolution.  
147 Consistent with the results of the nuclear PCA, the Uralian cave bear *rossicus* represents a deeply  
148 divergent and isolated phylogenetic lineage (Figure 1D), supporting its recognition as a third major  
149 cave bear group, which has been obscured until now due to reliance on mitochondrial DNA. Within  
150 the European cave bear group, three large bodied taxa, *spelaeus*, *ingressus* and *kanivetz*, form a  
151 clade that is sister to the smaller bodied cave bear *eremus*. This suggests a single increase in body  
152 size in their common ancestor, rather than two independent shifts as previously inferred from their  
153 mitochondrial relationships [15]. Moreover, the validity of the taxon *kanivetz* has itself been  
154 questioned by mitochondrial phylogeographic studies [15], which show it to be nested within the  
155 wider *ingressus* mitochondrial clade. Nuclear genome analysis, however, supports its  
156 distinctiveness and position as sister to the European taxa *spelaeus* and *ingressus*. Finally, the first  
157 three primary phylogenetic divisions of cave bears involve Uralian or Asian lineages, potentially  
158 reflecting an eastern origin for what has traditionally been regarded as a European radiation [21].

#### 159 160 ***Direct estimation of the genome-wide substitution rate***

161 The temporal gap between *praekudarensis* and its Late Pleistocene sister lineage *kudarensis*  
162 provides an opportunity to estimate the *Ursus* substitution rate and calibrate the revised nuclear  
163 phylogeny of cave bears. We calculated the difference in their respective genetic divergences to  
164 modern brown bear and polar bear outgroups using the 4,318,414 Consensify error-reduced nuclear  
165 positions, which provides an estimate of the number of nucleotide substitutions occurring during  
166 their sampling interval. Based on the median *praekudarensis* radiothermoluminescence date and the  
167 age estimates of the *kudarensis* samples (Table S2), this equates to 305,400 years and yields a  
168 genome-wide nuclear substitution rate of  $9.56 \times 10^{-10}$  substitutions/site/year (range  $7.39\text{--}13.56 \times 10^{-10}$   
169  $^{10}$  substitutions/site/year accommodating the radiothermoluminescence date uncertainty, Table S3).  
170 This estimate is substantially slower than ancient-DNA derived estimates for dogs and wolves  
171 ( $\sim 1.2 \times 10^{-8}$  [22]), but exceeds published estimates for humans and other great apes [23]. Notably,  
172 our estimated *Ursus* substitution rate is approximately double that estimated for humans ( $5 \times 10^{-10}$   
173 substitutions/site/year [24]), which aligns well with the difference in their respective generation  
174 times (brown and polar bears 11–12 years [25,26], humans 20–25 years [27,28]), suggesting their  
175 underlying per-generation nuclear mutation rate is approximately equal. Applying the same  
176 methodology to mitochondrial DNA (excluding the control region) produced an estimated *Ursus*  
177 mitochondrial substitution rate of  $1.81 \times 10^{-8}$  substitutions/site/year (range  $1.40\text{--}2.57 \times 10^{-8}$   
178 substitutions/site/year, Table S3). This estimate falls within the lower range of mitochondrial  
179 substitution rate estimates for other vertebrates [29]. It also overlaps with estimated rates for human  
180 mitochondrial DNA [30], deviating from the ratio predicted by generation times and suggesting  
181 some difference in the underlying rate of mitochondrial mutations between humans and bears.

182  
183 We used these newly estimated substitution rates to calculate absolute times of nuclear and  
184 mitochondrial divergence for all pairs of individuals (Figure 2, Tables S4 and S5), and calibrate

185 their respective phylogenies (Figure 3). We note that the divergence times of genetic lineages will  
 186 be older than the divergence of their respective populations as they likely include standing variation  
 187 in the ancestral population. However, since the lineages under study span a large evolutionary  
 188 timescale and show high levels of structuring (see D-statistic analysis below), the obtained times  
 189 most likely provide reasonable approximations. Median nuclear divergence time estimates of cave  
 190 bears and their sister clade, polar bears and brown bears, were found to be around 1.52 Ma (Figures  
 191 2A and 3). The mitochondrial divergence time is similar, around 1.48 Ma (Figures 2A and 3). These  
 192 estimates are more recent than most previous estimates based on mitochondrial DNA, but highly  
 193 consistent with the fossil record (see STAR Methods). Median estimates for the polar bear and  
 194 brown bear nuclear divergence are around 0.99 Ma (Figures 2B and 3), which is highly similar to  
 195 previous phylogenetic estimates [18]. Median nuclear divergence times of the three major cave bear  
 196 clades also fall around the same time, around 0.98 (Figures 2C and 3) and 0.87 Ma (Figures 2D and  
 197 3). Notably, these primary divergence events among cave bears, as well as between brown bears and  
 198 polar bears, coincide with the Middle Pleistocene Transition, 1.2–0.8 Ma, when glacial cycles  
 199 shifted from a ~40 to a ~100 ka periodicity causing extended glacial periods and more abrupt,  
 200 intense interglacials [31], which may have been a factor promoting their divergence. Within the  
 201 Caucasian cave bears, we find a comparatively deep divergence time between *kudarensis* and  
 202 *praekudarensis* (median estimate ~495 ka at the age of *praekudarensis*; Figures 2E and 3),  
 203 potentially arguing against the direct ancestor descendant-relationship suggested by morphology, or,  
 204 alternatively, for a genetically structured *praekudarensis* population. Among the European cave  
 205 bears, we find a comparatively rapid sequence of divergence events among the four sampled taxa  
 206 (median ages 427–344 ka, Figures 2F and 3), which are notably older than previous estimates based  
 207 on mitochondrial DNA [15,32].

#### 210 ***Mitochondrial transfer explains mito-nuclear discordance in cave bears***

211 Time calibration of the nuclear and mitochondrial cave bear phylogenies could reveal the  
 212 underlying causes of their discordance. Specifically, mitochondrial coalescence that considerably  
 213 post-dates the respective nuclear coalescence of taxa implies the transfer of mitochondrial DNA  
 214 through admixture. Examination of cave bear pairs exhibiting mito-nuclear discordance (defined as  
 215 pairs with different taxa descending from the ancestral nodes of their respective nuclear and  
 216 mitochondrial clades) reveals that all pairs but one (*spelaeus* and *ingressus*) show mitochondrial  
 217 coalescence that is more recent than their respective nuclear coalescence (Figure 2, Tables S3 and  
 218 S4), implicating mitochondrial transfer as the dominant factor shaping their mitochondrial  
 219 relationships.

221 Multiple alternative transfer scenarios could explain the observed mitochondrial relationships, and  
 222 selecting among them is challenging based on the available data. One notable set of comparisons  
 223 are those involving the Uralian cave bear *rossicus* and the European cave bears *ingressus* and  
 224 *kanivetz*, whose mitochondrial coalescence times post-date their respective nuclear coalescence by  
 225 more than 700 ka (Figure 2D). Since none of the sampled European or Uralian mitochondrial  
 226 lineage pairs approaches the nuclear coalescence of *rossicus* and the European cave bear group  
 227 (median estimate ~0.87 Ma), it seems most likely that *ingressus* or *kanivetz* transferred its  
 228 mitochondrial DNA to *rossicus*, although alternative scenarios such as multiple transfers or back  
 229 transfers cannot be conclusively excluded. Also notable are mitochondrial coalescence times of the  
 230 European cave bears *spelaeus* and *eremus*, and *ingressus* and *kanivetz*, which both considerably

231 post-date their respective nuclear divergences ( $> 290$  ka based on median substitution rate  
232 estimates; Figure 2F), implicating mitochondrial transfer also between lineages of these pairs.  
233 Determining the direction of these transfer events is challenging due to the rapid sequence of  
234 nuclear divergence events in European cave bears, around which many of their mitochondrial  
235 coalescence events also cluster (Figure 2F). The precise history of mitochondrial evolution is likely  
236 to remain uncertain until genetic data from samples pre-dating the inferred mitochondrial transfer  
237 events is obtained, which would require obtaining DNA sequences from multiple additional Middle  
238 Pleistocene specimens.

239

240

#### 241 ***Mitochondrial transfer is not strongly associated with nuclear introgression***

242 Since our analyses reveal numerous instances of mitochondrial transfer among cave bears, we  
243 investigated evidence of nuclear gene flow using D-statistic analysis of the error reduced genome  
244 sequences, which has been shown to represent a conservative approach that is more resistant to false  
245 positives than other methods [11]. Interestingly, we failed to detect nuclear gene flow among those  
246 cave bear taxa implicated in mitochondrial transfer events (Figure 4), suggesting that any residual  
247 nuclear introgression resulting from these gene flow events is below the detection threshold of our  
248 low coverage genome datasets. In fact, only a single nuclear gene flow event is detected among the  
249 sampled cave bear lineages, between the lineage leading to *kudarensis* and the lineage leading to the  
250 European cave bears (Figure 4). Notably, this nuclear admixture event is not accompanied by any  
251 evidence of mitochondrial transfer. Similarly, we have previously shown that gene flow occurred  
252 between cave bears and brown bears [10], again with no evidence of mitochondrial exchange.  
253 Replicating these tests including our novel cave bear genomes consistently supports this previous  
254 result, and moreover, our improved sampling pinpoints the common ancestor of the European and  
255 Uralian cave bears as the admixing cave bear lineage (Figure 4), with no evidence of gene flow  
256 following the divergence of its descendant clades.

257

258 This disparity between instances of nuclear and mitochondrial introgression within a recently  
259 diverging clade is puzzling. Enhanced mitochondrial introgression is documented in species with  
260 male-biased dispersal, such as bears, since this reduces the effective population size of maternally  
261 inherited loci in the incoming species at the contact zone [33]. However, this process does not  
262 explain the apparent absence of mitochondrial mixing among Caucasian and European cave bears,  
263 or among cave bears and brown bears. Here, some additional factor, such as a complete absence of  
264 females representing the incoming species, preferential mating of hybrids with their maternal  
265 species, or asymmetrical hybrid sterility [34], must have operated.

266

267

#### 268 ***Conclusions***

269 We have shown that petrous bones provide a way to extend both the time depth and magnitude of  
270 Middle Pleistocene genome sequencing. The palaeogenome of the Middle Pleistocene  
271 *praekudarensis* cave bear sequenced in this study has provided important insights into the evolution  
272 of this iconic group of extinct animals. Critically, by providing a means of calculating the genome  
273 substitution rate, it has calibrated their evolutionary history, revealed numerous instances of  
274 mitochondrial transfer, and suggested a potential link between a profound change in climate  
275 dynamics and the divergence of major evolutionary lineages.

276

277 Palaeogenomes provide the opportunity to study the process of evolution in real time. For many  
278 temperate zone species, however, the current time depth for palaeogenome recovery represents a  
279 comparatively small part of their total evolutionary history. The age of the *praekudarensis* genome  
280 pre-dates the origin of some cave bear taxa and encompasses an estimated 24% of the total  
281 evolutionary history of cave bears. Petrous bones may thus provide a means of extending the  
282 time depth of palaeogenome recovery over substantial fractions of the evolutionary histories of  
283 many temperate zone species.

284

285

## 286 **AUTHOR CONTRIBUTIONS**

287 In alphabetical order: Conceptualization AB, GB, MH; Data curation AB; Formal analysis AB,  
288 JLAP; Funding acquisition MH; Investigation FA, MP; Methodology AB; Project administration  
289 AB, LD, MH; Resources BG, EB, GB, GB-O, IF, LD, RP; Software AB, JLAP; Supervision AB,  
290 AP, GB, MH; Validation AB, JLAP; Visualization AB; Writing – original draft AB; Writing –  
291 review & editing all authors.

292

293

## 294 **ACKNOWLEDGMENTS**

295 We pay tribute to our coauthor Irina Foronova for her contributions to the field of palaeontology,  
296 who sadly passed away prior to the publication of this study. We thank Anthony J. Stuart for his  
297 help collecting radiocarbon dates of Ural cave bears. The authors would like to acknowledge  
298 support from the Science for Life Laboratory, the National Genomics Infrastructure (NGI), Sweden,  
299 the Knut and Alice Wallenberg Foundation and UPPMAX for providing assistance in massively  
300 parallel DNA sequencing and computational infrastructure. This work was funded by European  
301 Research Council (ERC) consolidator grant ‘gene flow’ 310763 to M.H. The study was fulfilled  
302 within a framework of the Federal theme of the Theriology laboratory of Zoological Institute of the  
303 Russian Academy of Sciences no. AAAA-A19-119032590102-7 "Phylogeny, morphology and  
304 systematics of placental mammals" and project "The evolution of the organic world. Role and  
305 influence of planetary processes (subprogram I. Development of life and biosphere processes)" and  
306 Program of the Russian Academy of Sciences Presidium and the Russian Ministry of Education and  
307 Science "Evolution of the organic world. The role and significance of planetary processes" (2019)  
308 (GB), and the Federal theme of the Laboratory of Biogeography of Institute of Geography of the  
309 RAS no. AAAA-A19-119021990093-8 "Evaluation of physical and geographical, hydrological, and  
310 biotic environmental changes and their effects for the base of sustainable environmental  
311 management" (AP) and supported partly by a RFBR grant to AP (17-01-00100-a); and on the state  
312 assignment of V.S. Sobolev Institute of Geology and Mineralogy, Siberian Branch of the RAS (IF).

