



## Palaeoecological and genetic analyses of Late Pleistocene bears in Asiatic Russia

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Brown bears are one of the few large carnivore species that survived the final Pleistocene wave of extinctions, perhaps in part owing to their wide ecological plasticity, variety of forms and polyphagia. Although the brown bear has become a well-studied system, many questions remain regarding the ecological, trophic and genetic diversity throughout their distribution. For example, knowledge about Asiatic Russian brown bears from the Late Pleistocene arctic tundra steppe, an ecosystem with no analogue in modern times, is sparse. Here we compared diets, morphometry and genetic affinities of Late Pleistocene bears based on broadly sampled subfossil remains from Asiatic Russia. Collecting sites included the Ural Mountains, the lower reaches of the Irtysh River, the upper reaches of the Ob River, the Altai Mountains of western Siberia, the Indigirka–Kolyma Lowlands and northwestern Chukotka. An extremely large bear specimen from the middle Indigirka (41 090 <sup>13</sup>C a BP) that lived in landscapes of treeless shrubs and wet meadows had a diet composed principally of large herbivorous mammals. A bear from western Chukotka (25 880 <sup>14</sup>C a BP), much smaller in size, had a diet close to that of modern brown bears. These two Late Pleistocene NE Russian brown bears may comprise a previously undiscovered, but extinct, genetic lineage. At the end of the Pleistocene (MIS 3 and MIS 2), the brown bears from the Ob River Valley and Urals lived in periglacial forest-steppes and those from the southern Urals in conditions of periglacial steppe. Brown bears from the Ob River valley and Urals, as well as ancient Altai bears, were characterized by a varied diet, from polyphagia to vegetarianism. In living brown bears, the proportions of different dietary foods are primarily related to food availability, which depends on the geographical zone and climatic conditions. We conclude that the same was true for Late Pleistocene brown bears of NE Siberia.

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As a large terrestrial carnivore with a wide Holarctic distribution and a notable subfossil record, the brown bear (*Ursus arctos*) has become a well-studied system of Northern Hemisphere Pleistocene–Holocene biogeography. A growing body of analyses of maternally inherited mitochondrial DNA (mtDNA) sequence data has demonstrated considerable phylogeographical structure, probably caused by maternal philopatry, with extant diversity falling into numerous lineages largely following geographical distribution, in addition to several extinct clades (Davison *et al.* 2011). Consequently, phylogeographical patterns from analyses of both extant and extinct bear lineages have left signatures of considerable extinction, refugial survival and extensive postglacial expansions of the brown bear species, indicating a complex biogeographical and palaeoecological history. Furthermore, the brown bear exhibits a dynamic spatial and temporal morphological and ecological diversity, also reflected by its large number of described subspecies and

contentious taxonomic classification (Meloro *et al.* 2017). Nevertheless, insight into the ecological and genetic diversity of the species throughout much of its evolutionary history and geographical range remains sparse.

In recent years, exceptionally large subfossil Pleistocene specimens of brown bear have been discovered in the territory of Yakutia, raising questions concerning the taxonomic, ecological, trophic and genetic positions of these large, ice age brown bears in NE Siberia (Boeskorov *et al.* 2019; Marciszak *et al.* 2019; Rey-Iglesia *et al.* 2019; Krylovich *et al.* 2020). However, the identification of bear bones, especially humeri, is often difficult because only Asian black bear (*Ursus thibetanus*), which constantly uses its forelimbs for climbing trees, has a humerus that differs markedly from that of other bear species (Gromova 1950; Meloro & de Oliveira 2019). This calls into question the species identification of bear postcranial remains based only on morphological features and requires the use of other methods (Gorlova *et al.* 2015).

Pleistocene and modern bears from Asiatic Russia are represented by several species: cave bear (*Ursus cf. kudarensis* Baryshnikov, 1985, and *Ursus savini nordostensis* Baryshnikov, 2011), Asian black bear (*U. thibetanus* Cuvier, 1823), polar bear (*Ursus maritimus* Phipps, 1774) and brown bear (*U. arctos* Linnaeus, 1758) (Aristov & Baryshnikov 2001; Boeskorov & Baryshnikov 2013; Kosintsev et al. 2016a,b). Cave bears have long been known from eastern slopes of the Urals and western Siberia (Gromov 1948; Alekseeva 1980; Vereshchagin & Baryshnikov 2000), whereas in NE Russia they were discovered relatively recently, isolated and confined to the deposits of the Olaryan suite (Early Pleistocene) that range in age from 1.5–0.5 Ma ago (Sher et al. 2011; Boeskorov et al. 2012). In addition, the predatory fauna of Olor suite in the Adycha basin was in recent years expanded to include the cave bears *Ursus cf. deningeri* (cf. *U. d. kudarensis*) from Oskhordokh (Knapp et al. 2009) and *U. savini* from Ulakhan Sullare (Boeskorov et al. 2012). Fossil remains of polar bears, on the other hand, are very rare and are confined mainly to the Holocene of coastal NE Russia (Boeskorov et al. 2018).

The brown bear has lived in Yakutia since at least the middle of the Middle Pleistocene, 400 000–300 000 years ago (Boeskorov & Baryshnikov 2013). Fossil remains of this species are known from the lower reaches of the Aldan, Lena and Kolyma Rivers, and the island of B. Lyakhovsky. Previous investigations of Quaternary brown bear remains from Yakutia suggested that a large brown bear in the Middle Pleistocene (Mindel-Riss, Riss), a form close to *Ursus arctos kamiensis* from the end of the Middle Pleistocene, was present. At the beginning of the Late Pleistocene, the smaller *Ursus arctos priscus* occupied this area, and at the end of the Pleistocene and in the Holocene an even smaller *U. arctos*, similar in size to the modern Yakutian brown bear, was present (Boeskorov & Baryshnikov 2013). There is now abundant evidence that the Late Pleistocene large brown bear form, *U. a. priscus*, was widespread not only in Yakutia but also in Chukotka, western Siberia and the Urals, as well as in eastern and western Europe (Vereshchagin 1973; Alekseeva 1980; Baryshnikov 2007; Doppes & Pacher 2014; Kosintsev & Bachura 2015; Marciszak et al. 2015, 2019; Boeskorov et al. 2019). Presumably, the large size of Late Pleistocene bears in Yakutia was caused by an increase in the amount of available food (Boeskorov et al. 2019). However, except for a recent comparison of stable isotopes of Pleistocene and modern Yakutian brown bears (Krylovich et al. 2020), a systematic comparison of the diets of fossil brown bears from different regions of Russia has not yet been carried out. Similarly, morphometric and genetic analyses comparing the large NE Russian Pleistocene bears with other contemporaneous and later bears from other regions are limited. Hence, questions still remain concerning the palaeogenetic and palaeoecological diversity of the NE Russian Pleistocene bears and their relationships to modern bears.

In this study, we analysed the trophic, morphometric and genetic diversity of Late Pleistocene bears in the Urals, western Siberia, eastern Siberia, Chukotka and the Russian Far East and compared them with Holocene and modern bears from these regions.

## Material and methods

### *Bear specimens studied*

The specific discrimination of bear remains is difficult because the morphological differences between them are often unclear. In the Late Pleistocene, five species of bears of the genus *Ursus* inhabited the Ural Mountains, the Altai Mountains and Siberia: the large and small cave bears, brown bear, polar bear and Asian black bear. There are marked differences in the upper arm bone, or humerus, between the Asian black bear and these other bears (Meloro & de Oliveira 2019). The humerus bones of other species were discriminated on the basis of published data (Petronio et al. 2003) and comparative skeletal collection from the zoological museum of the Institute of Plant and Animal Ecology (Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia).

A total of 60 fossil and modern bears were studied. Fossil bones were dated by different methods, including AMS radiocarbon dating of the bones themselves, dating by stratigraphical and biostratigraphical data, and dating by the degree of fossilization of bone tissue. The bones were collected from three regions of Russia (Table 1, Fig. 1):

- (i) From the NE and the Far East of Russia, 26 samples were studied for morphology ( $n = 5$ ), stable isotopes ( $n = 12$ ) and genetic ( $n = 19$ ) analysis. The samples included humerus bones of good preservation from two subfossil adult brown bears (Fig. 2), one very large from Middle Indigirka (F-2296) and another of medium size from Poginden River (F-2374). AMS radiocarbon dating was performed on three samples (F-1046, F-2296 and F-2374). Specimens are stored in National Alliance of Shidlovskiy 'Ice Age', Moscow, except for the modern specimens IK-1 and IK-2 from Ulunga River, which are held in a private collection.
- (ii) From the Altai region, eight subfossil bear specimens collected in either a riverbed or on the riverbank were all studied for stable isotopes, including a specimen thought to be of cave bear origin (*Spelearctos* sp.; F-0725), three purported brown bear specimens (*U. arctos*; F-0722, F-0729 and F-0754) and four specimens of either possible brown bear or unknown bear origin (F-1578, F-1579, F-0727 and F-0728). Specimens are held at the collection of National Alliance of Shidlovskiy 'Ice Age', Moscow.

Table 1. List of samples of fossils and modern bears from Asiatic Russia studied in this project.

