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# Phylogeography of ancient and modern brown bears from eastern Eurasia

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The brown bear (*Ursus arctos*) is an iconic carnivoran species of the Northern Hemisphere. Its population history has been studied extensively using mitochondrial markers, which demonstrated signatures of multiple waves of migration, arguably connected with glaciation periods. Among Eurasian brown bears, Siberian populations remain understudied. We have sequenced complete mitochondrial genomes of four ancient (~4.5–40 kya) bears from South Siberia and 19 modern bears from South Siberia and the Russian Far East. Reconstruction of phylogenetic relationships between haplotypes and evaluation of modern population structure have demonstrated that all the studied samples belong to the most widespread Eurasian clade 3. One of the ancient haplotypes takes a basal position relative to the whole of clade 3; the second is basal to the haplogroup 3a (the most common subclade), and two others belong to clades 3a1 and 3b. Modern Siberian bears retain at least some of this diversity; apart from the most common haplogroup 3a, we demonstrate the presence of clade 3b, which was previously found mainly in mainland Eurasia and Northern Japan. Our findings highlight the importance of South Siberia as a refugium for northern Eurasian brown bears and further corroborate the hypothesis of several waves of migration in the Pleistocene.

ADDITIONAL KEYWORDS: ancient DNA – Denisova – glacial refugium – mitochondrial genome – population structure.

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## INTRODUCTION

The brown bear (*Ursus arctos* Linnaeus, 1758) is one of the largest terrestrial predators on the planet, and its range covers the mountain and forest areas of the Northern Hemisphere. Owing to significant decline of habitats and excessive hunting, only small, isolated populations have remained in Southern and Central Europe, whereas larger populations exist in Eastern and Northern Europe, where the species is protected. Declining but still contiguous populations exist in the north-west of North America. Northern Asia represents the largest uninterrupted habitat, where the brown bear occupies continuous areas from the Ural Mountains to the Russian Far East.

Many brown bear population genetics studies have been based mostly on mitochondrial markers (Taberlet & Bouvet, 1994; Saarma *et al.*, 2007; Korsten *et al.*, 2009; Anijalg *et al.*, 2018), although some authors have also used nuclear genome markers (Tammeleht *et al.*, 2010; Norman *et al.*, 2013; Xenikoudakis *et al.*, 2015; Benazzo *et al.*, 2017; Barlow *et al.*, 2018; Tumendemberel *et al.*, 2019). The nomenclature for mitochondrial haplotypes was proposed by Leonard *et al.* (2000) and extended in subsequent studies (Barnes *et al.*, 2002; Miller *et al.*, 2006; Calvignac *et al.*, 2008; Davison *et al.*, 2011). According to recent analyses, mitogenomes of brown bears are presented as seven major haplogroups that are split further into subgroups. The geographical distribution of these groups in some areas putatively reflects stages of successive waves of migration (Matsuhashi *et al.*, 1999, 2001; Korsten *et al.*, 2009; Davison *et al.*, 2011; Hirata *et al.*, 2013; Anijalg *et al.*, 2018). The most abundant Holarctic haplogroup, 3a, is widespread in Eurasia; different studies have estimated the origin of the clade from ~53 kya (Anijalg *et al.*, 2018) to > 100 kya (Salis *et al.*, 2021). Among other clades, clade 3b is specific to mainland Eurasia (Gus'kov *et al.*, 2013; Hirata *et al.*, 2013, 2014; Salomashkina *et al.*, 2014; Salis *et al.*, 2021), and together with clade 4, it was also found on the islands of Japan (Hirata *et al.*, 2013, 2014). Representatives of clades 2c, 3a, 3b, 3c and 4 have been found among ancient samples of Eastern Beringia (Salis *et al.*, 2021).

Understanding brown bear phylogeography and migration patterns in north-eastern Eurasia requires more extensive sampling, especially from Siberia. In this study, we obtained nearly complete mitochondrial genomes of four ancient brown bears (~4.5–40 kyr old) from various palaeontological and archaeological excavations in Southern Siberia and supplemented these data with complete mitochondrial genomes of 19 modern bears from Siberia and the Russian Far East. We then performed phylogenetic analysis of these data together with previously published sequences to corroborate the hypothesis that ancient brown bears from mainland Eurasia represent multiple waves of dispersal to the islands of Japan, Eastern Beringia and North America.