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314

## 315 **DECLARATION OF INTERESTS**

316 The authors declare no competing interests.

## 317 MAIN TEXT FIGURE TITLES AND LEGENDS

318

### 319 **Figure 1. Cave bear relationships.**

320 A. Ordination of individual cave bears along the first and second principal components of a PCA  
321 based on 487,747 filtered transversion sites supports three major groups.  
322 B. Ordination of the same individuals along PC3–PC6, which separate, respectively: *eremus*,  
323 *kanivetz*, *kudarensis* and *praekudarensis*, and *ingressus* and *spelaeus*  
324 C. Mitochondrial phylogeny based on 16,383 bp of aligned sequence and rooted using the Asiatic  
325 black bear outgroup (not shown). Bootstrap support is 100% for all nodes.  
326 D. Nuclear phylogeny based on 39,122 filtered, error-reduced variable sites. Rooting and bootstrap  
327 support are as described for C. There are multiple instances of mito-nuclear discordance within the  
328 European cave bears, among the European and Uralian cave bears, and among brown bears and  
329 polar bears.

330

### 331 **Figure 2. Pairwise nuclear and mitochondrial divergence times.**

332 Points indicate ages estimated from substitution rates calculated using the median  
333 radiothermoluminescence age of the *praekudarensis* sample of 360 ka. Error bars represent the  
334 maximum and minimum ages reflecting the  $\pm 90$  ka uncertainty of the radiothermoluminescence  
335 age (See Tables S3–S5). Note that since the age uncertainties of the pairwise estimates are not  
336 independent, the rank order and relative separation of the point estimates will be maintained  
337 irrespective of the true *praekudarensis* age. Sample pairs showing mito-nuclear discordance are  
338 marked with asterisks. Results for specific clades discussed in the text are indicated (A–F).

339

### 340 **Figure 3. Calibrated nuclear and mitochondrial phylogenies.**

341 Branches terminate at the sample ages and nodes are centered on the mean of their respective  
342 pairwise estimates (See Figure 2 and Tables S3–S5). The complete mitochondrial evolutionary  
343 history of the European and Uralian cave bears is uncertain. The three recent mitochondrial transfer  
344 events that can be inferred from pairwise estimates (Figure 2) are indicated by vertical arrows.  
345 Shaded trapezoids connecting lineages indicate the two major episodes of nuclear gene flow  
346 identified by D-statistic analysis, and are coloured consistently with Figure 4.

347

### 348 **Figure 4. D-statistics tests of cave bear admixture.**

349 Results are expressed as D(P1,P2,P3,P4) with significant positive values (filled circles) indicating  
350 admixture between P2 and P3 subsequent to the divergence of P1 and P2, coloured consistently  
351 with the shaded trapezoids in Figure 3. To aid visualisation, the assignment of the P1 and P2  
352 individuals has been adjusted to make their respective D value positive. The results indicate two  
353 major episodes of nuclear gene flow among the sampled lineages: between brown bears and the  
354 ancestor of the European and Uralian cave bears, subsequent to their divergence from their  
355 respective sister lineages, polar bears D(polar,brown,cave,out) and the Caucasian cave bears  
356 D(Caucasian,European/Uralian,brown–polar,out); and between *kudarensis* and the ancestor of the  
357 European cave bears subsequent to their divergence from their respective sister lineages,  
358 *praekudarensis* D(prakudarensis,kudarensis,European/Uralian,out) and the Uralian cave bear  
359 *rossicus* D(Uralian,European,Caucasian,out).



## 360 STAR METHODS

361

## 362 RESOURCE AVAILABILITY

363

### 364 *Lead contact*

365 Further information and requests for resources should be directed to and will be fulfilled by the  
366 Lead Contact, Axel Barlow (axel.barlow.ab@gmail.com).

367

### 368 *Materials availability*

369 This study did not generate new unique reagents.

370

### 371 *Data and code availability*

372 The raw, unprocessed sequencing reads in fastq file format, in addition to the processed data  
373 mapped to each reference genome in bam file format, are available at the European Nucleotide  
374 Archive (ENA), accessions [submitted awaiting accessions]. Novel cave bear consensus  
375 mitochondrial sequences are available at the NCBI nucleotide database, accessions [submitted  
376 awaiting accessions].

377

## 378 EXPERIMENTAL MODEL AND SUBJECT DETAILS

379 The novel cave bear datasets generated for this study are: *praekudarensis* (KU1), *kudarensis*  
380 (HV72), *rossicus* (B05), and *kanivetz* (B04). These were generated from subfossil petrous bone  
381 samples. Details of sample localities and ages are shown in Table S2.

382

383 Several sources of evidence support a Middle Pleistocene age of layer 5c in Kudaro 1 Cave, from  
384 which the *praekudarensis* petrous bone was recovered: layer 5c has been dated using  
385 radiothermoluminescence at  $360,000 \pm 90,000$  yBp, and the overlying layer 5b has been dated  
386 using the same method at  $350,000 \pm 70,000$  yBp [12,35,36]; geomorphological studies of river  
387 terraces of the Caucasus [37] suggest the cave entrance of Kudaro 1 was opened 300–400 thousand  
388 years ago; archaeological material recovered from layer 5c belongs to the later Acheulean [36]; and  
389 other faunal material recovered from layer 5c is consistent with a Middle Pleistocene age [38],  
390 including *Macaca sp.*, *Canis mosbachensis*, *Panthera gombaszoegensis* and *Stephanorhinus*  
391 *hundsheimensis* (the rhinoceros was identified by [39] as *Dicerorhinus etruscus brachycephalus*).  
392 According to European markers, this faunal composition would suggest an even older age than  
393 indicated by the radiothermoluminescence dates. However, the Caucasus region is considered to  
394 represent a refugial area during the Pleistocene, which may explain the apparently more recent  
395 occurrence of these taxa in Kudaro 1 compared to Middle Pleistocene European deposits.

396

397 The *rossicus* and *kanivetz* samples have not been directly dated. For the purpose of molecular dating  
398 analyses, we used an indirectly estimated age for these samples based on the median age of  
399 radiocarbon dated cave bear bones from these sites (Table S6).

400

401

## 402 METHOD DETAILS

403

### 404 *Laboratory methods*

405 Six sequencing libraries (KU1\_1–KU1\_6) were prepared from the *praekudarensis* KU1 sample for  
406 this study. For the *kudarensis* sample HV72 we had previously prepared seven sequencing libraries  
407 (HV72\_1–HV72\_7) and carried out low level sequencing. Six of these libraries (HV72\_2 –  
408 HV72\_7) are described in [40]. We prepared one additional library (HV72\_8) from this sample for  
409 this study, which was used for deeper sequencing. Single libraries were prepared from the *rossicus*  
410 B05 and *kanivetz* B04 samples.

411

412 For each library, bone was sampled from the otic capsule of the cave bear petrous bones [41] and  
413 ground to a fine powder using a RETSCH mixer mill mm 400 at a frequency of 30 Hz for 10  
414 seconds. DNA was then extracted using a published protocol optimized for the recovery of short  
415 ancient DNA fragments [6], with the modifications described in [42]. 50 mg of bone powder was  
416 digested in 1 mL of extraction buffer (0.45 M EDTA, 0.25 mg/mL Proteinase K) overnight at 37°C,  
417 with rotation. Centrifugation was used to pellet any undigested material. The supernatant was  
418 removed, combined with 13 mL of binding buffer (5 M guanidine hydrochloride, 40% (vol/vol)  
419 isopropanol, 0.05% Tween-20, and 90 mM sodium acetate), and then passed through a commercial  
420 silica spin column (Qiagen MinElute) fitted with an extension reservoir (Zymo- spin V). Two wash  
421 steps were then carried out using PE buffer (Qiagen). Purified DNA was then eluted in two steps  
422 each using 12.5 µL TET buffer (10mM Tris-HCl, 1 mM EDTA, 0.05% Tween-20).

423

424 Illumina sequencing libraries were prepared from the DNA extracts using a published protocol  
425 based on single stranded DNA, optimised for the recovery of short ancient DNA fragments [43],  
426 with the modifications described in [42]. DNA was treated with the enzymes uracil-DNA  
427 glycosylase and Endonuclease VIII, to excise uracils resulting from the deamination of cytosine  
428 residues and to cleave DNA strands at abasic sites, in 44 µL reactions with the following reagent  
429 concentrations: 1.8x CircLigase buffer II, 4.5 mM MnCl<sub>2</sub>, 0.11 U/µL of uracil-DNA glycosylase,  
430 and 0.02 U/µL of endonuclease VIII. Residual phosphate groups were then removed from the  
431 template DNA fragment ends using 1 unit of FastAP. The DNA was then heat denatured, and oligo  
432 CL78 ligated to the 3' single-stranded fragments ends during overnight incubation in 80 µL  
433 reactions with the following reagent concentrations: 20% (vol/vol) PEG-4000, 0.125 mM CL78,  
434 and 2.5 units/µL CircLigase II. Ligation products were then immobilised on streptavidin beads  
435 (MyOne C1) to allow the removal of reagent mixtures in subsequent library preparation steps. The  
436 CL9 extension primer was annealed to the complementary CL78 oligo sequence and the strand  
437 complementary to the template single-stranded molecules filled-in using Bst 2.0 polymerase in 50  
438 µL reactions with the following reagent concentrations: 1x isothermal amplification buffer, 250 mM  
439 of each dNTP, 2 mM CL9 extension primer, and 0.48 U/µL Bst 2.0 polymerase. T4 DNA  
440 polymerase was then used to remove 3' overhangs, in 100 µL reactions with the following reagent  
441 concentrations: 1x Buffer Tango, 0.025% (vol/vol) Tween 20, 100 mM of each dNTP, and 0.05  
442 U/µL T4 DNA polymerase. The double-stranded adaptor (CL53/CL73) was then ligated to the  
443 blunt-ended molecules using T4 DNA ligase in 100 µL reactions with the following reagent  
444 concentrations: 1x T4 DNA ligase buffer, 5% (vol/vol) PEG-4000, 0.025% (vol/vol) Tween 20, 100  
445 mM double-stranded adaptor, and 0.1 U/µL T4 DNA ligase. The library strand complementary to  
446 the original single-stranded template molecule was then heat denatured and eluted in 25 µL TET  
447 buffer.

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449 Template library molecules were PCR amplified using AccuPrime Pfx polymerase, incorporating  
450 unique 8 base-pair (bp) index sequences within both P5 and P7 adapters, in 80 µL reactions with the

451 following reagent concentrations: 1x AccuPrime Pfx reaction mix, 0.4 mM each of P5 and P7  
452 indexing primers, and 0.025U/μL AccuPrime Pfx polymerase. Prior to library amplification, qPCR  
453 analysis of the unamplified library was used to identify the appropriate number of PCR cycles,  
454 corresponding to the cycle number at the point of inflection of the qPCR amplification curve,  
455 corrected for differing reaction volume and template amount in the subsequent library amplification  
456 PCR. The qPCR analysis involved 10 μL reactions with the following reagent concentrations: 1x  
457 SYBR green qPCR master mix, 0.2mM each of IS7 and IS8 amplification primers, and 0.2% of the  
458 unamplified library. After amplification, the indexed libraries were quantified using a TapeStation  
459 2200 instrument (Agilent) with D1000 screen tape and reagents, and a Qubit 2.0 instrument (Fisher)  
460 with the dsDNA HS Assay kit. Sequencing of the libraries was mostly performed on an Illumina  
461 NextSeq 500 sequencing platform, using the custom CL72 R1 sequencing primer [43] and the  
462 Gesaffelstein custom index 2 sequencing primer [44], following the procedures described in [44].  
463 Some *praekudarensis* libraries were additionally sequenced on an Illumina HiSeq 2500 sequencing  
464 platform using the same custom primers (see Table S1).

#### 466 ***Data processing***

467 Processing of the sequence reads was carried out within the BEARCAVE v.ce78f40 data analysis  
468 and storage environment (available at: <https://github.com/nikolasbasler/BEARCAVE>) and is  
469 reported in Table S1. BEARCAVE is freely available and can be used to obtain details of all  
470 software versions and parameter settings, as well as to replicate the described analyses. Data  
471 processing involved trimming adapter sequences and removing reads < 30 bp using CutAdapt [45],  
472 and merging overlapping paired-end reads using FLASH [46]. The specific BEARCAVE scripts  
473 used for these steps were: “*trim\_merge\_DS\_PE\_standard.sh*” for trimming and merging paired-end  
474 data generated from double stranded libraries (modern and some published ancient datasets);  
475 “*trim\_merge\_SS\_PE\_CL72.sh*” for trimming and merging paired-end data generated from single  
476 stranded libraries (ancient datasets); and “*trim\_SE.sh*” for trimming single-end data. Reads were  
477 then mapped to the reference genome assemblies of the polar bear [47] and the giant panda [48]  
478 using the bwa [49] aln algorithm and samtools [50], filtering for mapping quality (-q 30) and  
479 potential PCR duplicates (rmdup). For the *praekudarensis* data, duplicate removal was carried out  
480 separately for each library prior to merging them into a single genomic dataset. The specific  
481 BEARCAVE scripts used for mapping datasets to the polar bear reference were: “*map\_SE.sh*” for  
482 mapping ancient datasets with default bwa parameters, excluding unmerged read-pairs from paired-  
483 end datasets since they likely represent modern contamination, rendering these datasets effectively  
484 single-end and “*map\_modern\_PE.sh*” for mapping modern datasets with default bwa parameters  
485 including unmerged read pairs. The giant panda lineage is comparatively diverged from the  
486 investigated clade (around 12–19 million years [51,52]), requiring relaxation of the number of  
487 allowed mismatches between read and reference (-n 0.01, implemented using the corresponding  
488 BEARCAVE scripts) in order to achieve acceptable mapping performance [10]. Nonetheless, we  
489 were consistently able to map more data to the polar bear than the panda reference, reflecting its  
490 lower divergence from the investigated samples. For this reason, subsequent analyses investigating  
491 the broader scale patterns of divergence among the sampled genomes utilised the polar bear  
492 mapping reference in order to maximise the number of sampled genome positions. For analyses  
493 investigating patterns of admixture, the panda was used as mapping reference since these analyses  
494 may be biased by using the polar bear mapping reference, which represents an ingroup to the  
495 investigated clade [53,54].