Species	Collection no.	Bone	Site	Geological age <sup>1</sup>	Analysis <sup>2</sup>	Number on Fig. 1
Urals and western Siberia						
<i>Ursus arctos</i>	178/153	Lower jaw	Nizhnyaya Tavda	MIS 3	SI	6
<i>U. arctos</i>	178/154	Scapula	Nizhnyaya Tavda	MIS 3	SI	6
<i>U. arctos</i>	178/316	Radius	Nizhnyaya Tavda	MIS 3	SI	6
<i>U. arctos</i>	798/2536	Humerus	Shaitanskaya cave	MIS 2 (1)	M	2
<i>U. arctos</i>	858/21	Humerus	Evalga	MIS 2 (2)	SI	4
<i>U. arctos</i>	915/869	Humerus	Irtys River	MIS 3	M	7
<i>U. arctos</i>	915/1014	Humerus	Irtys River	MIS 3	M	7
<i>U. arctos</i>	915/1017	Humerus	Irtys River	MIS 3	M	7
<i>U. arctos</i>	915/2284	Ulna	Irtys River	MIS 3	SI	7
<i>U. arctos</i>	1028/325	Ulna	Usoltsevskaya cave	MIS 3	SI	3
<i>U. arctos</i>	2060/109	Humerus	Ostrolenskoe	MIS 3	SI; M	9
<i>U. arctos</i>	2060/113	Scapula	Ostrolenskoe	MIS 3	SI	9
<i>U. arctos</i>	2079/2	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/6	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/11	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/182	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/183	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/184	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/233	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/236	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/237	Humerus	Severnaya cave	MIS 3 (3)	M	1
<i>U. arctos</i>	2079/238	Humerus	Severnaya cave	MIS 3 (3)	SI; M	1
<i>U. arctos</i>	2079/239	Humerus	Severnaya cave	MIS 3 (3)	SI; M	1
<i>U. arctos</i>	2079/244	Humerus	Severnaya cave	MIS 3 (3)	SI; M	1
<i>U. arctos</i>	2149/1516	Vertebra	Merimy	MIS 3	SI	8
<i>U. arctos</i>	2305/1	Pelvis	Zykovo	MIS 2	SI	5
Altai region						
<i>U. arctos</i>	F-0722	Ulna	Chumysh River	Fossil	SI	12
<i>Spelaeoarctos</i>	F-0725	Tibia	Anuy River	Fossil	SI	13
<i>Ursus</i> sp.	F-0727	Femur	Charysh River	Fossil	SI	10
<i>Ursus</i> sp.	F-0728	Humerus	Charysh River	Fossil	SI	10
<i>Ursus</i> sp.	F-0729	Cranium	Charysh River	Fossil	SI	10
<i>U. arctos</i>	F-0754	Radius	Altai	Fossil	SI	14
<i>U. arctos?</i>	F-1578	Atlas	Kasmala River	Fossil	SI	11
<i>U. arctos?</i>	F-1579	Femur	Chumysh River	Fossil	SI	12
NE and Far East Russia						
<i>Ursus maritimus</i>	F(R)-1	Cranium	Alazeya River	Recent	G	16
<i>U. arctos</i>	F(R)-7/2	Lower jaw	NE Russia	Recent	G	27
<i>U. arctos</i>	F(R)-18	Hyoid	Maly Anyu River, W. Chukotka	Recent	G	22
<i>U. arctos</i>	F(R)-19	Hyoid	Maly Anyu River, W. Chukotka	Recent	G	22
<i>U. arctos</i>	F(R)-217	Lower jaw	NE Yakutia	Recent	G	28
<i>U. arctos</i>	F(R)-219	Cranium	Western Chukotka	Recent	G	26
<i>U. arctos</i>	F(R)-247	Hyoid	Eastern Siberian sea coast, 200 km from Ambarchik settlement	Recent	G	24
<i>U. arctos</i>	F(R)-248	Hyoid	Eastern Siberian sea coast, 200 km from Ambarchik settlement	Recent	G	24
<i>U. arctos</i>	F-275	Pelvis	Filipova River	Fossil	G; Iz	19
<i>U. arctos</i>	F(R)-276	Lower jaw	Letnyaya River	Recent	G	21
<i>U. arctos</i>	F-583	Ulna	Between Kolyma and Indigirka RR	Fossil	Iz	20
<i>U. maritimus</i>	F-1045	Cranium	Eastern Siberian Sea coast	Recent	G; SI	23
<i>U. maritimus</i>	F-1046	Cranium	Alazeya River	Holocene	G; SI; <sup>14</sup> C	16
<i>Ursus</i> sp.	F-1863	Ulna	Maly Anyu River	Fossil	G; SI	22
<i>Ursus</i> sp.	F-1864	Radius	Maly Anyu River	Fossil	SI	22
<i>U. arctos</i>	F-2296	Humerus	Indigirka River	Mis 3	G; SI; <sup>14</sup> C; M	15
<i>U. arctos</i>	F-2374	Humerus	Pogindin River	Mis 3	G; SI; <sup>14</sup> C; M	25
<i>Ursus</i> sp.	F-2723	Cranium	Pogindin River	Fossil	SI	25
<i>U. maritimus?</i>	F-3244	Pelvis	Rauchua River	Recent?	G; SI	17
<i>U. maritimus</i>	F-3549	Cranium	Hallercha tundra	?	G; SI	18
<i>U. arctos</i>	F-3677	Lower jaw	NE Yakutia	Fossil	SI	28

(continued)

Table 1. (continued)

Species	Collection no.	Bone	Site	Geological age <sup>1</sup>	Analysis <sup>2</sup>	Number on Fig. 1
<i>U. arctos</i>	F(R)-302	Humerus	W. Chukotka	Recent	M	26
<i>U. arctos</i>	F(R)-321	Humerus	W. Chukotka	Recent	M	26
<i>U. arctos</i>	IK-1	Lower jaw	Ulunga River	Recent	G	29
<i>U. arctos</i>	IK-2	Humerus	Ulunga River	Recent	M	29
<i>U. maritimus</i>	IK-3	Cranium	Rauchua River	Recent	G	17

<sup>1</sup>Without marks – this paper; (1) = Smirnov (1996); (2) = Kosintsev et al. (2005); (3) = Kosintsev & Bachura (2015).

<sup>2</sup>G = DNA; SI = stable isotopes; <sup>14</sup>C = radiocarbon dating; M = morphometry.

(iii) From caves on the eastern slope of the Urals and alluvial locations of western Siberia, 26 subfossil brown bear specimens were studied for morphology ( $n = 17$ ) and stable isotopes ( $n = 13$ ). The humeral bones of brown bears from the locations of the basins of the Lower Ob (the Vizhay and Idvel rivers), the Lower Irtysh (the Nizhnyaya

Tavda and Irtysh rivers) and the upper reaches of the Ural (the Gumbeyka River) were measured. The bear bones are expected to be from different geological ages as determined from previous studies. For example, previously dated bones from bears from Severnaya are of the following <sup>14</sup>C a BP dates:  $37\ 885 \pm 1400$  (SPb-1629),  $27\ 100 \pm 250$



Fig. 1. Geographical locations of the studied subfossil bear specimens.

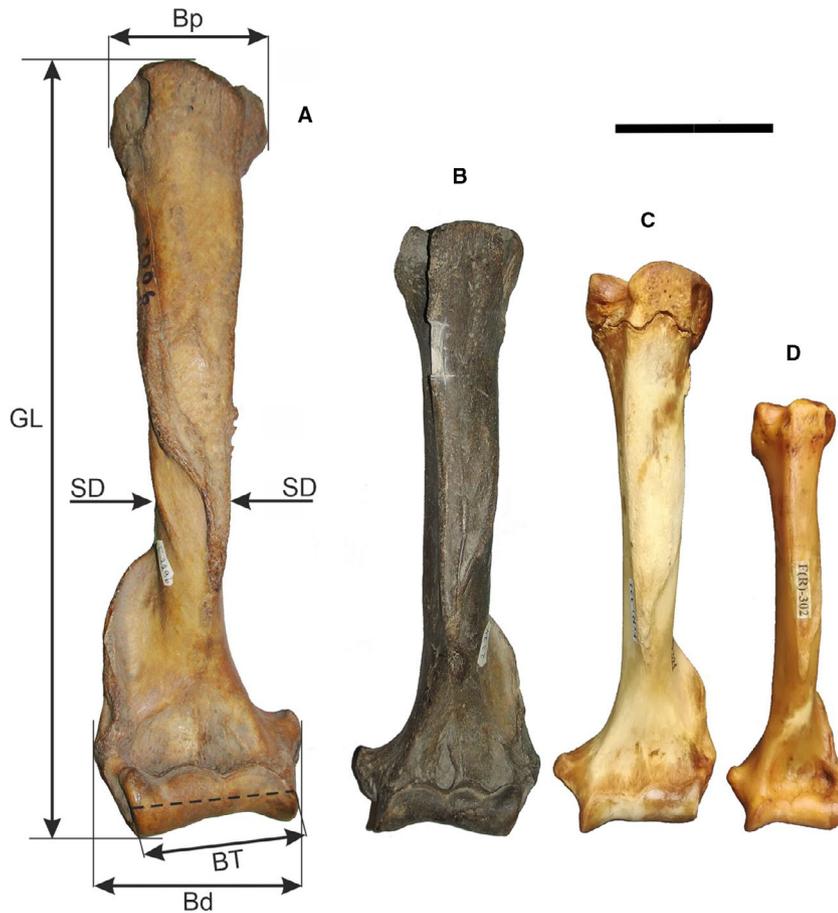


Fig. 2. Brown bear humeri from NE Russia. Pleistocene: A. specimen F-2296; B. specimen F-2374. Recent: C. specimen F(R)-321, male; D. specimen F(R)-302, female. National Alliance of Shidlovskiy 'Ice Age'. Scale: 10 cm. The difference in the sizes of samples C and D looks like sexual dimorphism; however, these may simply be local variations. GL = greatest length; Bp = greatest breadth of the proximal end; SD = smallest breadth of the diaphysis; Bd = greatest breadth of the distal end; BD = greatest breadth of the trochlea.

(SOAN-7916) and  $25\,547 \pm 800$  (SPb-1630) (Kosintsev & Bachura 2015), which correspond to the second half of the Karginsky interstadial or the second half of MIS 3. Mammalian bones from Evalga have been dated to the following  $^{14}\text{C}$  a BP dates:  $22\,890 \pm 910$  (SOAN-5198),  $19\,710 \pm 205$  (SOAN-4464),  $17\,050 \pm 160$  (SOAN-4844) and  $15\,640 \pm 220$  (SOAN-5198a) (Kosintsev *et al.* 2005), which correspond to the first half of the Sartan stadium or MIS 2. Bear bones from Shaitanskaya cave have been dated to MIS 2 (Smirnov 1996), whereas bones from Usoltsevskaya cave, Niznyaya Tavda, Irtys River, Merimy and Ostrolenskoe sites were dated to MIS 3 and those from Zykovo to MIS 2 (Volkova *et al.* 2002). The Pleistocene bears of the eastern slope of the northern Urals and western Siberia lived in conditions of periglacial forest-steppe (Volkova *et al.* 2002; Lapteva 2009) and the bears of the eastern slope of the southern Urals lived in the periglacial steppe (Lapteva, 2007). The samples

are stored in the Museum at the Institute of Plant and Animal Ecology (Ural Branch of the Russian Academy of Sciences).

#### Morphometric measurements

For comparison of sizes, the brown bear humeri that were most numerous in the collections were measured according to the scheme of von den Driesch (1976) by means of digital calipers, with an accuracy of 0.1 mm. The measurements included: greatest length (GL, when the whole bone was available), greatest breadth of the proximal end (Bp), smallest breadth of the diaphysis (SD), greatest breadth of the distal end (Bd) and greatest breadth of the trochlea (BD) (see Fig. 2).

#### Stable isotopes

All stable isotope measurements were performed on collagen extracted from bone samples. The Tübingen laboratory followed the protocol of Bocherens *et al.*

(1997) with precleaning of bones by ultrasonication in acetone and distilled water. A small aliquot (around 5 mg of bone powder) was separated to check the collagen content of the material by elemental analysis of nitrogen content (Bocherens *et al.* 2005). Stable isotope measurements of extracted collagen were performed at ICTA, Barcelona on a Thermo Flash 1112 (Thermo Scientific VC) elemental analyser and a Thermo Delta V Advantage mass spectrometer with a ConFlo II interface against V-PDB, AIR and IAEA-600 standards. In the IGRAS and ANSTO laboratories collagen was extracted and purified following the ultrafiltration protocol (Brown *et al.* 1988; Bronk Ramsey *et al.* 2004; Higham *et al.* 2006). Again, a small aliquot for each sample was tested to check the quality of the material and suitability for measurements judging by the collagen yield and C/N ratio. At ANSTO, elemental analyses were performed and stable isotopes were determined on an elemental analyzer isotope ratio mass spectrometry (EA-IRMS) (Elementar varioMICRO CUBE coupled to a Micromass Isoprime). Isotopic measurements of samples prepared at IGRAS were performed on an Elementar varioMICRO Cube coupled to an Isoprime Precision IRMS (Elementar, Germany / Ionplus, Switzerland).