## MATERIAL AND METHODS

### Sampling

Ancient samples, representing various palaeontological and archaeological excavations in Southern Siberia, were included in the study (Table 1). The most recent sample, U16 Kol, was excavated in 2010 from a 22nd–17th century BC settlement of ancient miners and metallurgists belonging to the Elunin Culture (Early Bronze Age), located on the north-eastern shore of lake Kolyvanskoe, 3.7 km from the village Savvushka

**Table 1.** Ancient brown bear samples and sequencing results

| Sample | Age (kya) | Location | Sample type | Total number of reads | Number of reads mapped | Percentage of mitogenome recovered | Mean coverage (number of unique reads per position) | NCBI accession number |
|---|---|---|---|---|---|---|---|---|
| U2 Chu | ~40 | Chumysh river (53.40°N, 85.74°E) | Tibia | 670 582 | 4427 | 97.4 | 21.8 | MW991397 |
| U3 Chi | ~30 | Chik river (55.00°N, 82.42°E) | Ulna | 537 611 | 1948 | 91.0 | 8.7 | MW991398 |
| U16 Kol | 4.2–3.6 | Kolyvan-1 (51.3745°N, 82.2144°E) | Tooth | 2 977 306 | 1398 | 96.7 | 6.7 | MW991399 |
| U17 DC4 | ~5.5 | Denisova Cave, layer 4 (51.3975°N, 84.6750°E) | Unidentifiable bone | 1 843 902 | 11 716 | 99.8 | 76.6 | MW991400 |

(Zmeinogorsky district, Altai Krai) (Grushin, 2015). Sample U17 DC4 was from the famous Denisova Cave, located on the river Anuy (Altai Krai). The bone was discovered in lithological layer 4, dated to ~3.5 kyr BCE (Derevyanko *et al.*, 2003). Samples U2 Chu and U3 Chi were excavated from sediments of the rivers Chumysh (Altai Krai) and Chik (Novosibirsk Oblast), respectively. These were the oldest samples; U2 Chu was radiocarbon dated to 40 kya (Vasiliev *et al.*, 2016: sample ID NSKA-01087), whereas U3 Chi was estimated to be ~30 kyr old based on previously dated samples from the same layer (Vasiliev *et al.*, 2016, 2018, 2020).

Nineteen samples of modern brown bears represented various regions of Siberia (Tomsk, Krasnoyarsk, Irkutsk and Yakutia) and the Russian Far East (Khabarovsk) (Fig. 1). The muscle samples were collected from legally hunted brown bears. A description of the modern samples is given in the Supporting Information (Table S1).

### Modern DNA extraction and sequencing

DNA from modern samples was isolated using DNeasy Blood & Tissue Kit (Qiagen, The Netherlands) and the High PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany), following the manufacturer's instructions.

Polymerase chain reaction (PCR), Sanger sequencing and reconstruction of the complete modern brown bear mitochondrial genomes were performed as described by Keis *et al.* (2013) and Anijalg *et al.* (2018).

### Ancient DNA extraction and enrichment

Ancient DNA was extracted as described by Druzhkova *et al.* (2013). Sequencing libraries were made using the TruSeq DNA Sample Preparation Kit (Illumina, USA), according to the manufacturer's protocol. Libraries were enriched using hybridization with contemporary *U. arctos* biotinylated mitochondrial DNA immobilized on Dynabeads Streptavidin magnetic beads (Life Technologies, USA), as in the study by Maricic *et al.* (2010).

Biotinylated probes for enrichment were generated from the modern brown bear sample (Irk; Supporting Information, Table S1) using PCR (Vorobieva *et al.*, 2020) with primers presented in the Supporting Information (Table S2). Enriched sequencing libraries