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## QUANTIFICATION AND STATISTICAL ANALYSIS

### *Assessment of ancient DNA authenticity*

We assessed the authenticity of the cave bear datasets by estimating the endogenous fragment length distribution and extent of cytosine deamination for 10 million randomly sampled reads mapping successfully to the polar bear reference, using the program mapDamage v2.08 [55] with Bayesian statistical estimation disabled and the merge reference sequences option enabled. All cave bear datasets showed evidence of DNA fragmentation and cytosine deamination consistent with the sample ages (Figure S1).

### *Nuclear genome PCA*

We investigated the broad scale patterns of divergence among the seven sampled cave bear taxa, using principal components analysis (PCA) of a single representative genome of each taxon. The polar bear was used as mapping reference for this analysis. A covariance matrix was calculated by sampling a single nucleotide at random from the read stack at each position of the reference genome using single base identity by state (IBS) in ANGSD v0.916 [56], only considering reads with a minimum mapping quality score of 30 (-minMapQ 30) and nucleotides with a minimum base quality score of 30 (-minQ 30). We further only considered sites from scaffolds > 1 Mb in length, with no missing data (-minInd N, where N = number of individuals), and which were below the upper 95<sup>th</sup> percentile of global coverage (-setMaxDepth, determined in advance using the -doDepth function in angsd). Transition (identified using genotype likelihoods) and singleton ( $1/N < -\text{minFreq}$  <  $2/N$ ) sites were also excluded. PCA of the covariance matrix was then carried out using the “eigen” function in R [57]. The exclusion of singleton sites in this analysis is an effective way of reducing sequencing errors, which frequently occur at high abundance in ancient datasets. However, since private alleles are also removed, this approach is sensitive to unbalanced sampling of clades, with the tendency to underestimate divergence for undersampled lineages [11]. This effect was observed in preliminary analyses including both *kudarensis* individuals, since all other cave bear taxa were represented by single individuals. The sampling of the *kudarensis* lineage was therefore reduced to the individual with the higher coverage (HV74) in order to achieve a less biased assessment of cave bear relationships. The ordination of individuals along PC1 and PC2 of this analysis is shown in Figure 1A, and along PC3 to PC6 in Figure 1B.

### *Generation of error-reduced genome sequences*

We prepared two error-reduced sets of genome sequences using Consensify: one for datasets mapped to the polar bear reference, which was used to estimate phylogeny and genetic distances; and one for datasets mapped to the panda reference for admixture tests using D-statistics.

For the first set of Consensify sequences, for each dataset we used angsd to count the observed frequency of mapped bases at each position of the polar bear reference (-doCounts function), filtering for mapping (-minMapQ 30) and base calling (-minQ 30) qualities. In order to achieve the most accurate estimates of genetic distances, we additionally excluded the terminal nucleotide of each mapped sequence to further reduce the probability of introducing errors resulting from cytosine deamination. The Consensify error-reduced sequence for each dataset was then generated from these base counts using the Consensify script (available from

543 <https://github.com/jlapaijmans/Consensify>), applying a maximum depth filter of the integer  $< 95\%$   
544 depth for each individual dataset, calculated in advance using the `-doDepth` function in `angsd`.  
545 Generation of the second set of sequences used the same methodology applied to datasets mapped  
546 to the panda reference, except that the terminal nucleotides of the mapped sequences were included  
547 in base counts. The Consensify error reduced sequences are summarised in Table S1.

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### 550 ***Phylogenetic analyses***

551 We estimated phylogenetic relationships among the 12 sampled nuclear genomes of cave bears,  
552 brown bears and polar bears, using the Consensify error-reduced sequences generated from datasets  
553 mapped to the polar bear reference. We used the ReDuCToR script (included in the Consensify  
554 distribution) to combine the Consensify sequences into a single alignment, removing all invariant  
555 columns and any containing missing data. Maximum-likelihood phylogenetic analysis was then  
556 carried out using RaxML v8.2.12 [58] under the GTR+GAMMA substitution model with 100 rapid  
557 bootstrap replicates and a thorough maximum likelihood search for the final tree (“-f a” option),  
558 which was rooted using the Asiatic black bear outgroup. Bootstrap values  $\geq 80\%$  were considered as  
559 statistically supported. The resulting phylogeny is shown in Figure 1D.

560

561 To estimate mitochondrial relationships, the *praekudarensis* mitochondrial genome sequence was  
562 generated from the datasets described in Table S1, except “6ux” and “j54”, which were sequenced  
563 at a later date. Adapter trimming was performed as described above (data processing section),  
564 except that reads  $< 28$  bp were discarded. Subsequent manual inspection of the mapped reads  
565 indicated that this lower minimum read length threshold was appropriate for reconstruction of the  
566 *praekudarensis* mitochondrial genome. The reads were mapped to the published reference  
567 mitochondrial sequence of the *kudarensis* cave bear HV74 [10] using the `bwa aln` algorithm,  
568 discarding reads with MapQuality score  $< 30$  with `samtools v1.3.1`, and removing duplicate reads  
569 using `MarkReadsByStartEnd.jar` ([https://github.com/dariober/Java-](https://github.com/dariober/Java-cafe/tree/master/MarkDupsByStartEnd)  
570 `cafe/tree/master/MarkDupsByStartEnd`). A consensus sequence was then generated from this  
571 alignment in `Geneious v7.0`, using a minimum sequence depth of 3x and a 75% majority rule for  
572 base calling. The consensus sequence was manually checked against the original alignment to  
573 exclude the possibility of erroneous or incorrect consensus base calls. Details of the mitochondrial  
574 genome reconstruction are shown in Table S7.

575

576 Mitochondrial genome sequences were also generated for the two brown bears (Ge, Uap), the two  
577 polar bears (SRS412584, SRS412585), the Asiatic black bear (ERS781634), and four of the Late  
578 Pleistocene cave bears (HV72, BO4, BO5, WK01), from the datasets described in Table S1. The  
579 mitochondrial genome sequences of the brown bear Uap and *eremus* cave bear WK01 have been  
580 published previously, based on much lower coverage [59]. We therefore recomputed them using the  
581 higher coverage datasets of [10] in order to achieve a more complete sequence. Mitochondrial  
582 reconstruction followed the methodology described above for *praekudarensis*, except that reads  $<$   
583 30 bp were discarded, reads from each dataset were mapped to a reference mitochondrial sequence  
584 selected as a close relative of the respective taxon, and consensus sequences were generated using a  
585 minimum sequence depth of 3x and a 90% majority rule for base calling. Details of the  
586 mitochondrial genome reconstruction are shown in Table S7.

587

588 The consensus sequences were aligned with published sequences of the *ingressus* (GS136),  
589 *spelaeus* (E-VD-1838), *eremus* (WK01) and *kudarensis* (HV74) individuals using the MUSCLE  
590 algorithm [60] implemented in MEGA X [61] with default parameters. The *Ursus* control region  
591 contains a microsatellite repeat which was removed as this cannot be reliably recovered using short  
592 read data. Maximum likelihood phylogenetic analysis was carried out as described above for the  
593 nuclear genome alignment. The resulting phylogeny is shown in Figure 1C.

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### 596 ***Molecular dating***

597 Genomic data from individuals sampled at different time points provides information on their  
598 genome-wide substitution rate. To estimate this rate for the cave, polar and brown bear clade, we  
599 compared the genomic divergences of the *praekudarensis* and *kudarensis* datasets to modern polar  
600 bears and brown bears, which are expected to be lower in the case of *praekudarensis* since its  
601 divergence time from the clade's common ancestor is considerably less. Thus, assuming a strict  
602 molecular clock, the difference in divergence divided by the median estimate of 305,400 years  
603 separating *praekudarensis* and *kudarensis* provides an estimate of the per-lineage substitution rate.  
604 The ReDuCToR alignment of Consensify sequences generated from datasets mapped to the polar  
605 bear reference was recomputed to include invariant positions. Pairwise genomic distances were then  
606 calculated from this alignment under the JC69 substitution model using the `dist.dna` function in the  
607 R package "ape", considering both transitions and transversions. Assuming the sites sampled using  
608 Consensify are a random and unbiased sample of the genome, these distances equate to whole  
609 genome divergences and can be used to estimate the genome-wide substitution rate. Six sets of  
610 substitution rate estimates were calculated using all combinations of the two *kudarensis* individuals,  
611 both polar bears and the modern Georgian brown bear (Table S3). The six estimates were highly  
612 consistent and their mean ( $9.56231 \times 10^{-10}$  substitutions/site/year) was used for subsequent  
613 divergence time estimations. The consistency of the rate estimates supports both the validity of our  
614 method, and the assumption of a strict molecular clock. We additionally estimated the genome-wide  
615 substitution rate assuming ages of the *praekudarensis* sample  $\pm 90$  ka, representing the uncertainty  
616 in its radiothermoluminescence date. We also repeated this entire set of calculations using pairwise  
617 mitochondrial distances to estimate the mitochondrial substitution rate.

618

619 We applied substitution rate estimates to calculate nuclear (Table S4) and mitochondrial (S5)  
620 divergence times from pairwise genetic distances among all individuals. Since the estimates reflect  
621 the per-lineage substitution rate, they were multiplied by two to obtain the rate of genetic  
622 divergence between sister lineages, assuming a strict molecular clock. Genetic divergences between  
623 individuals were then divided by this estimated rate of genetic divergence to obtain the divergence  
624 time in years (Tables S4 and S5). The resulting pairwise divergence times represent the total time  
625 taken for lineages to achieve the observed genetic divergence, and do not take into account the non-  
626 contemporaneous ages of the individuals. The median age of each pair of individuals was therefore  
627 added to their respective pairwise divergence time, which, assuming a strict molecular clock,  
628 provides their absolute time of divergence before the present day (Tables S4 and S5). This  
629 procedure resulted in multiple absolute age estimates for most nodes of the phylogeny, with each  
630 estimate based on a different combination of individuals. As for the substitution rate estimates, the  
631 consistency of these absolute node age estimates supports both the validity of our method, and the  
632 assumption of a strict molecular clock. To provide the calibrated trees in Figure 3, nodes were  
633 centered on the mean of their calculated median age estimates.

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### ***Comparison with other divergence estimates***

Based on a median radiothermoluminescence age of 360 ka for the *praekudarensis* sample, our nuclear genome analysis provided an estimated divergence time of cave bears and their sister clade, brown and polar bears, of 1.52 million years. The estimate provided by mitochondrial DNA is remarkably similar, at 1.48 million years. These estimates coincide with the last documented fossil occurrences ~1.6 million years ago of their accepted common ancestor, *Ursus etruscus* [62–64]. They also moderately pre-date the earliest documented fossil occurrences of the accepted ancestral cave bear, *Ursus deningeri*, towards the end of the Early Pleistocene (a review of the literature is provided in [65]). Finally, they moderately pre-date the earliest documented fossil showing *arctos*-like characteristics, which have been assigned to the brown bear lineage. These fossils also date towards the end of the Early Pleistocene, around 1.2 million years ago [63]. Our estimated divergence time is also considerably younger than a previous estimate based on complete mitochondrial genomes [51], which reported a divergence estimate of 2.75 million years (95% credibility interval (CI) 2.1–3.57) based on fossil calibration of the seal/bear divergence and of the *Ursus* lineage. A second study analysing ~4kb of mitochondrial sequence [66] utilised four calibration points within the bear clade. Although the divergence time of cave bears from their sister clade was not reported in this study, as first author AB carried out this analysis we can confirm the estimate was 2.20 million years (95% CI 1.58–3.00 million years). In contrast, an early study [67] based on control region and cytochrome b sequences produced a more recent estimate than ours, around 1.2 million years.

### ***Tests of nuclear admixture***

D-statistics were calculated from the Consensify error-reduced sequences generated from datasets mapped to the panda reference using the published C++ program D\_stat.cpp [10], and the results processed using the python scripts D\_stat\_parser.py and weighted\_block\_jackknife.py (available from <https://github.com/jacahill/Admixture>). Significance of the D-statistics was assessed by calculating the standard error using a weighted block jackknife analysis using 5 Mb genome windows, with D values deviating more than three standard-errors from zero (absolute Z-score > 3) considered as statistically significant. These tests used the Asiatic black bear as outgroup for allele polarisation, which has previously been shown to be a suitable outgroup taxon for testing for admixture within the brown-polar-cave bear clade [10]. The two brown bears included in this study have previously been shown to exhibit high genomic proportions of admixture with cave bears [10], and were chosen to maximise sensitivity in detecting the specific admixing cave bear lineage(s).