#### *AMS radiocarbon dating*

At ANSTO, radiocarbon measurements on extracted and purified collagen after converting the sample to graphite (Hua *et al.* 2001) were performed on a Vega 1 MV tandem accelerator (Wilcken *et al.* 2015). At IGRAS Radiocarbon Laboratory, graphitization and the pressing of targets for  $^{14}\text{C}$  AMS were conducted with the automated graphitization system AGE 3 (Wacker *et al.* 2010).  $^{14}\text{C}$  AMS measurements were performed at the Center for Applied Isotope Studies, University of Georgia (Athens, GA, USA) using the CAIS 0.5 MeV accelerator mass spectrometer. The sample ratios at ANSTO were normalized on the Ox I standard ratio and for IGRAS samples on the oxalic acid II (NBS SRM4990C) standard measured together with unknowns. The quoted uncalibrated dates are given in  $^{14}\text{C}$  years before AD 1950 (a BP) using the  $^{14}\text{C}$  half-life of 5568 years. The error is quoted as 1 standard deviation and reflects both statistical and experimental errors. The obtained  $^{14}\text{C}$  dates were calibrated according to IntCal20 and MARINE20 calibration curves (Heaton *et al.* 2020; Reimer, 2020) using the CALIB 8.2 program (<http://calib.org/calib/calib.html>). The polar bear sample was calibrated assuming a diet that is 95% marine and 5% terrestrial. Reservoir correction was assessed from the averaged determinations for the Laptev and Chukchi seas (<http://calib.org/marine/>).

#### *Genetic analyses*

Genomic DNA was extracted from 19 bone specimens from the NE and Far East of Russia, including two

ancient, radiocarbon-dated specimens (F-2296 and F-2374) and 17 modern/historical specimens (Table 1, Fig. 1). Because none of the bone samples had been intentionally preserved for subsequent extraction of DNA, they were regarded as non-modern (ancient) samples and thus all DNA extractions and the setting up of PCR amplifications were performed in a dedicated cleanroom facility, physically separated from any modern DNA laboratory and appropriate for ancient DNA research. The following protocol designed for ancient DNA extraction was used: 50–100 mg fine bone powder was obtained from each sample using a dental drill (HKM surgical hand piece, Pearson Dental, USA) and DNA from the bone powder was extracted following a silica column-based protocol (Dabney *et al.* 2013). Negative controls were prepared alongside all extractions. Amplification of DNA using bear-specific primers targeting the mtDNA control region and cytochrome b was performed to determine DNA preservation and clade affinity. PCR reactions followed a previously described protocol and primers (Lan *et al.* 2017). PCR products were Sanger sequenced directly using the same primers as in the PCR.

To target the entire mitochondrial genome (mitogenome), DNA extracts were processed by Daicel Arbor Biosciences (<https://arborbiosci.com/>) for preparation of Ion Torrent sequencing libraries and mitochondrial DNA enrichment and sequencing (see Lan *et al.* 2017 for details). Following sequencing, reads were de-multiplexed, quality trimmed and filtered using the default settings on the Ion Torrent Suite v. 4.4.3. Assembly of mitochondrial genomes was performed using the following strategy: species-specific mitochondrial reference genomes were selected from initial species identification based on phylogenetic analyses of amplicon mtDNA sequences (see above; results not shown). All Ion Torrent sequence reads were first aligned against these reference genomes using BWA aln (version 0.7.13; Li & Durbin 2010) and the default parameters, except for the parameter ‘-l1024’ to disable the seed and increase the number of high-quality hits for the damaged ancient DNA reads (Schubert *et al.* 2012). Then the remaining unmapped reads were aligned against the same reference using BWA mem with default parameters (for assembly statistics see Table S1). We filtered for human contamination by applying an edit-distance-based strategy (Schubert *et al.* 2012). All reads were mapped to a human mitochondrial genome reference (NCBI accession no. J01415.2) using the same BWA mapping method as described above. Reads with a higher mapping edit-distance to human mtDNA than to bear mitochondrial genomes were considered of likely human origin and were removed from the bear mitogenome mapping results. PCR duplicates were removed with the Mark Duplicates tool in the Picard software suite version 1.112 (<http://broadinstitute.github.io/picard/>) using a lenient validation stringency. Consensus calling was carried out using Samtools *mpileup* (Li 2011) with default settings.

The 19 new mitogenomes were aligned with 187 previously published mitogenomes and phylogenetic analyses included two mitogenomes from the cave bear (*Ursus spelaeus*) to root the trees (see Fig. S1). The dataset of complete mitogenomes was subjected to maximum likelihood phylogenetic analyses performed using RAxML-HPC BlackBox version 8.2.8 (Stamatakis 2014) in the CIPRES Science Gateway (Miller *et al.* 2010) under the GTR substitution model, which was identified as the best-supported model by jmodeltest2 (Guindon & Gascuel 2003; Durrin *et al.* 2012). A total of 1000 bootstrap replicates were conducted to evaluate branch support. Bayesian inference phylogenetic analyses and MCMC is defined as: Markov Chain Monte Carlo (MCMC)-based divergence time estimation was carried out including only dated samples (four polar bear samples were of unknown, possibly ancient, age) using BEAST version 1.10.4 (Suchard *et al.* 2018) and BEAGLE library version 2.1.2 (Ayres *et al.* 2011) for accelerated, parallel likelihood evaluation as implemented in the CIPRES Science Gateway. The strict clock and constant size coalescent prior were used. The median probability of calibrated radiocarbon dates was used to estimate ages for terminal nodes, including only historic/modern samples and the two ancient, radiocarbon-dated specimens from this study. Trees were sampled every 500 000 generations from a total of 500 million generations. Effective sampling size values greater than 200 were obtained for all parameters sampled from the MCMC and the posterior distributions were examined using Tracer version 1.6 (Drummond & Rambaut 2007). The maximum clade credibility tree was generated using Tree Annotator, implemented in the BEAST package, with 10% burn-in.

## Results

### *Morphometric measurements*

Measurements of the investigated brown bear humeri are shown in Table S2, which also includes additional published data from morphometric measurements of humeri from European Holocene and Pleistocene brown bears (Gromova 1950; Zalkin 1961, 1963; Paaver 1965; David, 1980; Torres 1988; Vasiliev & Grebnev 2009; Garcia-Vazquez 2015; Kuijper *et al.* 2016). When comparing the subfossil measurements with modern brown bears, the minimum sizes of the humerus bones of modern bears are generally smaller than the minimum sizes of the analogous Late Pleistocene bones studied here, and the maximum sizes do not reach the maximum sizes of the bones of the Late Pleistocene bears (Table S2). The most complete measurements of the studied subfossil specimens are of the width of the distal end and diaphysis of the humerus bones, which differ markedly but range within the variation of Iberian subfossil brown bears (Fig. 3A). Overall, the humerus bones form largely three main size groups. An outlier sample among the Russian specimens,

F(R)-302 (Fig. 3A, bottom left), which group among the smallest European bear humerus bones, belonged to a modern female brown bear killed by a hunter. There is no apparent association of size group with geographical location and geological age for the subfossil specimens. For example, specimens from one location (Severnaya Cave in the Urals) and of the same geological age (second half of MIS 3) are found in different size groups. The first group consisting of relatively smaller bears (Fig. 3A, middle) includes two modern male bears (F(R)-321 and IK-2) and six subfossils: five from northern Ural (2079/2, 2079/6, 2079/233, 2079/237 and 2079/238) and one from the Irtysh River (915/1017). The second group (Fig. 3A, top right) comprises the largest bones, all subfossils: two from NE Yakutia (F-2296 and F-2374) and three from the northern Ural (2079/244), southern Ural (2060/109) and the Irtysh River (915/869). No fossil bone is as small as the modern female bear F(R)-302. The two largest specimens were found in NE Russia (F-2296, Indigirka River) and in the Urals (2079/244, Severnaya Cave). It should be noted that the age difference between the giant brown bear F-2296 and the brown bear F-2374 is ~15 000 years (Table 2). Brown bears have a pronounced sexual dimorphism in body size, as males are much larger than females (Heptner *et al.* 1998). This is also manifested in the size of the skeleton (Koby 1949; Yoneda & Abe 1976; Petronio *et al.* 2003; Baryshnikov, 2007). It is possible that the humeri from the group of smaller bears belong to modern males and subfossil females, and the second group comprises larger subfossil males. However, depending on age and season, modern brown bear males can differ considerably in body size even within a population, as can also be seen with the range observed among the Iberian brown bears. Hence, a validation of body size sexual dimorphism among Pleistocene brown bears requires a larger sampling of subfossil remains that can be sex determined. In order to assess potential differences in morphology between the Iberian and Russian groups of bears, we also log-transformed the two variables, the width of the distal end and diaphysis, and plotted the regression lines obtained for the Iberian and Russian fossil bears (Fig. 3B).

### *Radiocarbon dating and stable isotopes*

The new radiocarbon dating results for three bones from bear specimens from NE Russia (F-2296, F-2374 and F-1046) are shown in Table 2. As expected, F-1046 returned a Holocene date, whereas the giant brown bear F-2296 and brown bear F-2374 returned dates of  $41\,090 \pm 570$  and  $25\,880 \pm 80$   $^{14}\text{C}$  a BP, respectively. The calibrated ranges (and median probabilities) for the two latter bears were 43 030 to 44 800 cal. a BP (44 000 cal. a BP) and 29 990 to 30 280 cal. a BP (30 110 cal. a BP), respectively (Table 2).

The results of the stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of 33 bear bones from NE Russia, the Altai region and Ural to western Siberia are given in Table S3 and Fig. 4.

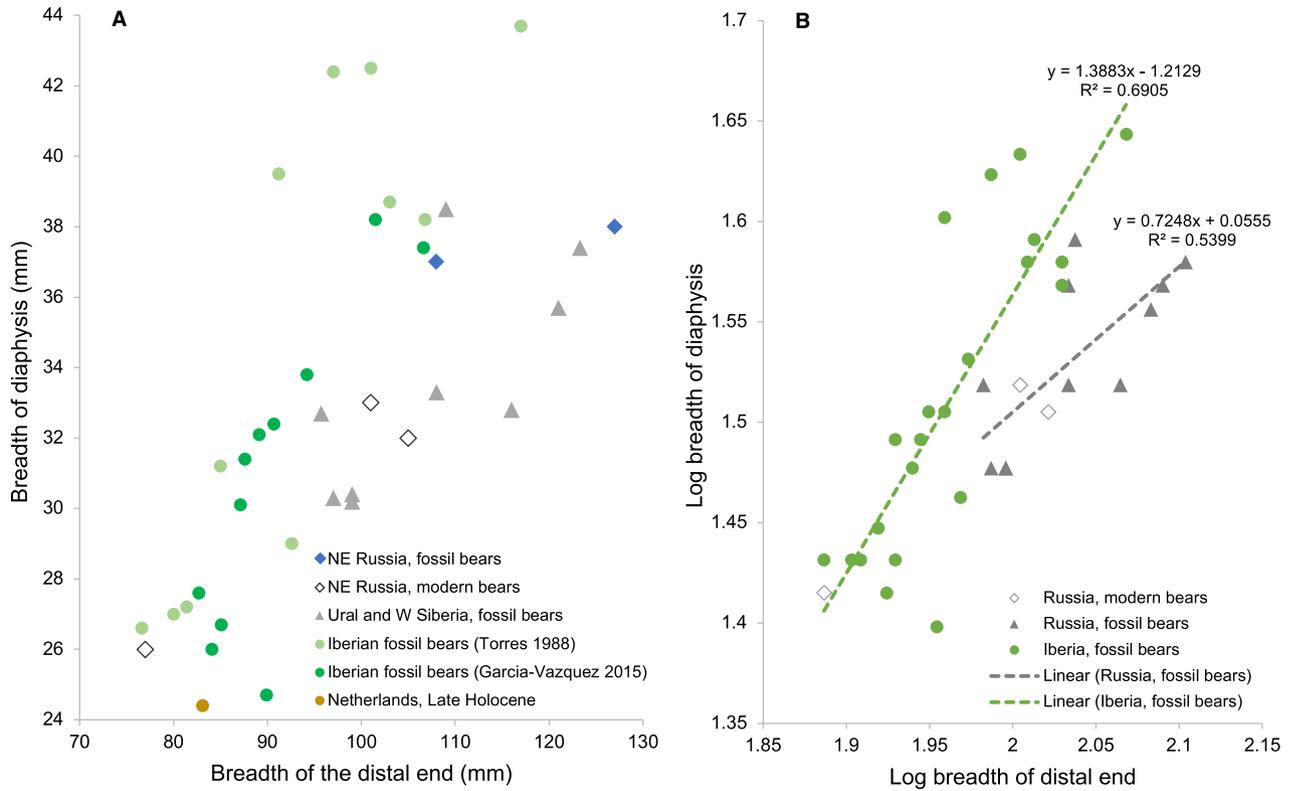


Fig. 3. A. Greatest breadth of the distal end (Bd) and smallest breadth of diaphysis (SD) humeri in *Ursus arctos* from measurements of specimens studied in this paper (see Table S2) and published studies. B. Log transformed values of Bd and SD humeri from fossil Russian and Iberian bears, as well as modern Russian bears. The linear regressions of the two groups of fossil bears are shown.