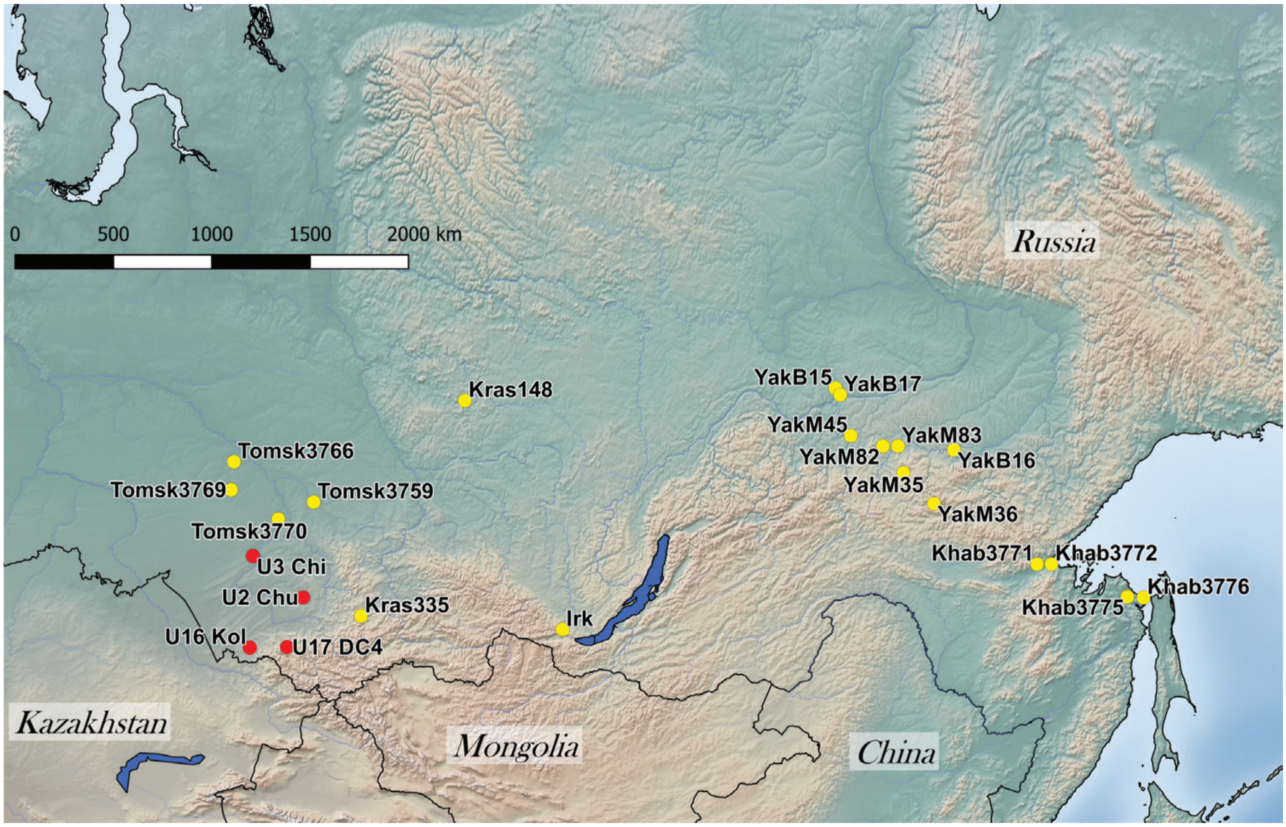

**Figure 1.** Map of sample collection sites. Colour code: yellow, modern samples; red, ancient samples.

were quantified using quantitative PCR in the presence of SYBR Green I.

### Ancient DNA sequencing and mitochondrial genome reconstruction

Paired-end sequencing was performed on an Illumina MiSeq using TruSeq Reagent Kits v.2, with 500 cycles.

Reference-based recovery of ancient bear mitochondrial genomes was performed using PALEOMIX v.1.2.13.2 BAM pipeline (Schubert *et al.*, 2014). Reads were trimmed and collapsed using AdapterRemoval v.2.1.7 (Lindgreen, 2012), then aligned to the reference brown bear mitochondrial genome (GenBank NC003427) with BWA MEM v.0.7.17-r118 (Li, 2013). Only hits with mapping quality > 20 were retained. The PCR duplicates were removed with a script incorporated in PALEOMIX. The C-to-T damage profiles were estimated, and base qualities were recalibrated with MapDamage v.2.0.8 (Jónsson *et al.*, 2013). Indel regions were realigned with GATK v.3.8-0-ge9d806836 (McKenna *et al.*, 2010-9). Contaminant reads were removed by alignment to a human mitochondrial genome (GenBank NC012920) and mapping quality comparison using a custom Python script (https://github.com/lca-imcb/lca-ngs/blob/master/contam_filter.py). Consensus sequences were reconstructed in Geneious (https://www.geneious.com), using the 75% majority rule, a minimum coverage of three and subsequent manual refinement.

### Phylogenetic analysis

Phylogenetic analysis was performed for sequences obtained in the present study, along with additional previously published bear mitochondrial sequences listed in the Supporting Information (Table S3).