We calculated D-statistics for all possible combinations of individuals congruent with their nuclear phylogeny (Figure 1D). We found significant evidence of differential admixture between brown bears and cave bears subsequent to the divergence of brown bears and polar bears; between brown bears and European+Uralian cave bears subsequent to their divergence from the Caucasian cave bears; and between European cave bears and the *kudarensis* lineage, subsequent to their divergence from their respective sister clades, the Uralian cave bear *rossicus* and the *praekudarensis* lineage (Figure 4). All other comparisons were not significantly different from zero.

679 **SUPPLEMENTAL ITEM TITLES**

680  
681 Figure S1. Assessment of ancient DNA authenticity. Related to STAR Methods.

682  
683 Table S1. Details of data processing and generation of error-reduced genome sequences. Related to  
684 STAR Methods.

685  
686 Table S2. Details of sample localities and ages. Related to STAR Methods.

687  
688 Table S3. Nuclear and mitochondrial substitution rate estimates based on the relative difference in  
689 genomic divergence of *kudarensis* (t1) and *praekudarensis* (t2) to a modern representative of the  
690 brown/polar bear clade (t3). Related to Figures 2 and 3, and STAR Methods.

691  
692 Table S4. Absolute times of nuclear divergence from the present day (node age) for all sample-pairs.  
693 Related to Figures 2 and 3, and STAR Methods.

694  
695 Table S5. Absolute times of mitochondrial divergence from the present day (node age) for all  
696 sample-pairs. Related to Figures 2 and 3, and STAR Methods.

697  
698 Table S6. Radiocarbon dates for Medvezhiya and Kizel cave bears used for indirect age estimates  
699 for the sequenced samples. Related to STAR Methods.



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**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Guanidine hydrochloride	Roth	Cat#0037.1
QIAGEN MinElute kit	Qiagen	Cat#28004
Critical Commercial Assays		
D1000 Screen Tape (Tapestation2200)	Agilent	Cat#5067-5582
dsDNA HS Assay Kit (Qubit 2.0)	Thermofisher	Cat#Q32851
Deposited Data		
7t5-KU1_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
qgj-KU1_2 unprocessed data, fastq format	This paper	[submitted awaiting accession]
ucp-KU1_2 unprocessed data, fastq format	This paper	[submitted awaiting accession]
x54-KU1_2 unprocessed data, fastq format	This paper	[submitted awaiting accession]
4z6-KU1_3 unprocessed data, fastq format	This paper	[submitted awaiting accession]
85j-KU1_3 unprocessed data, fastq format	This paper	[submitted awaiting accession]
e5e-KU1_3 unprocessed data, fastq format	This paper	[submitted awaiting accession]
vup-KU1_3 unprocessed data, fastq format	This paper	[submitted awaiting accession]
2pq-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
4id-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
65v-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
6xw-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
9s1-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
j54-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
siw-KU1_4 unprocessed data, fastq format	This paper	[submitted awaiting accession]
009-KU1_5 unprocessed data, fastq format	This paper	[submitted awaiting accession]
6mn-KU1_5 unprocessed data, fastq format	This paper	[submitted awaiting accession]
9j7-KU1_5 unprocessed data, fastq format	This paper	[submitted awaiting accession]
b3b-KU1_5 unprocessed data, fastq format	This paper	[submitted awaiting accession]
4w5-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
6ux-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
8ux-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
dLd-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
mhh-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
n3p-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]

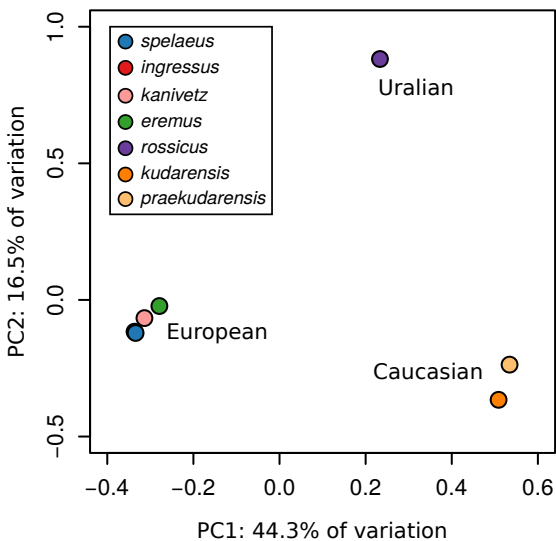
we6-KU1_6 unprocessed data, fastq format	This paper	[submitted awaiting accession]
ntw-HV72_8 unprocessed data, fastq format	This paper	[submitted awaiting accession]
w8o-HV72_8 unprocessed data, fastq format	This paper	[submitted awaiting accession]
2Ls-B05_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
vhf-B05_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
y3h-B05_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
9e1-B04_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
f9L-B04_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
vb5-B04_1 unprocessed data, fastq format	This paper	[submitted awaiting accession]
KU1 mapped reads, polar bear reference, bam format	This paper	[submitted awaiting accession]
KU1 mapped reads, panda reference, bam format	This paper	[submitted awaiting accession]
B05 mapped reads, polar bear reference, bam format	This paper	[submitted awaiting accession]
B05 mapped reads, panda reference, bam format	This paper	[submitted awaiting accession]
B04 mapped reads, polar bear reference, bam format	This paper	[submitted awaiting accession]
B04 mapped reads, panda reference, bam format	This paper	[submitted awaiting accession]
HV72 mapped reads, polar bear reference, bam format	This paper	[submitted awaiting accession]
HV72 mapped reads, panda reference, bam format	This paper	[submitted awaiting accession]
KU1 mitochondrial genome sequence	This paper	[submitted awaiting accession]
B05 mitochondrial genome sequence	This paper	[submitted awaiting accession]
B04 mitochondrial genome sequence	This paper	[submitted awaiting accession]
HV72 mitochondrial genome sequence	This paper	[submitted awaiting accession]
WK01 mitochondrial genome sequence	This paper	[submitted awaiting accession]
<b>Oligonucleotides</b>		
CL9 extension primer: GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	[43]	Sigma Aldrich
Double-stranded adapter Strand 1 (CL53): CGACGCTCTTC-ddC (ddC = dideoxycytidine) Strand 2 (CL73): [Phosphate]GGAAGAGCGTCGTGTAGGGAAAGAG*T* G*T*A (* = phosphothioate linkage)	[43]	Sigma Aldrich
CL78: AGATCGGAAG[C3Spacer] <sub>10</sub> [TEG-biotin] (TEG =triethylene glycol spacer)	[43]	Sigma Aldrich
P5 indexing primer: AATGATACGGCGACCAACGAGATCTACACnnnnnnnnA CACTCTTCCCTACACGACGCTCTT	[43]	Sigma Aldrich
P7 indexing primer: CAAGCAGAAGACGGCATACGAGATnnnnnnnnnGTGAC TGGAGTTCAGACGTGT	[43]	Sigma Aldrich
IS7 amplification primer: ACACTCTTCCCTACACGAC	[43]	Sigma Aldrich

IS8 amplification primer: GTGACTGGAGTTCAGACGTGT	[43]	Sigma Aldrich
CL72 R1 sequencing primer : ACACTCTTTCCCTACACGACGCTCTTCC	[43]	Sigma Aldrich
Gesaffelstein index 2 sequencing primer: GGAAGAGCGTCGTGTAGGGAAAGAGTGT	[44]	Sigma Aldrich
Software and Algorithms		
BEARCAVE ce78f40	N/A	<a href="https://github.com/nikolasbasler/BEARCAVE/">https://github.com/nikolasbasler/BEARCAVE/</a>
Cutadapt v1.12	[45]	<a href="https://cutadapt.readthedocs.io/en/stable/">https://cutadapt.readthedocs.io/en/stable/</a>
Flash v1.2.11	[46]	<a href="https://ccb.jhu.edu/software/FLASH/">https://ccb.jhu.edu/software/FLASH/</a>
BWA v0.7.15 and v0.7.8	[49]	<a href="http://bio-bwa.sourceforge.net/">http://bio-bwa.sourceforge.net/</a>
Samtools v1.3.1	[50]	<a href="https://sourceforge.net/projects/samtools/files/samtools/">https://sourceforge.net/projects/samtools/files/samtools/</a>
PreSeq	N/A	<a href="http://smithlabresearch.org/software/preseq/">http://smithlabresearch.org/software/preseq/</a>
MapDamage v2.0.8	[55]	<a href="https://ginolhac.github.io/mapDamage/">https://ginolhac.github.io/mapDamage/</a>
ANGSD v0.916	[56]	<a href="http://www.popgen.dk/angsd">http://www.popgen.dk/angsd</a>
Consensify v0.1	[11]	<a href="https://github.com/jlapajmans/Consensify">https://github.com/jlapajmans/Consensify</a>
ReDuCToR v0.1	[11]	<a href="https://github.com/jlapajmans/Consensify">https://github.com/jlapajmans/Consensify</a>
RaxML v8.2.12	[58]	<a href="https://github.com/stamatak/standard-RAxML">https://github.com/stamatak/standard-RAxML</a>
R version 3.6.3	[57]	<a href="https://www.r-project.org/">https://www.r-project.org/</a>
MarkReadsByStartEnd.jar	N/A	<a href="https://github.com/dariober/Java-cafe/tree/master/MarkDupsByStartEnd">https://github.com/dariober/Java-cafe/tree/master/MarkDupsByStartEnd</a>
MEGA X v10.1.7	[61]	<a href="https://www.megasoftware.net/">https://www.megasoftware.net/</a>
D_stat.cpp	[10]	<a href="https://github.com/jacahill/Admixture">https://github.com/jacahill/Admixture</a>
D-stat_parser.py	[10]	<a href="https://github.com/jacahill/Admixture">https://github.com/jacahill/Admixture</a>
weighted_block_jackknife.py	[10]	<a href="https://github.com/jacahill/Admixture">https://github.com/jacahill/Admixture</a>
Other		
Proteinase K	Promega	Cat#V3021
Zymo-spin V column extension reservoir	Zymo	Cat#C1016-50
Circligase II	Biozym	Cat#131402(CL9021 K)
Endonuclease VIII	NEB	Cat#A0299S
Uracil-DNA glycosylase (Afu UDG)	NEB	Cat#M0279S
FastAP	Thermo Fisher	Cat#EF0651
MyOne C1 streptavidin beads	Thermo Fisher	Cat#65001
Bst 2.0 polymerase	NEB	Cat#M0537S

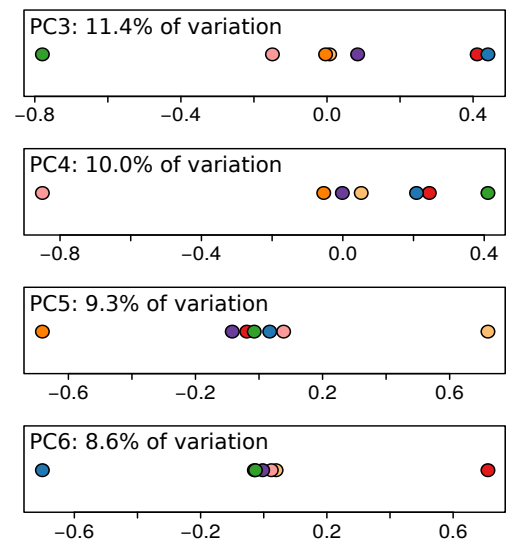


T4 DNA Polymerase	Thermo Fisher	Cat#EP0061
Buffer Tango (10x)	Thermo Fisher	Cat#BY5
T4 DNA ligase	Thermo Fisher	Cat#EL0011
Accuprime Pfx	Thermo Fisher	Cat#12344024
PEG-4000	Thermo Fisher	Cat#EP0061
Klenow fragment of DNA polymerase I	Thermo Fisher	Cat#EP0051
SYBR green PCR MasterMix	Thermo Fisher	Cat#4309155