The values fall into three main diet groups. (i) The first group is characteristic of a marine predator with high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and consists of bears from NE Russia, most of which are predicted to be polar bears. Initial morphological identification suggested three of the specimens to be either *U. arctos* (F-275) or questioned as being *Ursus* (F-1863 and F-1864), but stable isotope values and genetic analyses (F-275 and F-1863; see below) confirmed a polar bear identity. (ii) Another group consists of bears from all three regions with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ranging from SS and WW, respectively, indicating characteristics of mainly carnivorous (F-0754) to more omnivorous diets, suggestive of brown bears of varying diets, depending on region. (iii) The third group consists of five bears from the Altai region and two bears from the southernmost location in the

Urals, Ostrolenskoe, with low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values characteristic of a more plant-based diet, suggesting that these bears were either cave bears (Naito *et al.* 2020) or ‘herbivorous’ brown bears. It should be noted that the bones from the southern Ural have the structure of brown bear, not cave bear. On the other hand, the morphology of one bone from Altai (F-0725) suggests that it belongs to a cave bear, whereas other bones from Altai (F-0727, F-0729, F-1578 and F-1579) may be from either cave bear or brown bear. In Fig. 5, the new stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from this study are compared with data collected from previously published studies of bears (Rey-Iglesia *et al.* 2019; Krylovich *et al.* 2020) and other Late Pleistocene herbivorous and carnivorous species from the same regions (Bocherens *et al.* 1997; Iacumin *et al.* 2000; Krause *et al.* 2007; Barnett *et al.*

Table 2. Radiocarbon dating of a very large brown bear (F-2296), a medium-sized brown bear (F-3274) and a polar bear (F-1046).

ID	Laboratory code	$\delta^{13}\text{C}$ (‰)	$^{14}\text{C}$ age (a BP, $1\sigma$ )	Calibrated age (cal. a BP, $2\sigma$ )
F-2296	OZU341	-19.4±0.1	41 090±0.570	43 030–44 800 Median probability: 44 000
F-2374	IGAN <sub>AMS</sub> 6922	-19.7±0.1	25 880±80	29 990–30 280 Median probability: 30 110
F-1046	IGAN <sub>AMS</sub> 6915	-14.4±0.1	845±20	205–475 Median probability: 345

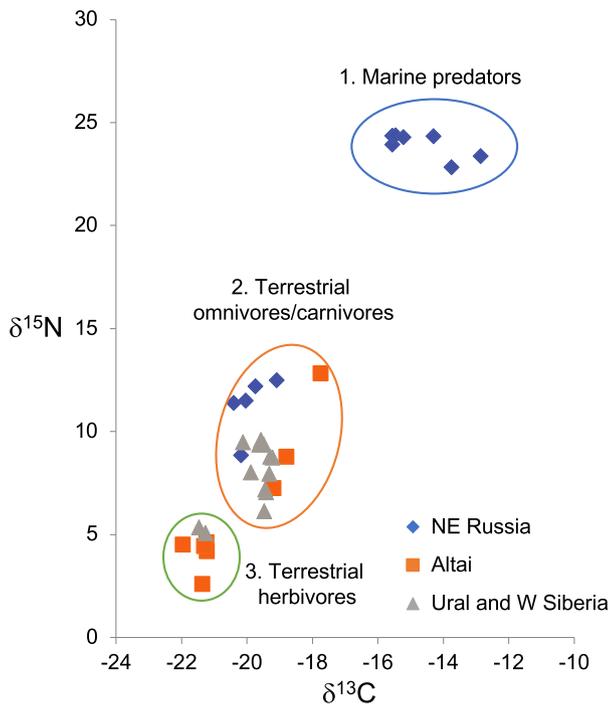


Fig. 4. Scatter plot of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of Late Pleistocene bears from this paper (see Table S3), according to their region of origin.

2009; Szpak *et al.* 2010; Dobrovolskaya & Tiunov 2013; Raghavan *et al.* 2014; Kirillova *et al.* 2015; Douka *et al.* 2019).

### Genetic analyses

Our mitochondrial genome sequencing generated 19 new bear mitogenomes at average depths of coverage from  $7\times$  to over  $300\times$  (Table S1). Phylogenetic analyses (Fig. 6, Fig. S1 and S2) established the identities of all 19 samples and confirmed brown bear (*U. arctos*) identities for the specimens F-2296 and F-2374. All of the phylogenetic analyses of complete mitogenomes placed, with strong support (posterior probability  $>0.99$  and bootstrap support 100%), the two large brown bear specimens from NE Russia (F-2296 and F-2374) in a single lineage sister to a strongly supported clade of modern brown bears that are found throughout Europe, northern Asia and into western Alaska (clade 3a; Figs 6, S1, S2).

The two ancient specimens do not group with another ancient brown bear from NE Russia (Rey-Iglesia *et al.* 2019), nor do they group with modern NE and Far East Russian brown bear samples. Instead, most extant NE Russian brown bears new to this study group within subclade 3a1, which comprises bears from Europe, western Russia, Sakhalin and western Alaska, showing the closest relationships with bears from the Magadan Oblast in East Russia, immediately south of Chukotka, where most of the analysed brown bear samples are from. The remaining brown bear individual from Ulunga River

(IK-1) groups with brown bears from Primorye and nearby Sakhalin. The sister lineage to clade 3a plus the two ancient Russian Far East brown bear samples is an individual (NCBI MG066702) sampled from the American Museum of Natural History's mammal collection (identified as a Tibetan brown bear, possibly of 'mixed breed'). A recent study based on expanded control region mtDNA amplicon sequences (Lan *et al.* 2017) found it to be closely related to individual brown bears from Turkey and Syria (Talbot & Shields 1996; Calvignac *et al.* 2009), suggesting that this may be a Syrian brown bear (*Ursus arctos syriacus*) that belongs to a clade distinct from clade 3a. In our analyses, the previously published large Pleistocene brown bear from NE Russia (Rey-Iglesia *et al.* 2019) groups as sister to clade 3b that comprises bears from Hokkaido and eastern North America.

The split between clade 3a and the two new Pleistocene Far East Russian brown bears is dated to *c.* 100 ka BP (highest probability density (HPD) 95%: 82–122 ka BP), while the divergence time estimate for the split between clades 3a1 and 3a2 is *c.* 51 ka BP (HPD 95%: 38–65 ka BP), which is within the range of previous estimates of *c.* 53 ka BP (Anijalg *et al.* 2018). The most recent common ancestor (MRCA) of the clade 3a1 crown group lived *c.* 41 ka BP (HPD 95%: 32–54 ka BP). A BEAST analysis excluding the previously published ancient Yakutian brown bear (the age of this undated sample was estimated using a BEAST tip-dating approach; Rey-Iglesia *et al.* 2019) produced comparable split date estimates (results not shown).

Eight of the samples analysed as part of this study were identified as polar bears (Fig. S2). Of these new polar bear mitogenomes, two polar bears (F(R)-1 and F-3549) are sisters to all other polar bear samples included in our analyses, suggesting likely maternal phylogeographical structure among polar bears along the Siberian coast. However, more samples from throughout the Siberian coast are necessary to confirm this. The remaining Russian polar bear samples group among extant polar bears from St Lawrence and Little Diomed Islands (Lindqvist *et al.* 2010) and from the Svalbard archipelago (Miller *et al.* 2012), displaying considerable maternal phylogeographical diversity among NE Russian polar bears.

### Discussion

Late Pleistocene steppe brown bears were distributed from western Europe to NE Asia, inhabiting open environments. Although this general Pleistocene range matches that of the modern northern Asiatic brown bear, the steppe brown bears were characteristically larger and differed markedly from their modern descendants by having larger skulls and large and unusually broad molars (Baryshnikov & Boeskorov 2004; Boeskorov *et al.* 2019; Marciszak *et al.* 2019). The minimum sizes recorded of the humerus bones of modern bears are also much smaller than the minimum