Sequences were aligned using MAFFT v.7.407 (Katoh & Standley, 2013). Stretches of tandem repeats in the hypervariable region were removed from the alignment before the analysis. The optimal alignment partitioning scheme and substitution models were chosen using PartitionFinder v.2 (Lanfear *et al.*, 2017). Phylogenies were reconstructed using Bayesian inference (BI) in MrBayes v.3.2.5 (Ronquist *et al.*, 2012) and using maximum likelihood (ML) in RAxML v.8.2.3 (Stamatakis, 2014). For BI, four Markov chains were run for five million iterations and sampled at intervals of 2500 generations, and the first 25% of chains were discarded as burn-in. Posterior probabilities were obtained from the 50% majority-rule consensus tree. For the ML method, the GTR+G substitution model was used instead of the ones indicated by PartitionFinder. A strategy of 'ML+thorough bootstrap' was implemented, with 10 000 replications in ten runs. Final tree manipulations were performed in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The median-joining network was reconstructed with with PopArt v.1.7.2 (http://popart.otago.ac.nz).

To estimate divergence times, we took a Bayesian approach implemented in BEAST v.1.8.4 (Suchard *et al.*, 2018). The tree prior was set to a constant-size coalescent model. The molecular clock was set as strict, and for approximation we used a previously estimated mitogenomic mutation rate of $2.48 \times 10^{-8}$ mutations per site per year (Anijalg *et al.*, 2018). The molecular clock was calibrated using our ancient samples (Table 1) and the 130- to 110-kyr-old ancient polar bear sample from Svalbard, Norway (Lindqvist *et al.*, 2010).

In order to compare our findings of clade 3b in continental Eurasia with previous studies, we obtained *COXII* and mitochondrial control region (CR) sequences for Central Asian brown bears from the study by Tumendemberel *et al.* (2019) and aligned those to our complete mitochondrial genome dataset using MAFFT. Then, we extracted 671-bp-long *COXII* and 263-bp-long CR alignments, concatenated those, and reconstructed the phylogenetic tree using FastTree.

### Population genetic analysis

Population genetic analysis of modern brown bear mitogenomes was performed with Arlequin v.3.5 (Excoffier & Lischer, 2010) and DnaSP v.5.10 (Librado & Rozas, 2009) software. Arlequin was used to calculate molecular diversity indexes and population average pairwise differences and to perform pairwise mismatch distribution analysis, AMOVA computations and neutrality tests. For pairwise mismatch distribution, we estimated parameters of the demographic expansion using pairwise difference as a metric and 1000 bootstrap replicates. Standard AMOVA (Excoffier *et al.*, 1992) was performed with 1000 permutations to identify differences between populations of Siberia, the Russian Far East (mainland), Kamchatka, Japan and Europe. Nucleotide diversity ($\pi$) was calculated using DnaSP with default parameters. The remaining calculations and tests were performed using Arlequin with default parameters and molecular distance set as pairwise differences.

## RESULTS

### Mitochondrial genomes

Mitochondrial genomes for four ancient and one modern brown bear were reconstructed from Illumina shotgun sequencing of DNA libraries enriched by hybridization capture. For ancient samples (listed in Table 1) aged 3.6–40 kyr, 0.04–0.66% reads were mapped to the reference genome, and with 0.5–3 million reads we were able to achieve 6.7- to 76.6-fold mitochondrial genome coverage. As a result, consensus sequences were successfully reconstructed almost completely (91–99.8% based on 3× coverage cut-off) for the ancient samples. We also ensured the lack of contamination with DNA from hybridization probes; we sequenced the modern sample used for probe generation (Irk) and checked manually that its characteristic variants were not present in ancient DNA data.

Complete mitochondrial genomes for 18 modern bears were reconstructed with Sanger sequencing as described by Anijalg *et al.* (2018). Here, we extended sampling geography by including samples ranging from Western Siberia to Khabarovsk.

### PHYLOGENETIC ANALYSIS

We performed phylogenetic analysis based on complete mitochondrial sequences of the four ancient and 19 modern bears from Siberia and the Russian Far East, together with 259 previously published mitochondrial genomes of brown and polar bears sampled worldwide (Supporting Information, Table S3). We were able to restore 91–99.8% of the ancient bear mitochondrial genomes and whole mitochondrial genomes for modern brown bear samples from Siberia (Irkutsk, Tomsk, Krasnoyarsk and Yakutia regions) and the Russian Far East (Khabarovsk region). Our results in general support the existence of clades delineated previously (Leonard *et al.*, 2000; Davison *et al.*, 2011; Hirata *et al.*, 2013), hence we will adhere to the nomenclature proposed there (Fig. 2).