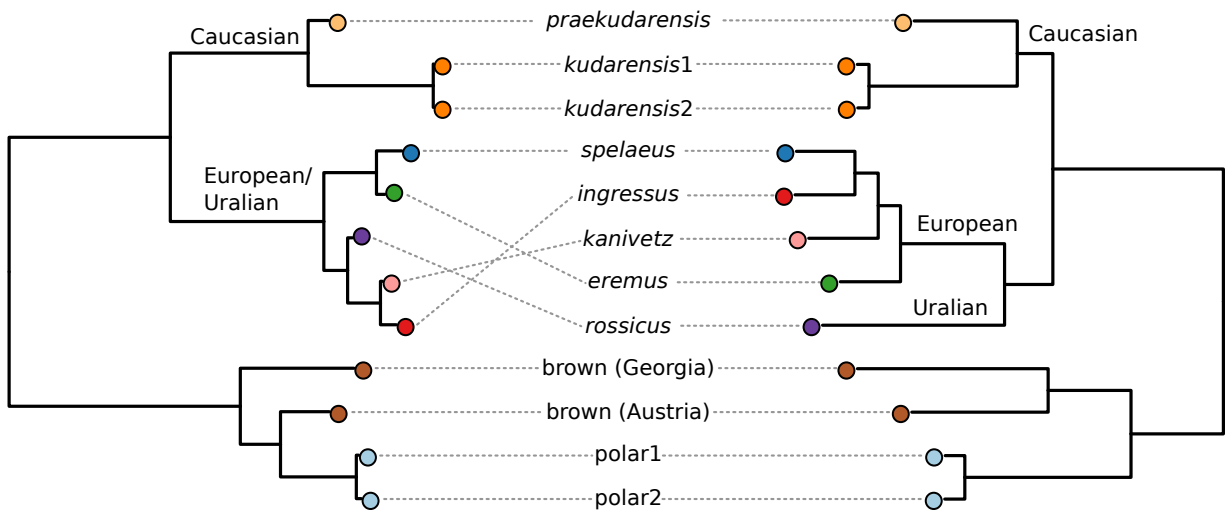
**Figure 1** **A – PCA axes 1–2**



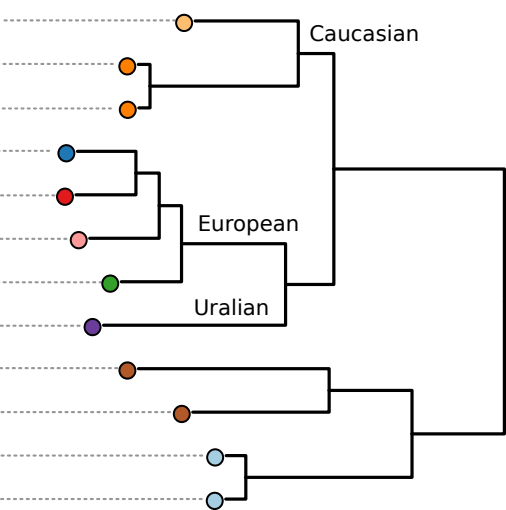
**B – PCA axes 3–6**

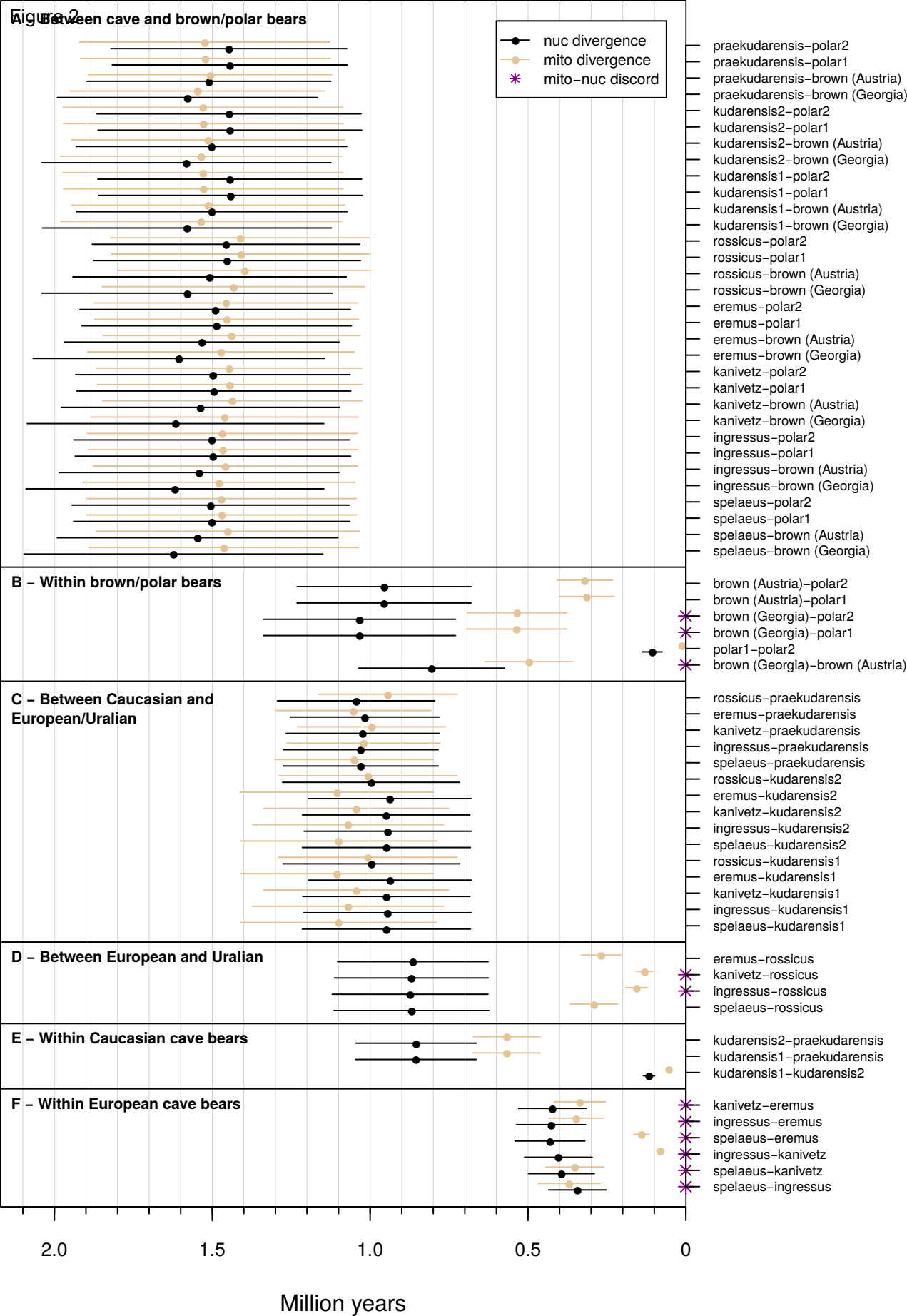


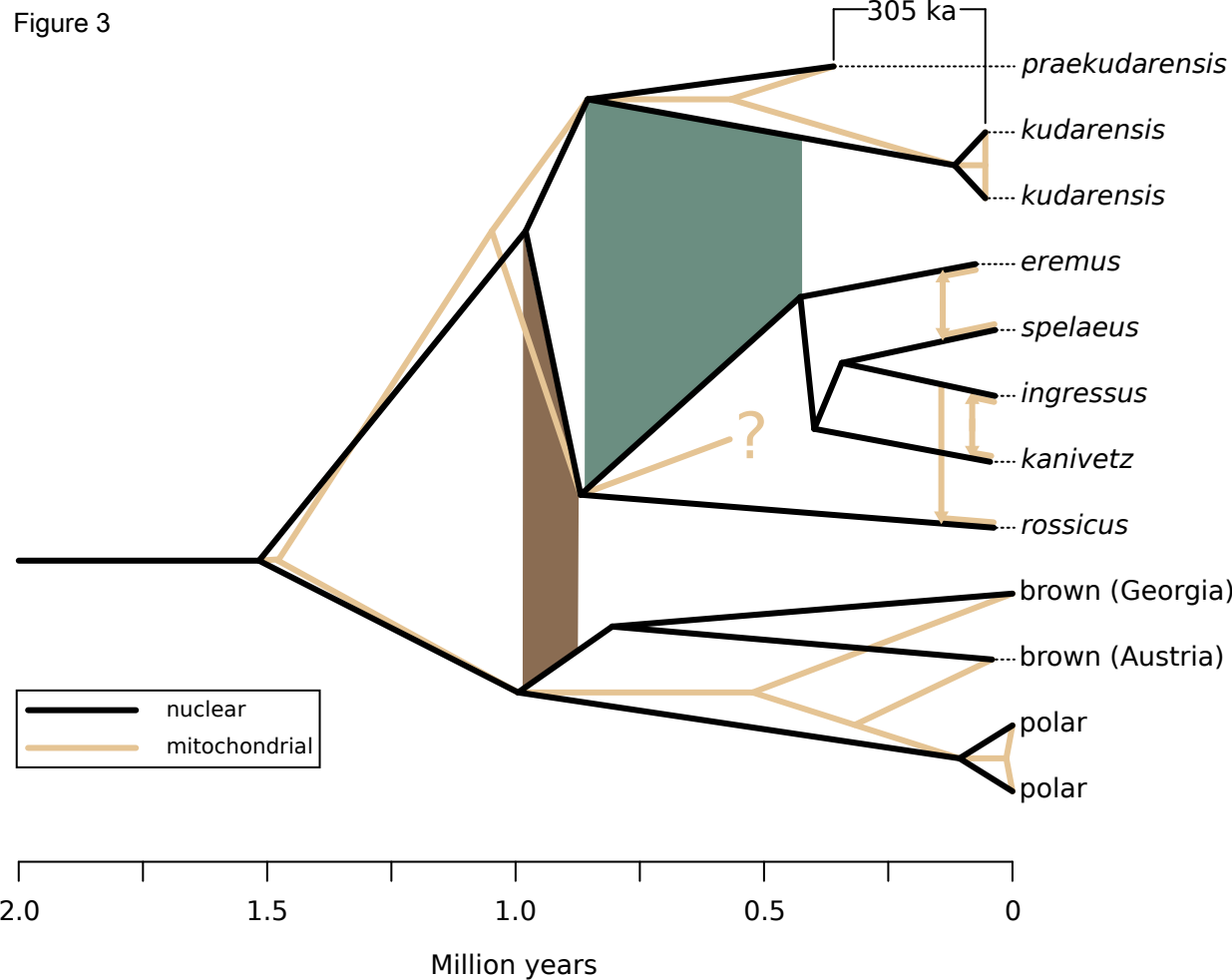
**C – Mitochondrial phylogeny**

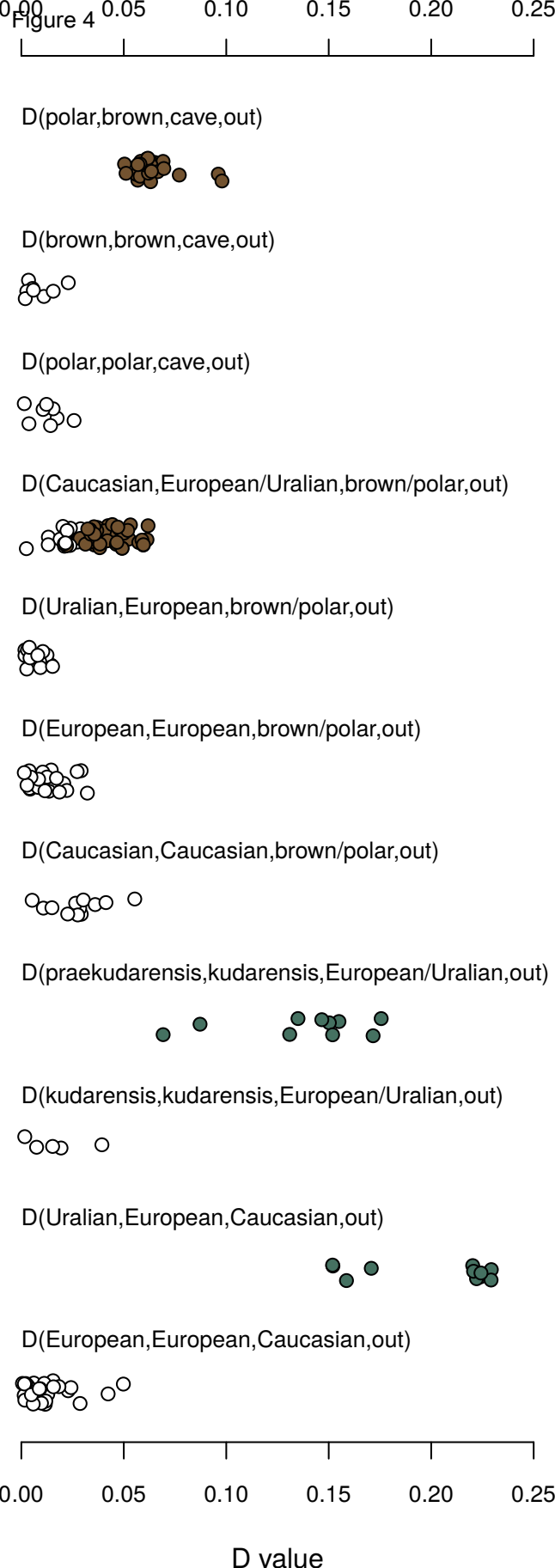


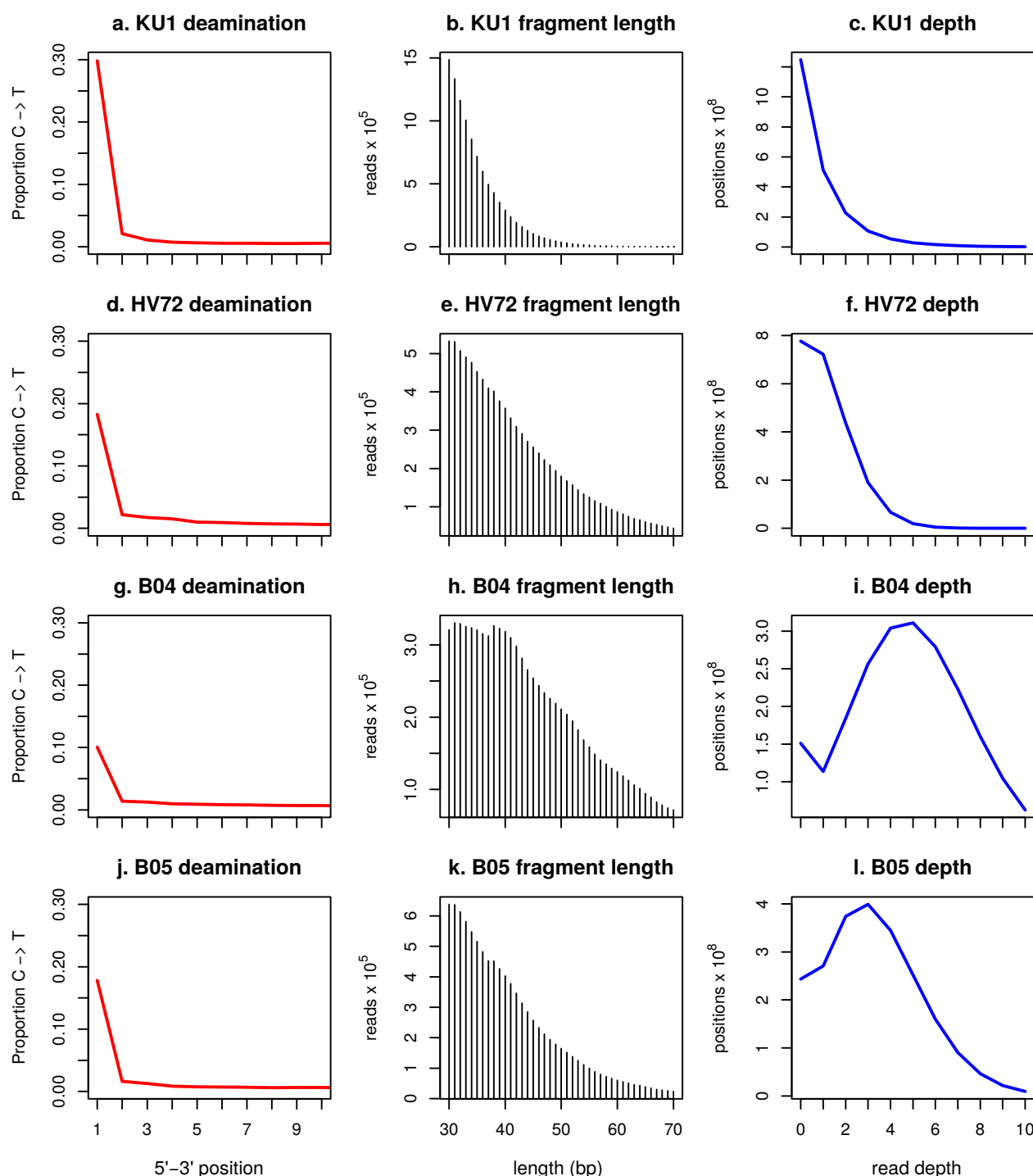
**D – Nuclear phylogeny**











**Figure S1. Assessment of ancient DNA authenticity. Related to STAR Methods.**

Estimated cytosine deamination (left panels), endogenous fragment length distributions (centre panels), and mapped read depth distributions (right panels) for cave bear samples analysed in this study: KU1 *praekudarensis* (a.–c.), HV72 *kudarensis* (d.–f.), B04 *kanivetz* (g.–i.), and B05 *rossicus* (j.–l.). Cytosine deamination and fragment length distributions are estimated from 10 million mapped reads per sample. For cytosine deamination, red lines show the frequency (y axes) of thymines in reads mapped to positions where the polar bear reference possesses a cytosine, for the first 10 bases of the 5' end of the sequenced fragments (x axes). The excess of C → T substitutions observed at the 5' end of the sequenced fragments indicates advanced cytosine deamination typical of ancient DNA. Note these are underestimates since the DNA extracts were treated with Endonuclease VIII to remove uracils, prior to library preparation. Endogenous fragment length

distributions represent the relative frequencies (y-axes) of reads mapping with alignment lengths between 30 bp and 70 bp (x-axes) to the polar bear reference. All distributions indicate advanced fragmentation typical of ancient DNA, with the *praekudarensis* dataset showing the greatest degree of fragmentation overall. Mapped read depth distributions show, for each complete dataset, the number (y-axes) of positions of the polar bear reference covered by 0–10 reads (x-axes).

Taxon	Run	Library	Mode <sup>a</sup>	Reads/read- pairs	Mapable reads <sup>b</sup>	Polar bear reference					Panda reference						
						Unique mapped <sup>c</sup>	Duplication <sup>d</sup> <sub>e</sub>	Endogenous	Mapped bp <sup>f</sup>	95% depth <sup>g</sup> bp <sup>h</sup>	Consensify bp <sup>h</sup>	Unique mapped <sup>c</sup>	Duplication <sup>d</sup> <sub>e</sub>	Endogenous	Mapped bp <sup>f</sup>	95% depth <sup>g</sup> bp <sup>h</sup>	Consensify bp <sup>h</sup>
<i>praekudarensis</i> (KU1) <sup>i</sup>	7f5	KU1_1	75 bp SE	929608	367745	74	0.03896	0.00020	3078			213	0.04911	0.00058	7542		
	qgj	KU1_2	75 bp SE	1376308	291063180	11981824	0.04687	0.04117	416274746			9851647	0.04562	0.03385	339757338		
	ucp		75 bp SE	392821957													
	x54		75 bp SE	129502135													
	4z6	KU1_3	75 bp SE	101656461	146383897	4355753	0.03298	0.02976	153706586			3553483	0.03193	0.02428	124381572		
	85j		75 bp SE	42946104													
	e5e		75 bp SE	1121766													
	vup		75 bp SE	49940554													
	2pq	KU1_4	75 bp SE	1373808	545309057	20008538	0.08420	0.03669	697468758			16338802	0.08286	0.02996	565343725		
	4id		75 bp SE	121346233													
	65v		75 bp SE	214714526													
	6xw		75 bp SE	44966523													
	9s1		75 bp SE	51895955													
	j54		100 bp PE	215258264													
	siw		75 bp SE	231802717													
	009	KU1_5	75 bp SE	50475622	150869957	5567830	0.03355	0.03690	194429321			4548639	0.03300	0.03015	157643168		
	6mn		75 bp SE	57832554													
	9j7		75 bp SE	134354691													
	b3b		75 bp SE	1577376													
	4w5	KU1_6	75 bp SE	42160302	508939093	17918152	0.07158	0.03521	628426966			14636130	0.07001	0.02876	509379472		
	6ux		100 bp PE	194458992													
	8ux		75 bp SE	37571245													
	dLd		75 bp SE	180488789													
	mhh		75 bp SE	196555394													
	n3p		75 bp SE	99325956													
	we6		75 bp SE	1087023													
	Total				2597540863	1642932929	59832171	0.06489	0.03642	2090309455	3	317286144	48928914	0.06357	0.02978	1696512817	3 <sup>j</sup>
<i>kudarensis</i> (HV72)	pk5	HV72_1 <sup>k</sup>	70 bp PE	2869873													
	nxu	HV72_2 <sup>l</sup>	70 bp PE	808632													
	zfb	HV72_3 <sup>m</sup>	70 bp PE	560954													
	et7	HV72_4 <sup>n</sup>	70 bp PE	541294													
	1oa	HV72_5 <sup>o</sup>	70 bp PE	617957													
	Ogw	HV72_6 <sup>p</sup>	70 bp PE	522654													
	xyw	HV72_7 <sup>q</sup>	70 bp PE	371421													
	ntw	HV72_8	75 bp SE	1978082													
	w8o	HV72_8	100 bp PE	408079285													
	Total			416350152	252663833	58829996	0.13835	0.23284	2662568631	3 <sup>j</sup>	598621119	45416300	0.13833	0.17975	2016578684	3 <sup>j</sup>	456578209
<i>rossicus</i> (B05)	2Ls	B05_1	75 bp SE	800911													
	vhf	B05_1	75 bp SE	229165973													
	y3h	B05_1	75 bp SE	345021971													