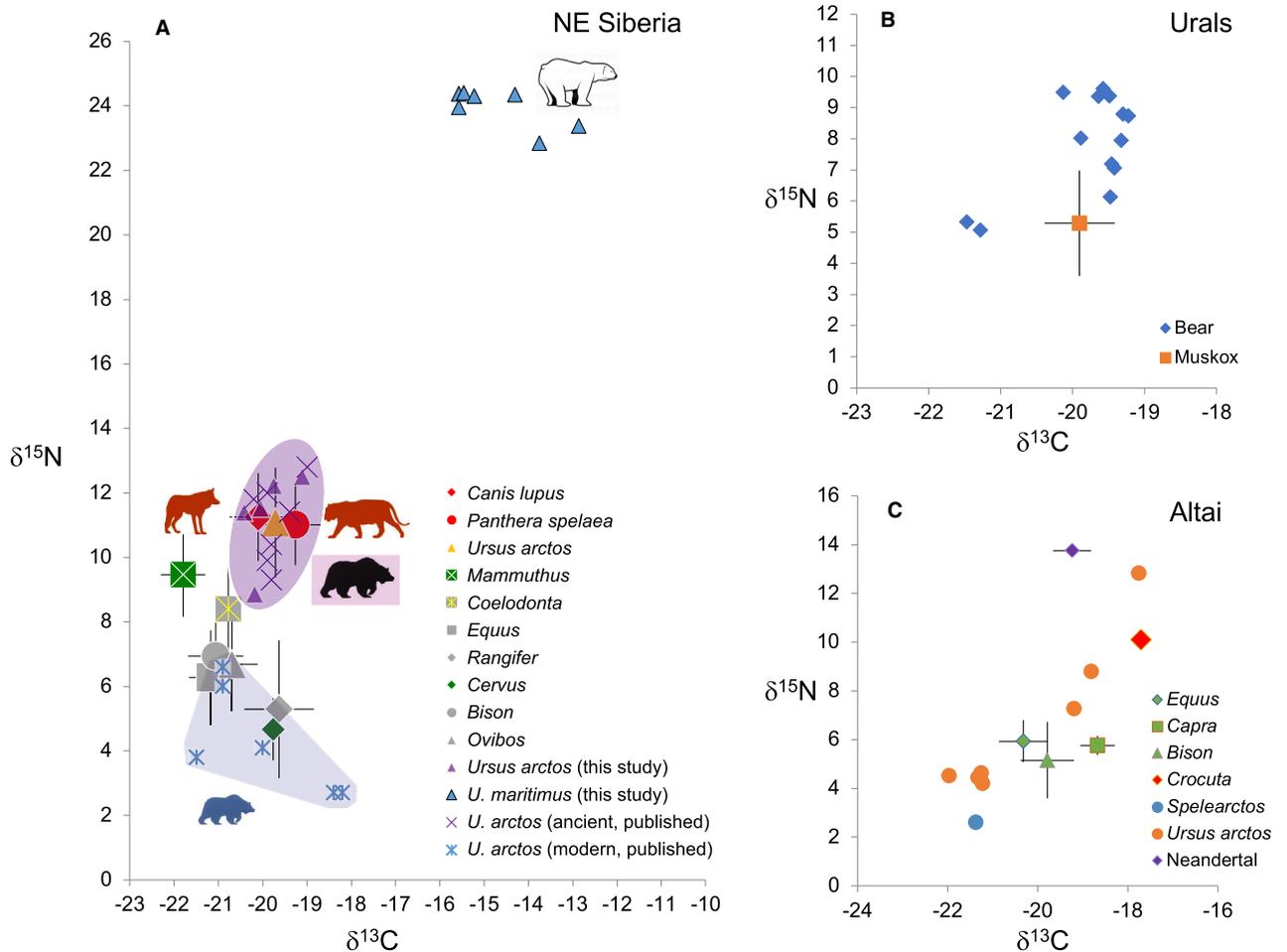
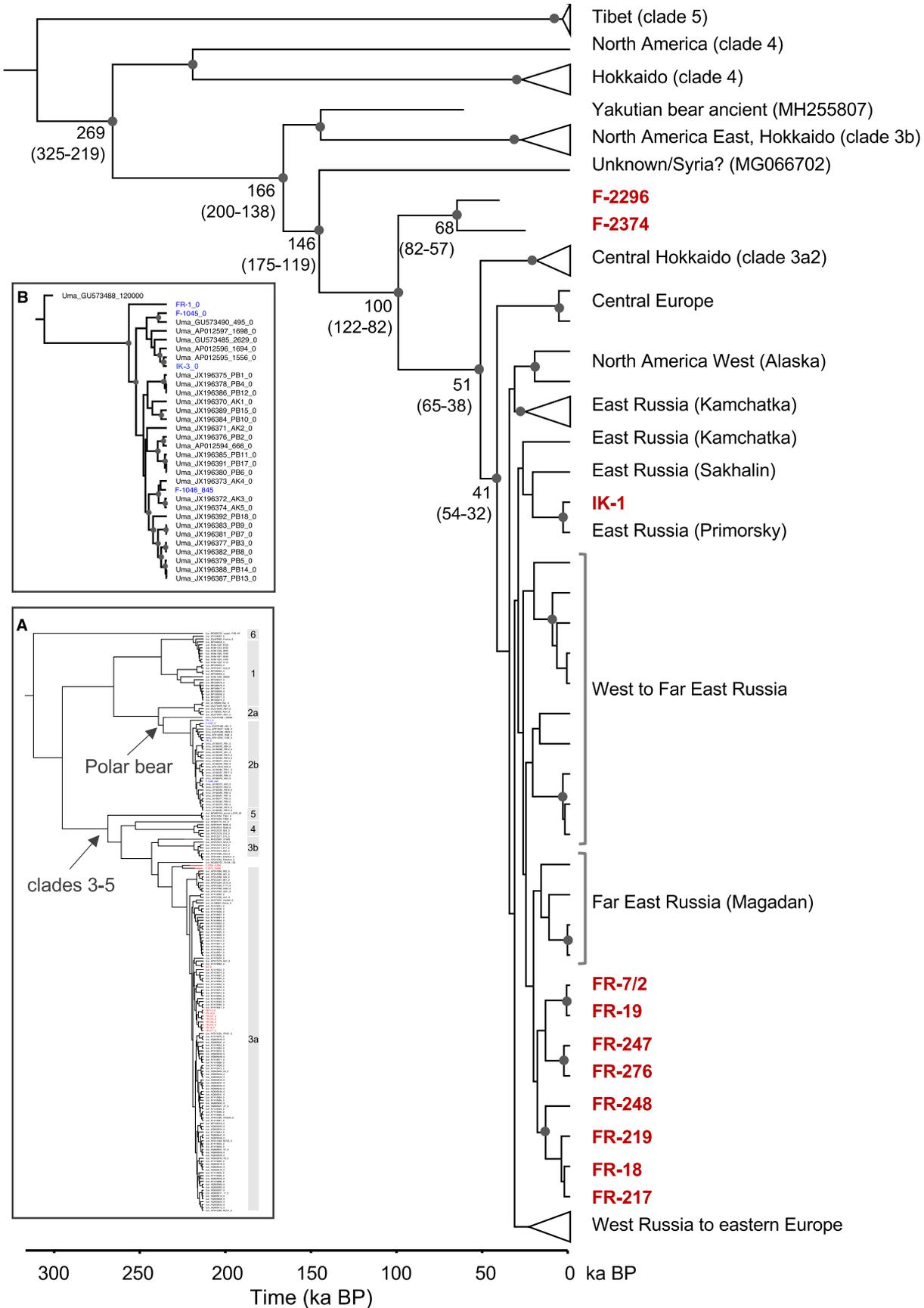


Fig. 5. A. Scatter plot of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of Late Pleistocene brown bears from NE Siberia from this study compared with previously published Pleistocene brown bears (Rey-Iglesia *et al.* 2019; Krylovich *et al.* 2020), modern brown bears (Krylovich *et al.* 2020) and Late Pleistocene polar bears from this study, as well as coeval Late Pleistocene herbivorous and carnivorous species (average values based on data from Bocherens *et al.* 1997; Iacumin *et al.* 2000; Barnett *et al.* 2009; Szpak *et al.* 2010; Raghavan *et al.* 2014; Kirillova *et al.* 2015). B. Scatter plot of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of Late Pleistocene brown bears from Urals from this study compared with previously published Late Pleistocene muskoxen from the same region (Raghavan *et al.* 2014). C. Scatter plot of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of Late Pleistocene bears from Altai from this study compared with previously published coeval Late Pleistocene herbivorous and carnivorous species (average values based on data from Krause *et al.* 2007; Dobrovolskaya & Tiunov 2013; Douka *et al.* 2019).

sizes of the Late Pleistocene analogous bones studied here and the maximum sizes of modern bears do not reach the maximum sizes of the bones of the Late Pleistocene bears. For example, sizes of the humeri of Holocene bears from the Netherlands (Kuijper *et al.* 2016), Upper Volga (Zalkin 1961), Middle Don (Zalkin 1963), Moldavia (David, 1980), the Baltic states (Paaver 1965) and the Kuznetsk Alatau (Vasiliev & Grebnev 2009) do not reach the maximum humerus bone sizes of Late Pleistocene bears.

Hence, the Late Pleistocene bears of northern Asia had on average a larger humerus than the Holocene and modern bears of Europe and Asia. It was previously shown that the Late Pleistocene bears in Europe and northern Asia had larger skull sizes than the Holocene bears (Baryshnikov, 2007; Boeskorov & Baryshnikov 2013; Doppes & Pacher 2014; Marciszak *et al.* 2015, 2019; Boeskorov *et al.* 2019). These observations show that the fossil bears of the Late Pleistocene were generally larger than modern ones in the

Fig. 6. Phylogenetic tree (only clades 3–5 are shown) of brown and polar bear mitochondrial genomes based on a tip-calibrated BEAST analysis with nodes centred on the estimated median divergence time (dates with 95% HPD range in parentheses are shown at select nodes). New Russian brown bear samples of known age are included and highlighted in red text (see Fig. S2 for the maximum likelihood tree including all 19 new Far East and NE Russian samples for which genetic data was generated). A. The full mitogenome BEAST tree with all clades 1–6 represented (see Fig. S1 for the tree with branches uncollapsed). B. The polar bear subclade (2b) with the new Russian samples of known age highlighted in blue text. Circles at clades indicate a posterior probability of >0.99 and bootstrap support of >90%. Assignments of recognized maternal brown bear clades are indicated with clade designation (following Leonard *et al.* 2000; Davison *et al.* 2011; Hirata *et al.* 2013).



same regions. Only modern brown bears from the Amur River (*Ursus arctos beringianus*), Kamchatka Peninsula (*Ursus arctos piscator*), Alaska (*Ursus arctos gyas* and *Ursus arctos dalli*) and the Kodiak Islands (*Ursus arctos middendorffi*) are close in size (Rausch 1963; Heptner *et al.* 1998; Baryshnikov, 2007) to the largest Late Pleistocene bears of northern Asia. It is interesting to note that Late Pleistocene and Holocene Iberian bears (Torres 1988; Garcia-Vazquez 2015; Garcia-Vazquez *et al.* 2015) divided largely into similar size groups to bears from northern Asia. We can assume that changes in body size among brown bears throughout the Late Pleistocene and Holocene were similar in Europe and northern Asia. The differences in slope of the regression lines between the Iberian and Russian fossil bears, however, suggest potential differences in body proportions and biomechanics between these groups. A larger sample size, including bears from similar geographical locations and geological ages, is needed to verify such potential differences.

The diets of modern bears depend on several factors: region, proximity to the sea, season, age and availability or abundance of food. The proportion of meat is determined by its availability (e.g. Vaisfeld & Chestin 1993; Bojarska & Selva 2012). In the coastal regions and in the valleys of the rivers that have access to the sea, marine fish and other animals are included in the diet. This fact has to be considered when assessing the diet of fossil bears. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the studied fossil brown bears bones from NE Russia are within the range of those previously published for Pleistocene bears from Yakutia (Rey-Iglesia *et al.* 2019). The  $\delta^{15}\text{N}$  values are similar to those of wolves and cave lions, and the  $\delta^{13}\text{C}$  values are intermediate with those of these two other predators (Fig. 5A). This suggests that these Late Pleistocene NE Russian brown bears were mostly carnivorous, with a choice of prey that overlaps with both wolves and cave lions, and possibly these bears were dominant predators or scavengers. Their large size would have allowed them to steal carcasses from other predators, in a similar way to what has been suggested for giant short-faced bears *Arctodus simus* in eastern Beringia (Matheus 1995). It is noteworthy that Late Pleistocene brown bears from western Europe also occupy a similar isotopic niche, suggesting a high amount of meat from megaherbivore carcasses in their diet (Bocherens *et al.* 2015).