Both ancient and modern samples from Siberia and the Russian Far East fell within clade 3 (Fig. 2). The most ancient samples in our set, U2 Chu and U3 Chi (both estimated to be ~30–40 kyr old), were identified as outgroups for subclade 3a and the whole of clade 3, respectively. The most recent sample, U16 Kol (~4.5 kyr old), was attributed to the current clade 3a1, which is widely distributed and whose closest relatives are modern bears from Eastern and Northern European Russia. The sample U17 DC (~5.5 kyr old) was found to belong to clade 3b and, together with a modern sample from Siberia (Kras148), formed a subclade distinct from the previously described group of bears from Eastern Hokkaido and Kuril Islands.

Previously, clade 3b samples were reported from Central Asia based on partial mitochondrial sequences (Tumendemberel *et al.*, 2019). Among these data, closely related sequences were found for each of three mitochondrial genomes of clade 3b in our study: *COXII* + CR sequence was identical between Irk (a modern bear from the Irkutsk region) and 60014Dor (a modern bear from Ikh Khyangan, Mongolia) belonging to clade 3b2, while Kras128 (a modern bear from the Krasnoyarsk region) and U17 (an ancient bear from Denisova Cave) were similar, but not identical to 200BU1 and 200S4 (from the Mongolian Altai), representatives of clade 3b1. In clade 3a1, samples from the study by Tumendemberel *et al.* (2019) (Khentii and Bogd Khan, Mongolia) clustered separately from the other samples in our study (Supporting Information, Fig. S1). No additional samples grouped together with U3, which retained its basal position relative to clade 3.

The two most ancient branches within subclade 3a1 have been described earlier and are represented by South-East European and Alaska–Kamchatka groups (Anijalg *et al.*, 2018) (Fig. 3). The remainder of clade 3a1 is divided tentatively into two poorly resolved subgroups that diverged ~35 kya. The first subgroup can be subdivided further into a more eastern one, where modern samples from Khabarovsk and Yakutia cluster together with samples from Primorsky Krai and Sakhalin, and a more western one, consisting mainly of bears originating from Europe. However, it also included two modern samples from Tomsk and Yakutia that were close to haplotypes typical for Northern Europe. The second subgroup consists of samples scattered across the vast area from the Russian Far East to the Ural Mountains. In the present study, we enriched this subgroup with three samples from Tomsk, three from Yakutia, two from Khabarovsk and a sample from Krasnoyarsk.

### ANALYSIS OF MODERN POPULATIONS

The relationships between mitochondrial haplotypes of northern Eurasian clades 3 and 4 were examined further using a median-joining network (Fig. 4). As expected, Hokkaido Island and Kuril Island samples fell into three distinct groups corresponding to clades 3a2, 3b (which also included Siberian samples Kras148 and Irk) and 4. The remaining diversity (clade 3a1) had a single origin, with a clear distinction between three groups: Kamchatka, Europe and Siberia together with the Russian Far East (mainland).

We estimated levels of genetic differentiation between populations of brown bears of Northern Europe (we excluded samples from Central and Southern Europe from this analysis), Siberia, the Russian Far East (mainland), Kamchatka and Hokkaido for a subset of completely recovered mitochondrial genomes that were available for 218 modern samples (Table 2). The genetic differentiation between the populations of Hokkaido and the other four is high (the fixation index [$F_{ST}$] between 0.2 and 0.5). European populations are also fairly differentiated from the Asian ones ($F_{ST}$ between 0.15 and 0.38). Kamchatka is significantly differentiated from both Europe and the Russian Far East (mainland) ($F_{ST} > 0.37$ for both), and less so from Siberia ($F_{ST} = 0.29$). Differentiation between the Russian Far East (mainland) and Siberia is very low. In terms of within-population nucleotide diversity (Supporting Information, Table S4), populations from