	Total			574988855	368057543	186891382	0.06894	0.50778	7762067882	6	1519452061	147543648	0.06852	0.40087	6056896419	6	1244838601
<i>kanivetz</i> (B04)	9e1	B04_1	75 bp SE	275211													
	f9L	B04_1	75 bp SE	236197693													
	vb5	B04_1	75 bp SE	254445960													
	Total			490918864	380166888	241762865	0.13300	0.63594	11867462564	9	1828930651	185653780	0.13037	0.48835	8981805339	8	1456996716
<i>kudarensis</i> (HV74) <sup>i</sup> [S2]	Total			243461078					4990765397	4	1113055402				3760751170	4	869048390



<i>eremus</i> (WK01) <sup>a</sup> [S2]	Total	499710778	7915956754	7	1569807355	6128843570	6	1210933302
<i>ingressus</i> (GS136ss) <sup>a</sup> [S3]	Total	432895345	9100724820	7	1659620719	6940781378	6	1266005835
<i>spelaeus</i> (E-VD-1838) <sup>a</sup> [S2]	Total	409117882	5818548166	5	1188238828	4552148667	5	971153181
Brown (Georgia, Ge) <sup>a</sup> [S2]	Total	213218024	29072595580	18	1966369913	14518303667	14	1482046107
Brown (Austria, Uap) <sup>a</sup> [S2]	Total	217267718	3512273973	3	767590358	2651330420	3	591504908
Polar (SRS412584) <sup>a</sup> [S4]	Total	105516047	12144369821	9	1704519042	6812026125	7	1118483213
Polar (SRS412585) <sup>a</sup> [S4]	Total	112291778	10050050358	8	1596434788	6021930179	6	1025241632
Asiatic black (ERS781634) <sup>a</sup> [S5]	Total	169470434	22574306140	15	1964247967	13436353408	12	1523577868

**Table S1. Details of data processing and generation of error-reduced genome sequences. Related to STAR Methods.**

<sup>a</sup>75 bp single-end datasets were sequenced on the NextSeq 500 platform, 100 bp paired-end datasets were sequenced on the HiSeq 2500 platform, and 70 bp paired-end datasets were sequenced on the MiSeq platform;

<sup>b</sup>“mapable reads” are the number of trimmed (and merged for paired-end data) reads > 30 bp used for mapping using bwa;

<sup>c</sup>number of mapped reads remaining after duplicate removal;

<sup>d</sup>proportion of mapped reads removed as duplicates;

<sup>e</sup>estimated proportion of endogenous molecules, calculated as “unique mapped” / “mapable reads”;

<sup>f</sup>number of mapped base-pairs;

<sup>g</sup>maximum depth filter applied for Consensify;

<sup>h</sup>total bp of error reduced sequence generated using Consensify;

<sup>i</sup>data from the six *praekudarensis* libraries were processed separately and then combined;

<sup>j</sup>for these datasets, the 95th percentile of coverage was between 2–3 reads, and so 3 reads was used as the maximum allowed depth;

<sup>k</sup>data from Dabney/double-stranded treatment in [S1];

<sup>l</sup>data from combined/double-stranded treatment in [S1];

<sup>m</sup>data from combined/single-stranded treatment in [S1];

<sup>n</sup>data from Dabney/double-stranded treatment in [S1];

<sup>o</sup>data from Dabney/single-stranded treatment in [S1];

<sup>p</sup>data from Rohland/double-stranded treatment in [S1];

<sup>q</sup>data from Rohland/single-stranded treatment in [S1];

<sup>r</sup>published dataset, see cited reference for details.

Sample [reference]	taxon	locality	Age (years Bp)	Dating method	Date reference
KU1 [this study]; ZIN 31896 <sup>1</sup>	<i>praekudarensis</i>	Kudaro-1 cave (layer 5c), South Ossetia, Southern Caucasus	360,000 ± 90,000	Layer 5c dated by Radiothermoluminescence. Also see STAR Methods.	[S7]
HV72 [this study]	<i>kudarensis</i> 1	Hovk-1 cave, Armenia, Southern Caucasus	54,600 ± 5,700	Layer dated by optically stimulated luminescence	[S8]
HV74 [S2]	<i>kudarensis</i> 2	Hovk-1 cave, Armenia, Southern Caucasus	54,600 ± 5,700; > 49,000	Layer dated by optically stimulated luminescence; radiocarbon dating	[S8,S1]
B05 [this study] ZIN 28601-9a/51 <sup>1</sup>	<i>rossicus</i>	Kizel cave, Ural Mountains, Russia	37,698 (indirect estimate)	Median of available radiocarbon dates for Kizel cave bears. See Table S6.	This study
WK01 [S2]	<i>eremus</i>	Windischkopf cave, Austria	71,992 (95% CI 54,640– 91,860)	Mitochondrial tip dating	[S6]
B04 [this study] ZIN n/n <sup>1</sup>	<i>kanivetz</i>	Medvezhiya cave, Ural Mountains, Russia	45,043 (indirect estimate)	Median of available radiocarbon dates for Medvezhiya cave bears. See Table S6.	This study
GS136 [S2]	<i>ingressus</i>	Gamssulzen cave, Austria	35,062 ± 966	Radiocarbon dating	[S6]
E-VD-1838 [S2]	<i>spelaeus</i>	Eiros cave, Spain	34,806 ± 931	Radiocarbon dating	[S6]
Ge [S2]	brown	Georgia, Great Caucasus	Modern	N/A	[S2]
Uap [S2]	brown	Winden cave, Austria	41,201 ± 895	Radiocarbon dating	[S6]
SRS412584 <sup>2</sup> [S4]	polar1	North Beaufort Sea	Modern	N/A	[S4]
SRS412585 <sup>2</sup> [S4]	polar2	Wrangel Island	Modern	N/A	[S4]
ERS781634 <sup>2</sup> [S5]	Asiatic black	Zoo	Modern	N/A	[S5]

**Table S2. Details of sample localities and ages. Related to STAR Methods.**

<sup>1</sup>Zoological Institute RAS Collection Number.