Our stable isotope data for Late Pleistocene bears from the Urals (Severnaya cave, Usoltsevskaia cave) and western Siberia (Zykova, Evalga, Nizhnyaya Tavda, Merimy, Irtysh River) are the first to be generated. Very few isotopic data on Late Pleistocene mammals from this area have been published so far, precluding a detailed dietary reconstruction. However, compared with the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of muskoxen (Raghavan *et al.* 2014), it appears that a large majority of the studied bears had an omnivorous or more carnivorous diet, and only two specimens had lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in a position similar to those of ungulates and cave bears; hence, they

were probably vegetarian (Fig. 5B). Large (2079/244) and small (2079/238) specimens, most likely males and females, as well as bears from the different ages, MIS 3 (Severnaya cave, Usoltsevskaia cave, Nizhnyaya Tavda, Irtysh River and Merimy) and MIS 2 (Zykova and Evalga), appear to have had identical diets. The stable isotopic signature of several samples, including two brown bear specimens from the southernmost location Ostrolenskoe (2060/109, 2060/113), indicates a predominantly plant-based diet. Geographical differences in the diet of contemporaneous brown bears are noted among the Ural specimens. The ‘herbivorous’ brown bears of the southernmost Ostrolenskoe locality lived in the periglacial steppe (Lapteva, 2007), while the northern bears lived in the periglacial forest-steppe (Volkova *et al.* 2002; Lapteva 2009).

The isotopic values of the Late Pleistocene brown bears from the Altai have been compared with published data on ungulates and hyena from Denisova Cave (Douka *et al.* 2019) and with those of Neandertals from Okladnikov (Krause *et al.* 2007; Dobrovolskaya & Tiunov 2013). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the analysed brown bears range widely from low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values similar to those of one speloid bear, representing a vegetarian diet, to values that are intermediate between those of ungulates and hyena, probably representing an omnivorous diet. The value of one specimen is almost as high as that of the Neandertals, pointing out a possible dietary competition for carcasses with the local hominins (Fig. 5C). Therefore, as in the Urals and in contrast to Yakutia, the brown bears from the Altai had variable diets.

These noted differences in diet from bears across the Asian continent are probably associated with climatic and environmental variation, but further studies are needed to explore this hypothesis. Geographical differences are also noted in the diet of the NE Russian and Ural brown bears. In general, the NE Russian bears have elevated  $\delta^{15}\text{N}$  values, similar to observations in a recent study of large-sized Yakutian Pleistocene brown bears (Krylovich *et al.* 2020). The markedly higher  $\delta^{15}\text{N}$  in these Pleistocene bears compared with modern bears from Yakutia indicate differences in their trophic niche probably associated with climatic and environmental change (Krylovich *et al.* 2020).

Extensive matrilineal molecular phylogenetic studies, and even nuclear genomic analyses (e.g. Miller *et al.* 2012; Cahill *et al.* 2015), have been published of the closely related brown bear and polar bear in recent years; however, several questions concerning Late Pleistocene evolutionary history and biogeography remain largely unresolved. For example, studies (Keis *et al.* 2013; Anijal *et al.* 2018) based on complete mitogenomes of Eurasian brown bears from the most widely and continuously distributed brown bear ‘3a’ subclade, which stretches from eastern Europe, through northern Asia and into western Alaska, have addressed issues of relatively recent post-glacial expansion from Late Pleistocene glacial refugia following the last glaciation. Analyses including prehis-

toric and radiocarbon dated Russian subfossil bears, however, are still limited (but see Rey-Iglesia *et al.* 2019). It was recently suggested that the geographical distribution of the maternal subclade 3a1 may have had a wide distribution during the Late Pleistocene but retreated to glacial refugia, possibly in the Carpathian and Altai–Sayan regions (Anijalg *et al.* 2018), as the climate cooled. From these refugia, bears may have migrated NE towards Beringia and Kamchatka starting around 37 ka ago, later followed by a westward migration into West Asia (Anijalg *et al.* 2018). However, the large time range between the divergence of clades 3b and 3a and the MRCA of clade 3a (c. 166–51 ka) illustrates a considerable gap in knowledge of brown bear evolutionary history during a critical time period when Earth underwent warming during the Eemian Interglacial (130–115 ka; Dahl-Jensen *et al.* 2013) followed by dramatic cooling leading up to the Last Glacial Maximum (26–19 ka; Clark *et al.* 2009).

The mitogenome phylogenetic analyses reported here of two new Yakutian Late Pleistocene samples indicate that the split between their lineage and the rest of clade 3a may be as old as 100 ka. The close genetic relationship between the two bears, although separated by about 15 ka, suggests that this, probably now extinct, NE Russian lineage represents bears that occupied a Beringian refugium, from which bears later migrated throughout Asia and colonized North America. Fossil evidence has suggested that the first wave of brown bears entered North America through Beringia around 70 ka (Craighead & Mitchell 1982; Davison *et al.* 2011), which supports this scenario. Alternatively, this NE Russian lineage may represent a relictual lineage that became isolated and diverged as the climate cooled following the Eemian interglacial, and possibly went extinct. A previous study of another ancient Yakutian brown bear (Rey-Iglesia *et al.* 2019), its age estimated from phylogenetic dating to around 61 ka, was found to be closely related to the extinct subclade clade 3c (Barnes *et al.* 2002). However, the two ancient Yakutian brown bears reported here are distantly related to this other Yakutian bear, which in our analyses is resolved as a sister lineage to subclade 3b. Although our results suggest significant, now extirpated, genetic diversity among NE Siberian Pleistocene brown bears, to determine whether all these ancient Yakutian bears belong to subclade clade 3c would require complete mitogenomes from the extinct clade 3c North American brown bears. Nevertheless, these two new ancient NE Russian bears increase the age estimate for the MRCA of clade 3a or its divergence from a close relative, and this study provides an important contribution to filling the gaps in our knowledge of brown bear evolutionary and biogeographic history.

## Conclusions

Our research introduces new information on Late Pleistocene brown bears from the Asian part of Russia, both

very large morphotypes and ‘normal’ sizes. Very large representatives of the species survived to the Late Pleistocene. In general, the tendency towards reduction of the size of these animals is valid only from the time interval after ~45 000 years ago, when they, at least in Yakutia, were known to have reached their maximum size. Already after ~20 ka ago, their dimensions were close to the sizes of modern bears, although they were more robust. Body sizes also differed by region, which was probably associated with availability of type of foods and their abundance, as is also seen among brown bears today. The diet of Pleistocene brown bears in NE Russia was largely meat, whereas Altai brown bears, as well as cave bears, were mainly vegetarian, similarly to cave bears in Europe. The fossil brown bears of the Urals and western Siberia were omnivorous, but meat made up a significant part of the diet, although two individuals were more herbivorous. A marine diet typically associated with polar bears was found for samples also determined to be polar bears by genetic analyses. The brown bear remains genetically analysed here characterized three distinct maternal lineages, including a possibly new, but probably extinct, lineage comprising two Late Pleistocene Yakutian brown bears.

In addition to the value of morphometric, palaeoecological and genetic analyses of Pleistocene specimens to increase insights into and understanding of bear evolutionary history, such studies are also important for the identification of museum specimens, particularly those of poor preservation. Precise identifications are essential for record keeping of museum objects and their subsequent study. For example, the remains of two specimens from NE Russia analysed in this study (F-1863 and F-1864) resembled a large predator that, based on the locality and geological time, could only be *Panthera* or *Ursus*. Another specimen (F-275) had an identification as brown bear based on initial morphological assessment. As a result of our study, stable isotope and genetic analyses determined a polar bear identity for all three specimens.

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*Author contributions.* – This study was conceived and organized by PAK, IVK and CL. PAK and IVK led the sample collection and produced and analysed the morphometric data. HB conducted and analysed the stable isotope data. EPZ and VAL performed the radiocarbon dating. SST performed the palaeogeographical reconstructions. TL and CL produced and analysed the genetic data. The manuscript was written by PAK, BH, IVK and CL with input from all authors.

*Data availability statement.* – The mitochondrial genome sequences generated in this study are available in the NCBI GenBank database with accession numbers OK001262 to OK001280.