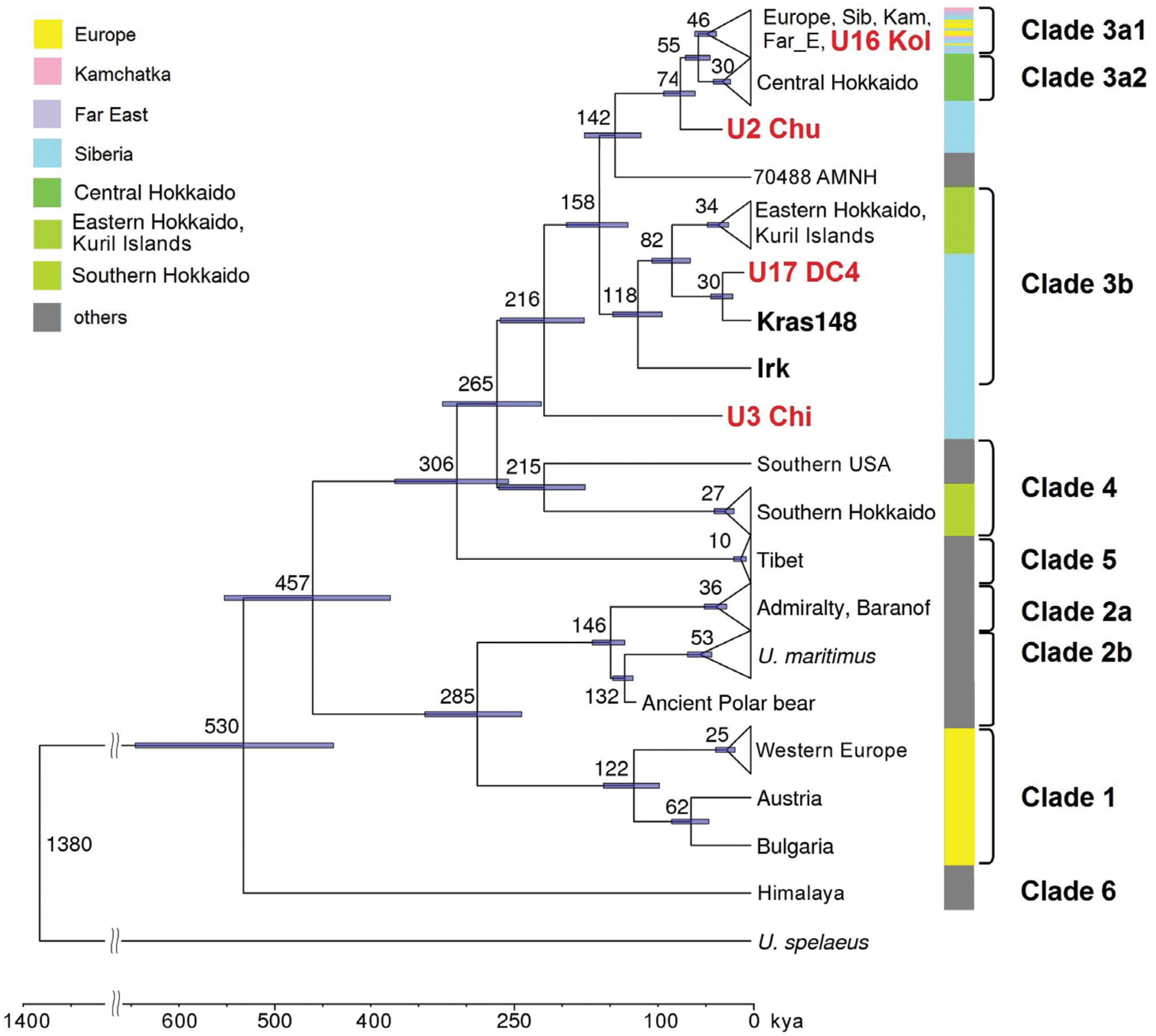

**Figure 2.** BEAST phylogeny for complete mitochondrial genomes of 282 brown bears. Some subtrees have been collapsed for brevity. Red labels indicate ancient samples, and bold black labels indicate modern samples from the present study. Node numbers and blue lines are the mean and 95% confidence interval of divergence time (in thousands of years). Numbers over branches are the posterior Bayesian probabilities. Outgroups: India, *Ursus thibetanus* MG066704 and *Ursus spelaeus* NC_011112.

Hokkaido (π = 0.007) and Siberia (π = 0.0023) are much more diverse compared with Europe, the Russian Far East (mainland) and Kamchatka (π = 0.001), which reflects the presence of remnants from two (Siberia) or three (Japan) waves of migration.