<sup>2</sup>Sample code is European Nucleotide Archive accession number.

t1	t2	t3	Divergence t1:t3 <sup>a</sup>	Divergence t2:t3 <sup>a</sup>	t1:t3 – t2:t3 <sup>a</sup>	median sub rate	min sub rate	max sub rate
<u>Nuclear DNA</u>								
HV72	KU1	Brown (Ge)	2.97016 x 10 <sup>-3</sup>	2.67496 x 10 <sup>-3</sup>	2.95198 x 10 <sup>-4</sup>	9.66596 x 10 <sup>-10</sup>	7.46581 x 10 <sup>-10</sup>	1.37047 x 10 <sup>-9</sup>
HV72	KU1	Polar (SRS412584)	2.70656 x 10 <sup>-3</sup>	2.41774 x 10 <sup>-3</sup>	2.88822 x 10 <sup>-4</sup>	9.45718 x 10 <sup>-10</sup>	7.30456 x 10 <sup>-10</sup>	1.34086 x 10 <sup>-9</sup>
HV72	KU1	Polar (SRS412585)	2.71051 x 10 <sup>-3</sup>	2.42401 x 10 <sup>-3</sup>	2.86501 x 10 <sup>-4</sup>	9.38116 x 10 <sup>-10</sup>	7.24584 x 10 <sup>-10</sup>	1.33009 x 10 <sup>-9</sup>
HV74	KU1	Brown (Ge)	2.97341 x 10 <sup>-3</sup>	2.67496 x 10 <sup>-3</sup>	2.98453 x 10 <sup>-4</sup>	9.77253 x 10 <sup>-10</sup>	7.54813 x 10 <sup>-10</sup>	1.38558 x 10 <sup>-9</sup>
HV74	KU1	Polar (SRS412584)	2.70982 x 10 <sup>-3</sup>	2.41774 x 10 <sup>-3</sup>	2.92076 x 10 <sup>-4</sup>	9.56371 x 10 <sup>-10</sup>	7.38684 x 10 <sup>-10</sup>	1.35597 x 10 <sup>-9</sup>
HV74	KU1	Polar (SRS412585)	2.71516 x 10 <sup>-3</sup>	2.42401 x 10 <sup>-3</sup>	2.91149 x 10 <sup>-4</sup>	9.53335 x 10 <sup>-10</sup>	7.36339 x 10 <sup>-10</sup>	1.35166 x 10 <sup>-9</sup>
Mean						9.56231 x 10 <sup>-10</sup>	7.38576 x 10 <sup>-10</sup>	1.35577 x 10 <sup>-9</sup>
<u>Mitochondrial DNA</u>								
HV72	KU1	Brown (Ge)	5.46936 x 10 <sup>-2</sup>	4.95862 x 10 <sup>-2</sup>	5.10732 x 10 <sup>-3</sup>	1.67234 x 10 <sup>-8</sup>	1.29168 x 10 <sup>-8</sup>	2.37109 x 10 <sup>-8</sup>
HV72	KU1	Polar (SRS412584)	5.44350 x 10 <sup>-2</sup>	4.86811 x 10 <sup>-2</sup>	5.75381 x 10 <sup>-3</sup>	1.88403 x 10 <sup>-8</sup>	1.45519 x 10 <sup>-8</sup>	2.67122 x 10 <sup>-8</sup>
HV72	KU1	Polar (SRS412585)	5.44996 x 10 <sup>-2</sup>	4.87458 x 10 <sup>-2</sup>	5.75381 x 10 <sup>-3</sup>	1.88403 x 10 <sup>-8</sup>	1.45519 x 10 <sup>-8</sup>	2.67122 x 10 <sup>-8</sup>
HV74	KU1	Brown (Ge)	5.46936 x 10 <sup>-2</sup>	4.95862 x 10 <sup>-2</sup>	5.10732 x 10 <sup>-3</sup>	1.67234 x 10 <sup>-8</sup>	1.29168 x 10 <sup>-8</sup>	2.37109 x 10 <sup>-8</sup>
HV74	KU1	Polar (SRS412584)	5.44350 x 10 <sup>-2</sup>	4.86811 x 10 <sup>-2</sup>	5.75381 x 10 <sup>-3</sup>	1.88403 x 10 <sup>-8</sup>	1.45519 x 10 <sup>-8</sup>	2.67122 x 10 <sup>-8</sup>
HV74	KU1	Polar (SRS412585)	5.44996 x 10 <sup>-2</sup>	4.87458 x 10 <sup>-2</sup>	5.75381 x 10 <sup>-3</sup>	1.88403 x 10 <sup>-8</sup>	1.45519 x 10 <sup>-8</sup>	2.67122 x 10 <sup>-8</sup>
Mean						1.81346 x 10 <sup>-8</sup>	1.40069 x 10 <sup>-8</sup>	2.57118 x 10 <sup>-8</sup>

**Table S3. Nuclear and mitochondrial substitution rate estimates based on the relative difference in genomic divergence of *kudarensis* (t1) and *praekudarensis* (t2) to a modern representative of the brown/polar bear clade (t3). Related to Figures 2 and 3, and STAR Methods.**

<sup>a</sup>Values are the genetic divergence measured as the proportion of positions in the ReDuCToR alignment.

t1	t2	Age t1	Age t2	Genetic divergence	Divergence Time	Median node age	min node age	max node age
spelaeus	ingressus	34806	35062	$5.90264 \times 10^{-4}$	308641	343575	252620	434530
spelaeus	kanivetz	34806	45043	$6.77406 \times 10^{-4}$	354206	394131	289748	498514
ingressus	kanivetz	35062	45043	$6.95485 \times 10^{-4}$	363659	403712	296543	510881
spelaeus	eremus	34806	74696	$7.18663 \times 10^{-4}$	375779	430530	319790	541270
ingressus	eremus	35062	74696	$7.11478 \times 10^{-4}$	372022	426901	317268	536534
kanivetz	eremus	45043	74696	$6.94558 \times 10^{-4}$	363175	423044	316018	530070
spelaeus	rossicus	34806	37698	$1.59278 \times 10^{-3}$	832844	869096	623661	1114532
ingressus	rossicus	35062	37698	$1.60067 \times 10^{-3}$	836970	873350	626699	1120001
kanivetz	rossicus	45043	37698	$1.58373 \times 10^{-3}$	828112	869482	625442	1113523
eremus	rossicus	74696	37698	$1.54614 \times 10^{-3}$	808455	864652	626404	1102901
kudarensis (HV72)	kudarensis (HV74)	54600	54600	$1.18571 \times 10^{-4}$	61999	116599	98328	134870
kudarensis (HV72)	praekudarensis	54600	360000	$1.23921 \times 10^{-3}$	647965	855265	664313	1046218
kudarensis (HV74)	praekudarensis	54600	360000	$1.23782 \times 10^{-3}$	647237	854537	663799	1045275
spelaeus	kudarensis (HV72)	34806	54600	$1.72832 \times 10^{-3}$	903713	948416	682096	1214736
ingressus	kudarensis (HV72)	35062	54600	$1.72112 \times 10^{-3}$	899951	944782	679571	1209994
kanivetz	kudarensis (HV72)	45043	54600	$1.71880 \times 10^{-3}$	898737	948559	683705	1213413
eremus	kudarensis (HV72)	74696	54600	$1.66821 \times 10^{-3}$	872282	936930	679872	1193987
rossicus	kudarensis (HV72)	37698	54600	$1.81629 \times 10^{-3}$	949712	995861	715985	1275737
spelaeus	kudarensis (HV74)	34806	54600	$1.72832 \times 10^{-3}$	903713	948416	682096	1214736
ingressus	kudarensis (HV74)	35062	54600	$1.71973 \times 10^{-3}$	899223	944054	679057	1209051
kanivetz	kudarensis (HV74)	45043	54600	$1.72019 \times 10^{-3}$	899466	949287	684219	1214356
eremus	kudarensis (HV74)	74696	54600	$1.66913 \times 10^{-3}$	872767	937415	680215	1194616
rossicus	kudarensis (HV74)	37698	54600	$1.81861 \times 10^{-3}$	950926	997075	716841	1277309
spelaeus	praekudarensis	34806	360000	$1.59186 \times 10^{-3}$	832359	1029762	784469	1275054
ingressus	praekudarensis	35062	360000	$1.59162 \times 10^{-3}$	832237	1029768	784512	1275025
kanivetz	praekudarensis	45043	360000	$1.57074 \times 10^{-3}$	821317	1023838	781800	1265877
eremus	praekudarensis	74696	360000	$1.53013 \times 10^{-3}$	800084	1017432	781651	1253213
rossicus	praekudarensis	37698	360000	$1.61738 \times 10^{-3}$	845706	1044555	795330	1293781
brown (Ge)	Brown (Uap)	0	41201	$1.50113 \times 10^{-3}$	784918	805518	574206	1036830
Polar (SRS412584)	Polar (SRS412585)	0	0	$2.03806 \times 10^{-4}$	106567	106567	75162	137972
brown (Ge)	Polar (SRS412584)	0	0	$1.97764 \times 10^{-3}$	1034078	1034078	729340	1338816
brown (Ge)	Polar (SRS412585)	0	0	$1.97717 \times 10^{-3}$	1033835	1033835	729169	1338502
Brown (Uap)	Polar (SRS412584)	41201	0	$1.78913 \times 10^{-3}$	935511	956112	680421	1231803
Brown (Uap)	Polar (SRS412585)	41201	0	$1.78843 \times 10^{-3}$	935147	955748	680164	1231332
spelaeus	brown (Ge)	34806	0	$3.07060 \times 10^{-3}$	1605572	1622975	1149820	2096130
spelaeus	Brown (Uap)	34806	41201	$2.88530 \times 10^{-3}$	1508685	1546688	1102086	1991291
spelaeus	Polar (SRS412584)	34806	0	$2.83881 \times 10^{-3}$	1484376	1501779	1064340	1939218
spelaeus	Polar (SRS412585)	34806	0	$2.84555 \times 10^{-3}$	1487900	1505303	1066826	1943781
ingressus	brown (Ge)	35062	0	$3.06130 \times 10^{-3}$	1600709	1618240	1146518	2089961
ingressus	Brown (Uap)	35062	41201	$2.87577 \times 10^{-3}$	1503701	1541833	1098699	1984967
ingressus	Polar (SRS412584)	35062	0	$2.83114 \times 10^{-3}$	1480365	1497896	1061639	1934153
ingressus	Polar (SRS412585)	35062	0	$2.83835 \times 10^{-3}$	1484133	1501664	1064296	1939031
kanivetz	brown (Ge)	45043	0	$3.04828 \times 10^{-3}$	1593900	1616422	1146707	2086137
kanivetz	Brown (Uap)	45043	41201	$2.85811 \times 10^{-3}$	1494464	1537586	1097174	1977998
kanivetz	Polar (SRS412584)	45043	0	$2.81627 \times 10^{-3}$	1472586	1495108	1061143	1929072
kanivetz	Polar (SRS412585)	45043	0	$2.82208 \times 10^{-3}$	1475625	1498146	1063286	1933006
eremus	brown (Ge)	74696	0	$2.99898 \times 10^{-3}$	1568127	1605475	1143355	2067595
eremus	Brown (Uap)	74696	41201	$2.82208 \times 10^{-3}$	1475625	1533573	1098713	1968433
eremus	Polar (SRS412584)	74696	0	$2.77118 \times 10^{-3}$	1449009	1486357	1059340	1913373
eremus	Polar (SRS412585)	74696	0	$2.77954 \times 10^{-3}$	1453384	1490732	1062426	1919037
rossicus	brown (Ge)	37698	0	$2.98457 \times 10^{-3}$	1560590	1579439	1119540	2039338
rossicus	Brown (Uap)	37698	41201	$2.80999 \times 10^{-3}$	1469305	1508754	1075757	1941752
rossicus	Polar (SRS412584)	37698	0	$2.74328 \times 10^{-3}$	1434425	1453274	1030556	1875993
rossicus	Polar (SRS412585)	37698	0	$2.74910 \times 10^{-3}$	1437463	1456312	1032698	1879926
kudarensis (HV72)	brown (Ge)	54600	0	$2.97016 \times 10^{-3}$	1553053	1580353	1122675	2038031
kudarensis (HV72)	Brown (Uap)	54600	41201	$2.78094 \times 10^{-3}$	1454113	1502013	1073493	1930534
kudarensis (HV72)	Polar (SRS412584)	54600	0	$2.70656 \times 10^{-3}$	1415224	1442524	1025464	1859585
kudarensis (HV72)	Polar (SRS412585)	54600	0	$2.71051 \times 10^{-3}$	1417290	1444590	1026921	1862259
kudarensis (HV74)	brown (Ge)	54600	0	$2.97341 \times 10^{-3}$	1554755	1582055	1123876	2040234
kudarensis (HV74)	Brown (Uap)	54600	41201	$2.78187 \times 10^{-3}$	1454599	1502500	1073836	1931163
kudarensis (HV74)	Polar (SRS412584)	54600	0	$2.70982 \times 10^{-3}$	1416926	1444226	1026664	1861787
kudarensis (HV74)	Polar (SRS412585)	54600	0	$2.71516 \times 10^{-3}$	1419721	1447021	1028635	1865406
praekudarensis	brown (Ge)	360000	0	$2.67496 \times 10^{-3}$	1398698	1578698	1166508	1990888
praekudarensis	Brown (Uap)	360000	41201	$2.50510 \times 10^{-3}$	1309880	1510481	1124465	1896496
praekudarensis	Polar (SRS412584)	360000	0	$2.41774 \times 10^{-3}$	1264203	1444203	1071648	1816758
praekudarensis	Polar (SRS412585)	360000	0	$2.42401 \times 10^{-3}$	1267483	1447483	1073962	1821005