## References

- Alekseeva, E. V. 1980: *Pleistocene Mammals of Southeastern Western Siberia*. 188 pp. Nauka Press, Moscow (in Russian).
- Anijalg, P., Ho, S. Y. W., Davison, J., Keis, M., Tammelaht, E., Bobowik, K., Tumanov, I. L., Saveljev, A. P., Lyapunova, E. A., Vorobiev, A. A., Markov, N. I., Kryukov, A. P., Kojola, I., Swenson, J. E., Hagen, S. B., Eiken, H. G., Paule, L. & Saarma, U. 2018: Large-scale migrations of brown bears in Eurasia and to North America during the Late Pleistocene. *Journal of Biogeography* 45, 394–405.
- Aristov, A. A. & Baryshnikov, G. F. 2001: *The Mammals of Russia and adjacent territories. Carnivores and pinnipeds*. 560 pp. Zoological Institute Press, St. Petersburg (in Russian).
- Ayres, D. L., Darling, A., Zwickl, D. J., Beerli, P., Holder, M. T., Lewis, P. O., Huelsenbeck, J. P., Ronquist, F., Swofford, D. L., Cummings, M. P., Rambaut, A. & Suchard, M. A. 2011: BEAGLE: An Application Programming Interface and High-Performance Computing Library for Statistical Phylogenetics. *Systematic Biology* 61, 170–173.
- Barnes, I., Matheus, P., Shapiro, B., Jensen, D. & Cooper, A. 2002: Dynamics of Pleistocene extinctions in Beringian brown bears. *Science* 295, 2267–2270.
- Barnett, R., Shapiro, B., Barnes, I., Ho, S. Y. W., Burger, J., Yamaguchi, N., Higham, T. F. G., Wheeler, T., Rosendahl, W., Sher, A. V., Sotnikova, M., Kuznetsova, T., Baryshnikov, G. F., Martin, L. D., Harington, R., Burns, J. A. & Cooper, A. 2009: Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a Late Pleistocene reduction in genetic diversity. *Molecular Ecology* 18, 1668–1677.
- Baryshnikov, G. F. 2007: *Ursidae. Fauna of Russia and Neighboring Countries. Mammals I(5)*. 542 pp. Nauka Press, St. Petersburg (in Russian).
- Baryshnikov, G. F. & Boeskorov, G. G. 2004: Skull of the Pleistocene brown bear (*Ursus arctos*) from Yakutia, Russia. *Russian Journal of Theriology* 3, 71–75.
- Bocherens, H., Billiou, D., Patou-Mathis, M., Bonjean, D., Otte, M. & Mariotti, A. 1997: Paleobiological implications of the isotopic signatures (<sup>13</sup>C, <sup>15</sup>N) of fossil mammal collagen in Scladina Cave (Sclayn, Belgium). *Quaternary Research* 48, 370–380.
- Bocherens, H., Drucker, D., Billiou, D. & Moussa, I. 2005: Une nouvelle approche pour évaluer l'état de conservation de l'os et du collagène pour les mesures isotopiques (datation au radiocarbone, isotopes stables du carbone et de l'azote). *L'anthropologie* 109, 557–567.
- Bocherens, H., Drucker, D. G., Germonpré, M., Lázničková-Galetová, M., Naito, Y., Wißing, C., Brůžek, J. & Oliva, M. 2015: Reconstruction of the Gravettian food-web at Předmostí I using isotopic tracking of bone collagen. *Quaternary International* 359–360, 211–228.
- Boeskorov, G. G. & Baryshnikov, G. F. 2013: *Late Quaternary Carnivora of Yakutia*. 199 pp. Nauka Press, St. Petersburg (in Russian).
- Boeskorov, G. G., Baryshnikov, G. F., Tikhonov, A. N., Protopopov, A. V., Klimovsky, A. I., Grigoriev, S. E., Cheprasov, M. Y., Novgorodov, G. P., Shchelchkova, M. V. & van der Plicht, J. 2019: New data on the large brown bear (*Ursus arctos* L., 1758, Ursidae, Carnivora, Mammalia) from the Pleistocene of Yakutia. *Doklady Earth Sciences* 486, 685–690 (in Russian).
- Boeskorov, G. G., Grigoriev, S. E. & Baryshnikov, G. F. 2012: New evidence of the existence of cave bears in the Pleistocene of the Siberian Arctic. *Doklady Biological Sciences* 445, 239–243 (in Russian).
- Boeskorov, G. G., Tikhonov, A. N., Protopopov, A. V., Stepanov, A. D., Plotnikov, V. V., van der Plicht, J., Shchelchkova, M. V. & Baryshnikov, G. F. 2018: New records of Holocene polar bear and walrus (Carnivora) in the Russian Arctic. *Russian Journal of Theriology* 17, 68–77.
- Bojarska, K. & Selva, N. 2012: Spatial patterns in brown bear *Ursus arctos* diet: the role of geographical and environmental factors. *Mammal Review* 142, 120–143.
- Bronk Ramsey, C., Higham, T. & Leach, P. 2004: Towards High-Precision AMS: Progress and Limitations. *Radiocarbon* 46, 17–24.
- Brown, T. A., Nelson, D. E., Vogel, J. S. & Southon, J. R. 1988: Improved collagen extraction by modified Longin method. *Radiocarbon* 30, 171–177.
- Cahill, J. A., Stirling, I., Kistler, L., Salamzade, R., Ersmark, E., Fulton, T. L., Stiller, M., Green, R. E. & Shapiro, B. 2015: Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Molecular Ecology* 24, 1205–1217.
- Calvignac, S., Hughes, S. & Hänni, C. 2009: Genetic diversity of endangered brown bear (*Ursus arctos*) populations at the crossroads of Europe, Asia and Africa. *Diversity and Distributions* 15, 742–750.
- Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B., Mitrovica, J. X., Hostetler, S. W. A. & McCabe, M. 2009: The Last Glacial Maximum. *Science* 325, 710–714.
- Craighead, J. J. & Mitchell, J. A. 1982: Grizzly bear (*Ursus arctos*). In Chapman, J. A. & Feldhamer, G. A. (eds.): *Wild Mammals of North America*, 515–586. Johns Hopkins University Press, Baltimore.
- Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L. & Meyer, M. 2013: Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences* 110, 15758–15763.
- Dahl-Jensen, D., Albert, M. R., Aldahan, A., Azuma, N., Balslev-Clausen, D., Baumgartner, M., Berggren, A.-M., Bigler, M., Binder, T. & Blunier, T. 2013: Eemian interglacial reconstructed from a Greenland folded ice core. *Nature* 493, 489–494.
- Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. 2012: jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772–772.
- David, A. I. 1980: *The mammal fauna of the Pleistocene of Moldavia*. 187 pp. Stiintsa, Kishinev (in Russian).
- Davison, J., Ho, S. Y. W., Bray, S. C., Korsten, M., Tammelaht, E., Hindrikson, M., Østbye, K., Østbye, E., Lauritzen, S. E., Austin, J., Cooper, A. & Saarma, U. 2011: Late-Quaternary biogeographic scenarios for the brown bear (*Ursus arctos*), a wild mammal model species. *Quaternary Science Reviews* 30, 418–430.
- Dobrovolskaya, M. V. & Tiunov, A. V. 2013: The Neanderthals of Okladnikov Cave Altai: Environment and diet based on isotopic analysis. *Archaeology, Ethnology and Anthropology of Eurasia* 41, 78–88.
- Doppes, D. & Pacher, M. 2014: 10,000 years of *Ursus arctos* in the Alps – A success story? Analyses of the Late Glacial and Early Holocene brown bear remains from Alpine caves in Austria. *Quaternary International* 339–340, 266–274.
- Douka, K., Slon, V., Jacobs, Z., Ramsey, C. B., Shunkov, M. V., Derevianko, A. P., Mafessoni, F., Kozlikin, M. B., Li, B., Grün, R., Comesky, D., Deviese, T., Brown, S., Viola, B., Kinsley, L., Buckley, M., Meyer, M., Roberts, R. G., Pääbo, S., Kelso, J. & Higham, T. 2019: Age estimates for hominin fossils and the onset of the Upper Palaeolithic at Denisova Cave. *Nature* 565, 640–644.
- von den Driesch, A. 1976: A guide to the measurement of animal bones from archaeological sites. *Peabody Museum Bulletin* 1, 138 pp. Peabody Museum Press, Cambridge.
- Drummond, A. J. & Rambaut, A. 2007: BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7, 214. <https://doi.org/10.1186/1471-2148-7-214>.
- García-Vázquez, A. 2015: *Caracterización del oso pardo (Ursus arctos L.) fósil en el NW de la Península Ibérica: datos morfométricos y moleculares*. Tesis Doctoral, Universidad de A Coruña, 426 pp.
- García-Vázquez, A., Pinto Llon, A. C., Gonzalez-Fortes, G. M. & Grandal-D'Anglade, A. 2015: Distribución y cronología del oso pardo (*Ursus arctos* L.) en la Península Ibérica durante el Pleistoceno Superior y Holoceno. [Distribution and chronology of brown bear (*Ursus arctos* L.) in the Iberian Peninsula during Upper Pleistocene and Holocene]. *Spanish Journal of Palaeontology* 30, 161–184.

- Gorlova, Y. N., Krylovich, O. A., Tiunov, A. V., Khasanov, B. F., Vasyukov, D. D. & Savinetskiy, A. B. 2015: Isotopic analysis as a method of taxonomical identification of archeozoological materials. *Archaeology, Ethnology & Anthropology of Eurasia* 43, 110–121 (in Russian).
- Gromov, V. I. 1948: Paleontological and archaeological substantiation of the stratigraphy of continental deposits of the Quaternary period on the territory of the USSR (Mammals, Paleolithic). *Proceedings of the Institute of Geological Sciences* 64, 520 pp. Publishing House of the Academy of Sciences of the USSR, Moscow (in Russian).
- Gromova, V. I. 1950: Key to the mammals of the USSR based on skeletal bones. *Trudy Komissii Po Izucheniyu Chetvertichnogo Perioda* 1, 1–240 (in Russian).
- Guindon, S. & Gascuel, O. 2003: A simple, fast, and accurate method to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52, 696–704.
- Heaton, T. J., Köhler, P., Butzin, M., Bard, E., Reimer, R. W., Austin, W. E. N., Bronk Ramsey, C., Grootes, P. M., Hughen, K. A., Kromer, B., Reimer, P. J., Adkins, J., Burke, A., Cook, M. S., Olsen, J. & Skinner, L. C. 2020: Marine20—The Marine Radiocarbon Age Calibration Curve (0–55,000 cal BP). *Radiocarbon* 62, 779–820.
- Heptner, V. G., Naumov, N. P., Yurgenson, P. B., Sludskii, A. A., Chirkova, A. F. & Bannikov, A. G. 1998: *Mammals of the Soviet Union 2(1a)*. P. M. Rao Sirenian and Carnivora (Sea Cows; Wolves and Bears). 734 pp. Smithsonian Institution Libraries and the National Science Foundation Press, Washington, D.C.
- Higham, T. G., Jacobi, R. M. & Bronk Ramsey, C. 2006: AMS radiocarbon dating of ancient bone using ultrafiltration. *Radiocarbon* 48, 179–195.
- Hirata, D., Mano, T., Abramov, A., Baryshnikov, G., Kosintsev, P., Vorobiev, A., Raichev, E. G., Tsunoda, H., Kaneko, Y., Murata, K., Fukui, D. & Masuda, R. 2013: Molecular phylogeography of the brown bear (*Ursus arctos*) in northeastern Asia based on analyses of complete mitochondrial DNA sequences. *Molecular Biology and Evolution* 30, 1644–1652.
- Hua, Q., Jacobsen, G. E., Zoppi, U., Lawson, E. M., Williams, A. A., Smith, A. M. & McManis, M. J. 2001: Progress in Radiocarbon Target Preparation at the ANTARES AMS Centre. *Radiocarbon* 43, 275–282.
- Iacumin, P., Nikolaev, V. & Ramigni, M. 2000: C and N stable isotope measurements on Eurasian fossil mammals, 40 000 to 10 000 years BP: herbivore physiologies and palaeoenvironmental reconstruction. *Palaeogeography, Palaeoclimatology, Palaeoecology* 163, 33–47.
- Keis, M., Remm, J., Ho, S. Y. W., Davison, J., Tammelaht, E., Tumanov, I. L., Saveljev, A. P., Männil, P., Kojola, I., Abramov, A. V., Margus, T. & Saarma, U. 2013: Complete mitochondrial genomes and a novel spatial genetic method reveal cryptic phylogeographical structure and migration patterns among brown bears in north-western Eurasia. *Journal of Biogeography* 40, 915–927.
- Kirilova, I. V., Tiunov, A. V., Levchenko, V. A., Chernova, O. F., Yudin, V. G., Bertuch, F. & Shidlovskiy, F. K. 2015: On the discovery of a cave lion from the Malyy Anyui River (Chukotka, Russia). *Quaternary Science Reviews* 117, 135–153.
- Knapp, M., Rohland, N., Weinstock, J., Baryshnikov, G., Sher, A., Nagel, D., Rabeder, G., Pinhasi, R., Schmidt, H. A. & Hofreiter, M. 2009: First DNA sequences from Asian cave bear fossils reveal deep divergences and complex phylogeographic patterns. *Molecular Ecology* 18, 1225–1238.
- Koby, F. E. 1949: Le dimorphisme sexuel des canines d' *Ursus arctos* et d' *Ursus spelaeus*. *Revue Suisse De Zoologie; Annales De La Société Zoologique Suisse Et Du Muséum D'histoire Naturelle De Genève* 56, 675–687.
- Kosintsev, P. A. & Bachura, O. P. 2015: A mass burial of brown bears (*Ursus arctos* L., 1758) from the Upper Pleistocene of the Northern Urals. *Doklady Biological Sciences* 462, 128–130.
- Kosintsev, P. A., Bachura, O. P., Serikov, Y. B. & Orlova, L. A. 2005: Large mammals of the north of the Middle Trans-Urals at the end of the Late Pleistocene. *Proceeding of the IV all-Russian conference on Quaternary Research*, 203–205. Geoprint, Syktyvkar.
- Kosintsev, P. A., Gasilin, V. V., Gimranov, D. O. & Bachura, O. P. 2016a: Carnivores (Mammalia, Carnivora) of the Urals in the Late Pleistocene and Holocene. *Quaternary International* 420, 145–155.
- Kosintsev, P. A., Tiunov, M. P., Gimranov, D. O. & Panov, V. S. 2016b: The first finding of Asian black bear (Carnivora, Ursidae, *Ursus (Euarctos) thibetanus* G. Cuvier, 1823) in the Late Pleistocene of Northern Eurasia. *Doklady Biological Sciences* 471, 266–268.
- Krause, J., Orlando, L., Serre, D., Viola, B., Prüfer, K., Richards, M. P., Hublin, J. J., Hänni, C., Derevianko, A. P. & Pääbo, S. 2007: Neanderthals in Central Asia and Siberia. *Nature* 449, 902–904.
- Krylovich, O. A., Boeskorov, G. G., Shchelchkova, M. V. & Savinetskiy, A. B. 2020: The trophic position of pleistocene and modern brown bears (*Ursus arctos*) of Yakutia based on stable isotope analyses. *Biology Bulletin* 47, 1013–1021.
- Kuijper, W. J., Verheijen, I. K. A., Ramcharan, A., van der Plicht, H. & van Kolfschoten, T. 2016: One of the last wild brown bears (*Ursus arctos*) in the Netherlands (Noordwijk). *Lutra* 59, 49–64.
- Lan, T., Gill, S., Bellemain, E., Bischof, R., Nawaz, M. A. & Lindqvist, C. 2017: Evolutionary history of enigmatic bears in the Tibetan Plateau-Himalaya region and the identity of the Yeti. *Proceedings of the Royal Society B* 284, 20171804, <https://doi.org/10.1098/rspb.2017.1804>.
- Lapteva, E. G. 2007: *The development of the vegetation and climate of the eastern slope of the Urals in the second half of the Late Neopleistocene and in the Holocene*. Abstract of Ph.D. thesis, 24 pp. Moscow University Press, Moscow (in Russian).
- Lapteva, E. G. 2009: Landscape-climatic changes on the Eastern macroslope of the Northern Urals over the past 50000 years. *Russian Journal of Ecology* 40, 267–273.
- Leonard, J. A., Wayne, R. K. & Cooper, A. 2000: Population genetics of ice age brown bears. *Proceedings of the National Academy of Sciences* 97, 1651–1654.
- Li, H. 2011: A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27, 2987–2993.
- Li, H. & Durbin, R. 2010: Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 26, 589–595.
- Lindqvist, C., Schuster, S. C., Sun, Y., Talbot, S. L., Qi, J., Ratan, A., Tomsho, L. P., Kasson, L., Zeyl, E., Aars, J., Miller, W., Ingólfsson, O., Bachmann, L. & Wiig, Ø. 2010: Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. *Proceedings of the National Academy of Sciences of the United States of America* 107, 5053–5057.
- Marciszak, A., Schouwenburg, C. H., Lipecki, G., Talamo, S., Shpansky, A., Malikov, D. & Gornig, W. 2019: Steppe brown bear *Ursus arctos* 'priscus' from the Late Pleistocene of Europe. *Quaternary International* 534, 158–170.
- Marciszak, A., Stefaniak, K., Mackiewicz, P. & Ridush, B. 2015: *Ursus arctos* L., 1758 from Bukovynka Cave (W Ukraine) in an overview on the fossil brown bears size variability based on cranial material. *Quaternary International* 357, 136–148.
- Matheus, P. E. 1995: Diet and co-ecology of Pleistocene short-faced bears and brown bears in Eastern Beringia. *Quaternary Research* 44, 447–453.
- Meloro, C., Guidarelli, G., Colangelo, P., Ciucci, P. & Loy, A. 2017: Mandible size and shape in extant Ursidae (Carnivora, Mammalia): A tool for taxonomy and ecogeography. *Journal of Zoological Systematics and Evolutionary Research* 55, 269–287.
- Meloro, C. & de Oliveira, A. M. 2019: Elbow Joint Geometry in Bears (Ursidae, Carnivora): a tool to infer palaeobiology and functional adaptations of Quaternary fossils. *Journal of Mammalian Evolution* 26, 133–146.
- Miller, M. A., Pfeiffer, W. & Schwartz, T. 2010: Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), 1–8, New Orleans, USA. <https://doi.org/10.1109/GCE.2010.5676129>.
- Miller, W., Schuster, S. C., Welch, A. J., Ratan, A., Bedoya-Reina, O. C., Zhao, F., Kim, H. L., Burhans, R. C., Drautz, D. I., Wittekindt, N. E., Tomsho, L. P., Ibarra-Laclette, E., Herrera-Estrella, L., Peacock, E., Farley, S., Sage, G. K., Rode, K., Obbard, M., Montiel, R., Bachmann, L., Ingólfsson, O., Aars, J., Mailund, T., Wiig, Ø., Talbot, S. L. & Lindqvist, C. 2012: Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proceedings of the National Academy of Sciences of the United States of America* 109, E2382–E2390.