## DISCUSSION

Until recently, it was believed that a group of brown bears (*U. arctos*) from northern Eurasia expanded through the territory of modern continental Eurasia and to North America in the Late Pleistocene (37–29 kya) (Korsten *et al.*, 2009; Anijalg *et al.*, 2018). Here, we provide additional evidence supporting the hypothesis of Eurasian origin for the entire clade 3 based on the analysis of full mitochondrial DNA. Although mitochondrial lineages represent only maternal dispersal, our data demonstrate the presence of basal haplotypes among ancient pre-Last Glacial Maximum samples from Siberia.

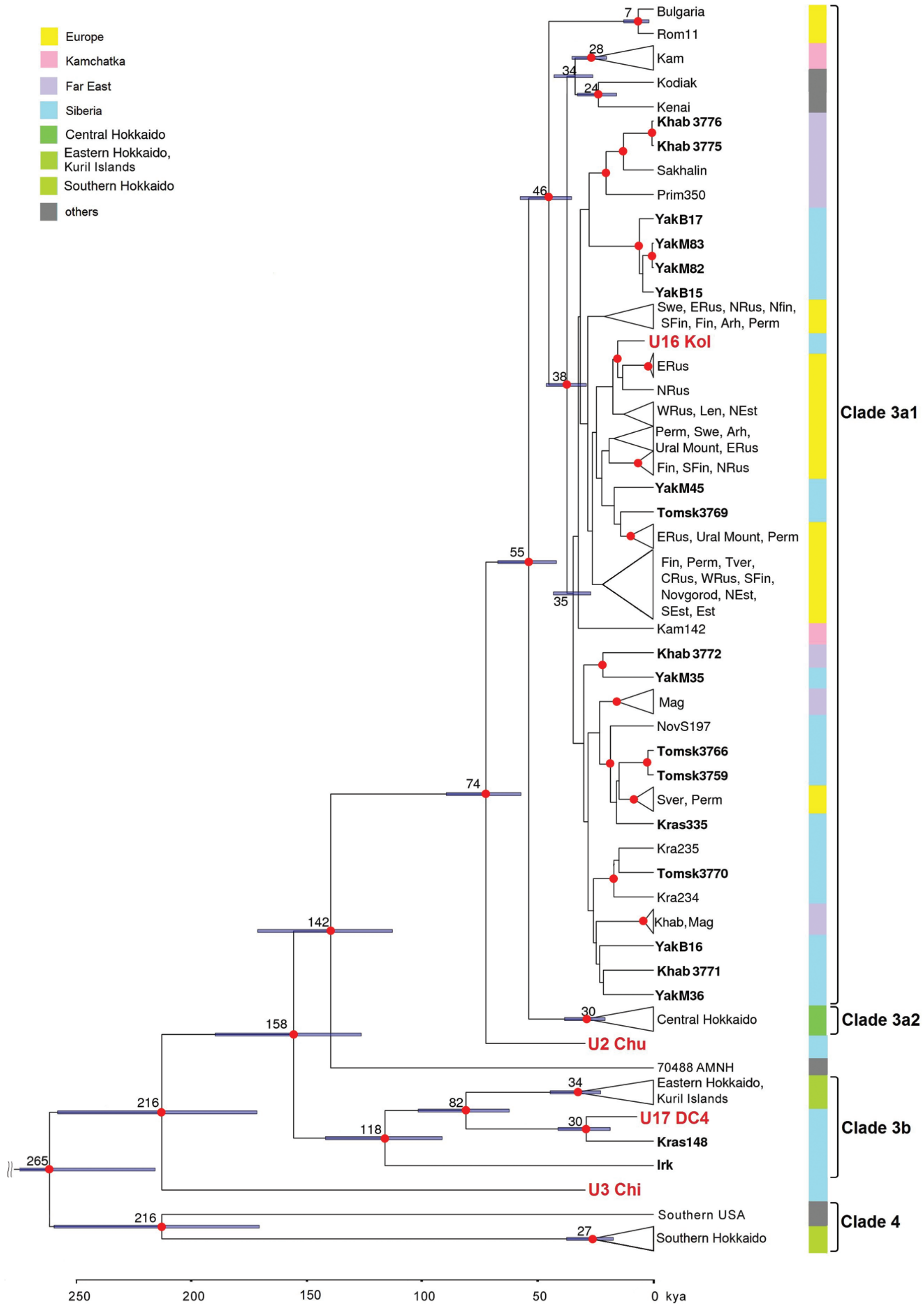

**Figure 3.** Expanded view of clade 3 from Figure 2 representing phylogenetic placements of modern (shown in bold) and ancient (red) samples obtained in the present study. For labels of Published samples are described in Supporting Information (Table S3).