**Table S4. Absolute times of nuclear divergence from the present day (node age) for all sample-pairs. Related to Figures 2 and 3, and STAR Methods.**

Node ages between pairs of individuals (t1, t2) are calculated from their pairwise divergence time (genetic divergence/estimated divergence rate) combined with their respective ages (Age t1, Age t2). Median, maximum and minimum node ages are calculated, respectively, from their corresponding substitution rate estimates shown in Table S3.

t1	t2	Age t1	Age t2	Genetic divergence	Divergence Time	Median node age	Lower node age	Upper node age
spelaeus	ingressus	34806	35062	$1.21541 \times 10^{-2}$	335108	370042	271287	468797
spelaeus	kanivetz	34806	45043	$1.13137 \times 10^{-2}$	311936	351860	259934	443786
ingressus	kanivetz	35062	45043	$1.48694 \times 10^{-3}$	40997	81050	68968	93131
spelaeus	eremus	34806	74696	$3.10318 \times 10^{-3}$	85560	140311	115097	165525
ingressus	eremus	35062	74696	$1.06025 \times 10^{-2}$	292328	347207	261060	433355
kanivetz	eremus	45043	74696	$1.00207 \times 10^{-2}$	276286	336155	254735	417576
spelaeus	rossicus	34806	37698	$9.24489 \times 10^{-3}$	254896	291148	216031	366265
ingressus	rossicus	35062	37698	$4.33152 \times 10^{-3}$	119427	155807	120612	191001
kanivetz	rossicus	45043	37698	$3.23248 \times 10^{-3}$	89125	130495	104230	156760
eremus	rossicus	74696	37698	$7.69330 \times 10^{-3}$	212116	268313	205804	330823
kudarensis (HV72)	kudarensis (HV74)	54600	54600	0	0	54600	54600	54600
kudarensis (HV72)	praekudarensis	54600	360000	$1.30592 \times 10^{-2}$	360063	567363	461254	673472
kudarensis (HV74)	praekudarensis	54600	360000	$1.30592 \times 10^{-2}$	360063	567363	461254	673472
spelaeus	kudarensis (HV72)	34806	54600	$3.82726 \times 10^{-2}$	1055234	1099937	788964	1410910
ingressus	kudarensis (HV72)	35062	54600	$3.71735 \times 10^{-2}$	1024932	1069763	767720	1371806
kanivetz	kudarensis (HV72)	45043	54600	$3.60745 \times 10^{-2}$	994630	1044451	751338	1337564
eremus	kudarensis (HV72)	74696	54600	$3.77554 \times 10^{-2}$	1040974	1105622	798852	1412393
rossicus	kudarensis (HV72)	37698	54600	$3.48461 \times 10^{-2}$	960762	1006911	723779	1290044
spelaeus	kudarensis (HV74)	34806	54600	$3.82726 \times 10^{-2}$	1055234	1099937	788964	1410910
ingressus	kudarensis (HV74)	35062	54600	$3.71735 \times 10^{-2}$	1024932	1069763	767720	1371806
kanivetz	kudarensis (HV74)	45043	54600	$3.60745 \times 10^{-2}$	994630	1044451	751338	1337564
eremus	kudarensis (HV74)	74696	54600	$3.77554 \times 10^{-2}$	1040974	1105622	798852	1412393
rossicus	kudarensis (HV74)	37698	54600	$3.48461 \times 10^{-2}$	960762	1006911	723779	1290044
spelaeus	praekudarensis	34806	360000	$3.09672 \times 10^{-2}$	853813	1051216	799601	1302831
ingressus	praekudarensis	35062	360000	$2.98681 \times 10^{-2}$	823511	1021042	778357	1263726
kanivetz	praekudarensis	45043	360000	$2.87691 \times 10^{-2}$	793208	995730	761975	1229485
eremus	praekudarensis	74696	360000	$3.03207 \times 10^{-2}$	835988	1053336	806974	1299698
rossicus	praekudarensis	37698	360000	$2.70235 \times 10^{-2}$	745081	943930	724358	1163502
brown (Ge)	Brown (Uap)	0	41201	$1.72614 \times 10^{-2}$	475925	496525	356272	636778
Polar (SRS412584)	Polar (SRS412585)	0	0	$4.52547 \times 10^{-4}$	12477	12477	8800	16154
brown (Ge)	Polar (SRS412584)	0	0	$1.94595 \times 10^{-2}$	536530	536530	378417	694642
brown (Ge)	Polar (SRS412585)	0	0	$1.93949 \times 10^{-2}$	534747	534747	377160	692335
Brown (Uap)	Polar (SRS412584)	41201	0	$1.06672 \times 10^{-2}$	294111	314711	228038	401385
Brown (Uap)	Polar (SRS412585)	41201	0	$1.08611 \times 10^{-2}$	299458	320059	231810	408308
spelaeus	brown (Ge)	34806	0	$5.24308 \times 10^{-2}$	1445600	1463003	1036991	1889014
spelaeus	Brown (Uap)	34806	41201	$5.12671 \times 10^{-2}$	1413515	1451518	1034962	1868075
spelaeus	Polar (SRS412584)	34806	0	$5.26894 \times 10^{-2}$	1452730	1470133	1042020	1898245
spelaeus	Polar (SRS412585)	34806	0	$5.27541 \times 10^{-2}$	1454512	1471915	1043277	1900553
ingressus	brown (Ge)	35062	0	$5.30127 \times 10^{-2}$	1461642	1479173	1048434	1909912
ingressus	Brown (Uap)	35062	41201	$5.15257 \times 10^{-2}$	1420645	1458776	1040119	1877434
ingressus	Polar (SRS412584)	35062	0	$5.25601 \times 10^{-2}$	1449165	1466696	1039633	1893758
ingressus	Polar (SRS412585)	35062	0	$5.26248 \times 10^{-2}$	1450947	1468478	1040891	1896066
kanivetz	brown (Ge)	45043	0	$5.21722 \times 10^{-2}$	1438470	1460991	1037081	1884902
kanivetz	Brown (Uap)	45043	41201	$5.05560 \times 10^{-2}$	1393907	1437029	1026251	1847808
kanivetz	Polar (SRS412584)	45043	0	$5.15904 \times 10^{-2}$	1422427	1444949	1025766	1864132
kanivetz	Polar (SRS412585)	45043	0	$5.16550 \times 10^{-2}$	1424210	1446731	1027023	1866439
eremus	brown (Ge)	74696	0	$5.20429 \times 10^{-2}$	1434905	1472253	1049393	1895113
eremus	Brown (Uap)	74696	41201	$5.01034 \times 10^{-2}$	1381430	1439378	1032277	1846480
eremus	Polar (SRS412584)	74696	0	$5.13964 \times 10^{-2}$	1417080	1454428	1036821	1872035
eremus	Polar (SRS412585)	74696	0	$5.14611 \times 10^{-2}$	1418862	1456210	1038078	1874343
rossicus	brown (Ge)	37698	0	$5.12671 \times 10^{-2}$	1413515	1432364	1015807	1848920
rossicus	Brown (Uap)	37698	41201	$4.92630 \times 10^{-2}$	1358258	1397707	997435	1797979
rossicus	Polar (SRS412584)	37698	0	$5.04267 \times 10^{-2}$	1390342	1409191	999464	1818919
rossicus	Polar (SRS412585)	37698	0	$5.04913 \times 10^{-2}$	1392125	1410974	1000721	1821227
kudarensis (HV72)	brown (Ge)	54600	0	$5.46936 \times 10^{-2}$	1507987	1535287	1090890	1979684
kudarensis (HV72)	Brown (Uap)	54600	41201	$5.31420 \times 10^{-2}$	1465207	1513108	1081318	1944897
kudarensis (HV72)	Polar (SRS412584)	54600	0	$5.44350 \times 10^{-2}$	1500857	1528157	1085861	1970453
kudarensis (HV72)	Polar (SRS412585)	54600	0	$5.44996 \times 10^{-2}$	1502639	1529939	1087118	1972760
kudarensis (HV74)	brown (Ge)	54600	0	$5.46936 \times 10^{-2}$	1507987	1535287	1090890	1979684
kudarensis (HV74)	Brown (Uap)	54600	41201	$5.31420 \times 10^{-2}$	1465207	1513108	1081318	1944897
kudarensis (HV74)	Polar (SRS412584)	54600	0	$5.44350 \times 10^{-2}$	1500857	1528157	1085861	1970453
kudarensis (HV74)	Polar (SRS412585)	54600	0	$5.44996 \times 10^{-2}$	1502639	1529939	1087118	1972760
praekudarensis	brown (Ge)	360000	0	$4.95862 \times 10^{-2}$	1367170	1547170	1144271	1950069
praekudarensis	Brown (Uap)	360000	41201	$4.73882 \times 10^{-2}$	1306565	1507166	1122127	1892205
praekudarensis	Polar (SRS412584)	360000	0	$4.86811 \times 10^{-2}$	1342215	1522215	1126670	1917760
praekudarensis	Polar (SRS412585)	360000	0	$4.87458 \times 10^{-2}$	1343998	1523998	1127928	1920068

**Table S5. Absolute times of mitochondrial divergence from the present day (node age) for all sample-pairs. Related to Figures 2 and 3, and STAR Methods.**

Node ages between pairs of individuals (t1, t2) are calculated from their pairwise divergence time (genetic divergence/estimated divergence rate) combined with their respective ages (Age t1, Age t2). Median, maximum and minimum node ages are calculated, respectively, from their corresponding substitution rate estimates shown in Table S3.

Sample	Cave	ZIN cat #	Description	Date code	radiocarbon age	Delta 13C	Maximum calibrated age*	Minimum calibrated age*	Calibrated median age*	Notes
USP-01	Medvezhiya	34991-6	<i>kanivetz</i> partial skull	OxA-19568	45,150 ± 600	-20.84	47,984	45,320	46,652	Date may extend out of calibration range
USP-03	Medvezhiya	34991-19	<i>kanivetz</i> mandible	OxA-19608	42,000 ± 450	-20.1	44,251	42,616	43,434	
UKZ-05	Kizel	28601-44	<i>rossicus</i> right maxilla	OxA-19565	46,250 ± 700	-20.94	...	46,332	...	Date may extend out of calibration range
UKZ-06	Kizel	28601-32	<i>rossicus</i> right maxilla	OxA-19566	39,040 ± 330	-22.68	41,461	40,452	40,957	
-	Kizel	-	-	OxA-16964	36,390 ± 270	-21.842	39,646	38,462	39,054	From [S9]
UKZ-01	Kizel	28601-29	<i>rossicus</i> right maxilla	OxA-19561	35,330 ± 220	-21.55	38,543	37,387	37,965	
UKZ-02	Kizel	28601-13a	<i>rossicus</i> right maxilla	OxA-19562	35,110 ± 230	-21.08	38,301	37,095	37,698	
UKZ-07	Kizel	28601-41	<i>rossicus</i> right maxilla	OxA-19567	34,610 ± 230	-21.43	37,752	36,646	37,199	
UKZ-04	Kizel	28601-24	<i>rossicus</i> left maxilla	OxA-19564	32,940 ± 190	-21.1	35,832	34,423	35,128	
UKZ-03	Kizel	28601-12a	<i>rossicus</i> left maxilla	OxA-19563	32,630 ± 180	-20.96	35,240	34,136	34,688	
-	Kizel	-	-	OxA-16960	31,870 ± 190	-21.462	34,271	33,361	33,816	From [S9]

**Table S6. Radiocarbon dates for Medvezhiya and Kizel cave bears used for indirect age estimates for the sequenced samples. Related to STAR Methods.**

\*Calibrated using OxCal 4.2 online, based on the IntCal-13 curve.

Taxon (sample)	Reference taxon (GenBank Acc.)	Mapable reads	Mapped	Unique mapped	% Duplication	Mapped bp	Read depth*	% Covered > 2 reads
<i>praekudarensis</i> (KU1)	<i>kudarensis</i> (MH605139)	1399428212	40576	35942	11.42	1175197	69.89	97.38
brown (Ge)	<i>U. arctos</i> (EU497665)	61792045	5861	5354	8.6504	794631	47.43	96.60
brown (Uap)	<i>U. arctos</i> (EU497665)	140333181	73626	67049	8.93299	3037226	181.29	99.13
black (ERS781634)	<i>U. thibetanus</i> (NC_009971)	167698034	138701	31563	77.2439	2824722	168.19	99.23
polar (SRS412584)	<i>U. maritimus</i> (NC_003428)	31203972	32947	20507	37.7576	2001068	117.59	95.85
polar (SRS412585)	<i>U. maritimus</i> (NC_003428)	20357905	16691	13225	20.7657	1239153	72.82	95.84
<i>kanivetz</i> (B04)	<i>spelaeus</i> (EU327344)	380166888	169096	129959	23.1448	5925633	352.51	98.81
<i>rossicus</i> (B04)	<i>spelaeus</i> (EU327344)	368057543	242012	189019	21.8968	8010665	476.54	98.89
<i>eremus</i> (WK01)	<i>spelaeus</i> (EU327344)	335628787	157015	137148	12.6529	5817044	346.05	98.64
<i>kudarensis</i> (HV72)	<i>kudarensis</i> (MH605139)	252663833	133306	106436	20.1566	5894257	350.56	99.55

**Table S7. Details of mitochondrial genome reconstruction. Related to STAR Methods.**

\*Mapped bp divided by reference genome size.

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