- Naito, Y. I., Meleg, I. N., Robu, M., Vlaicu, M., Drucker, D. G., Wißing, C., Hofreiter, M., Barlow, A. & Bocherens, H. 2020: Heavy reliance on plants for Romanian cave bears evidenced by amino acid nitrogen isotope analysis. *Scientific Reports* 10, 6612, <https://doi.org/10.1038/s41598-020-62990-0>.
- Paaver, K. L. 1965: *Formation of Teriofauna and Variations of Mammals in Baltic States During the Holocene*. 494 pp. Academy of Science of the Estonian SSR Press, Tallinn.
- Petronio, C., di Canzio, E. & Di Stefano, G. 2003: Morphological and biometrical differences in the limb bones of *Ursus arctos* and *Ursus spelaeus* and phylogenetical considerations on the two species. *Palaeontographica, Abteilung A, Stuttgart* 269, 137–152.
- Raghavan, M., Themudo, G. E., Smith, C. I., Zazula, G. & Campos, P. F. 2014: Musk ox (*Ovibos moschatus*) of the mammoth steppe: tracing palaeodietary and palaeoenvironmental changes over the last 50,000 years using carbon and nitrogen isotopic analysis. *Quaternary Science Reviews* 102, 192–201.
- Rausch, R. L. 1963: Geographic variation in size in North American brown bears, *Ursus arctos* L., as indicated by condylobasal length. *Canadian Journal of Zoology* 41, 33–45.
- Reimer, P. J. and 41 others 2020: The IntCal20 Northern Hemisphere Radiocarbon Age Calibration Curve (0–55 cal kBP). *Radiocarbon* 62, 725–757.
- Rey-Iglesia, A., García-Vázquez, A., Treadaway, E. C., van der Plicht, J., Baryshnikov, G. F., Szpak, P., Bocherens, H., Boeskorov, G. G. & Lorenzen, E. D. 2019: Evolutionary history and palaeoecology of brown bear in North-East Siberia re-examined using ancient DNA and stable isotopes from skeletal remains. *Scientific Reports* 9, 4462, <https://doi.org/10.1038/s41598-019-40168-7>.
- Schubert, M., Ginolhac, A., Lindgreen, S., Thompson, J. F., Al-Rasheid, K. A., Willerslev, E., Krogh, A. & Orlando, L. 2012: Improving ancient DNA read mapping against modern reference genomes. *BMC Genomics* 13, 178, <https://doi.org/10.1186/1471-2164-13-178>.
- Sher, A. V., Weinstock, J., Baryshnikov, G. F., Davydov, S. P., Boeskorov, G. G., Zazhigin, V. S. & Nikolskiy, P. A. 2011: The first record of ‘spelaeoid’ bears in Arctic Siberia. *Quaternary Science Reviews* 30, 2238–2249.
- Smirnov, N. G. 1996: Small mammal diversity of Northern Urals in Late Pleistocene and Holocene. In Smirnov, N. G. (ed.): *Materialy i issledovaniya po istorii sovremennoj fauny Urala: Sbornik nauchnykh trudov*, 39–83, Ekaterinburg Press, Ekaterinburg (in Russian).
- Stamatakis, A. 2014: RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313.
- Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J. & Rambaut, A. 2018: Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4, vey016, <https://doi.org/10.1093/ve/vey016>.
- Szpak, P., Gröcke, D. R., Debruyne, R., MacPhee, R. D. E., Guthrie, R. D., Froese, D., Zazula, G. D., Patterson, W. P. & Poinar, H. N. 2010: Regional differences in bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of Pleistocene mammoths: implications for palaeoecology of the mammoth steppe. *Palaeogeography, Palaeoclimatology, Palaeoecology* 286, 88–96.
- Talbot, S. L. & Shields, G. F. 1996: Phylogeography of brown bears (*Ursus arctos*) of Alaska and parapatry within the Ursidae. *Molecular Phylogenetics and Evolution* 5, 477–494.
- Torres, T. 1988: Osos (Mammalia, Carnivora, Ursidae) del Pleistoceno Iberico (*U. deningeri* Von Reichenau, *U. spelaeus* Rosenmüller-Heinroth, *U. arctos* Linneo): III. Estudio anatómico y métrico del miembro torácico, carpo y metacarpo. *Boletín Geológico y Minero* 99, 44–100.
- Vaisfeld, M. A. & Chestin, I. E. (eds.) 1993: *Bears, Brown bear, Polar Bear, Black Asian Bear. Distribution, Ecology, Use and Protection. Game Animals of Russia and Adjacent Countries and Their Environment*. 519 pp. Nauka Press, Moscow (in Russian).
- Vasiliev, S. K. & Grebnev, I. E. 2009: The skeletal bone morphology of the Holocene brown bear (*Ursus arctos* L., 1758) of the Kuznetsk Alatau. *Yenisei Province* 4, 68–76 (in Russian).
- Vereshchagin, N. K. 1973: Craniological characteristics of modern and fossil bears. *Zoological Journal* 52, 920–930 (in Russian).
- Vereshchagin, N. & Baryshnikov, G. 2000: Small cave bear *Ursus (Spelearctos) rossicus uralensis* from the Kizel Cave in the Ural (Russia). *Geoloski Zbornik Ljubljana* 15, 53–66.
- Volkova, V. S., Arkhipov, S. A., Babushkin, A. E., Kulkova, I. A., Guskov, S. A., Kuzmina, O. B., Levchuk, L. K., Mikhailova, I. V. & Sukhorukova, S. S. 2002: *Stratigraphy of Oil and Gas Basins of Siberia. Cenozoic of Western Siberia*. 246 pp. SO RAN Publication, ‘GEO’ branch, Novosibirsk (in Russian).
- Wacker, L., Némec, M. & Bourquin, J. 2010: A revolutionary graphitisation system: fully automated, compact and simple. *Nuclear Instruments and Methods in Physics Research B* 268, 931–934.
- Wilcken, K., Hotchkis, M., Levchenko, V., Fink, D., Hauser, T. & Kitchen, R. 2015: From carbon to actinides: A new universal IMV accelerator mass spectrometer at ANSTO. *Nuclear Instruments and Methods in Physics Research B* 361, 133–138.
- Yoneda, M. & Abe, H. 1976: Sexual dimorphism and geographic variation in the skull of the Ezo Brown Bear (*Ursus arctos yesoensis*). *Memoirs Faculty of Agriculture Hokkaido University* 9, 265–276.
- Zalkin, V. I. 1961: The mammals of the Oka river basin and of the Upper Volga at the beginning of the present era. *Bulletin of Moscow Society of Naturalists. Biological Series* 66, 23–39 (in Russian).
- Zalkin, V. I. 1963: On the history of mammals in forest steppe of Eastern Europe. *Bulletin of Moscow Society of Naturalists. Biological Series* 68, 43–62 (in Russian).

## Supporting Information

Additional Supporting Information may be found in the online version of this article at <http://www.boreas.dk>.

**Fig. S1.** Phylogenetic tree based on a tip-calibrated BEAST analysis. This tree is similar to Fig. 6 in the main text but here shown with all branches uncollapsed.

**Fig. S2.** Maximum likelihood tree generated with RAxML, including all 19 new Far East and NE Russian samples for which genetic data were generated.

**Table S1.** Mitochondrial genome sequencing statistics and species ID determined from genetic analyses.

**Table S2.** Measurements of brown bears’ humeri from different sites (see also Fig. 2).

**Table S3.**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  stable isotope values in studied samples.