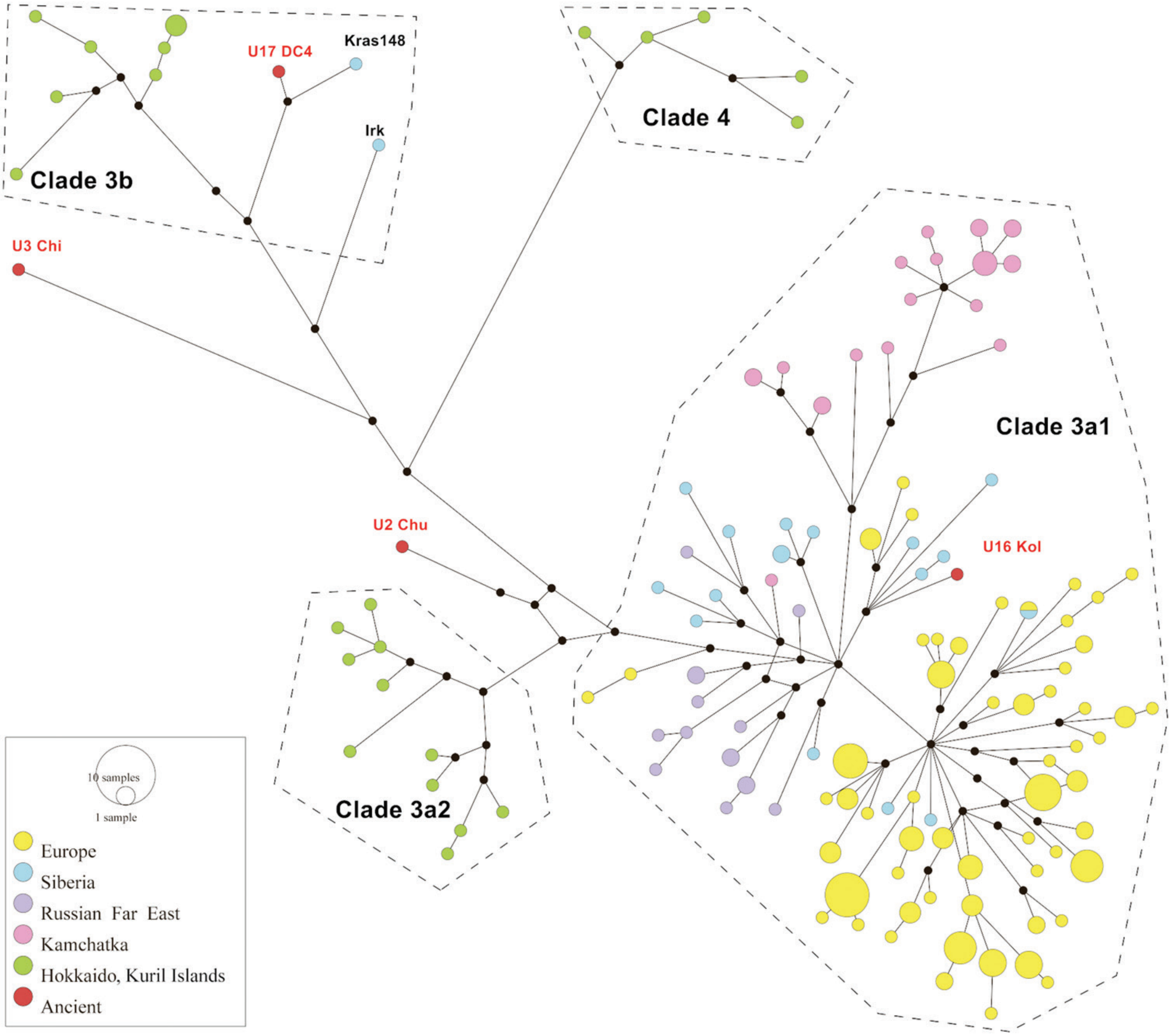

**Figure 4.** Phylogenetic network of 136 modern bear mitochondrial genomes belonging to clades 3 and 4. Circle size corresponds to the number of identical samples. Colour code: yellow, Europe; blue, Siberia; green, Japan; red, Kamchatka; purple, the Russian Far East.

**Table 2.** Brown bear population differentiation

| | | $F_{ST}$ *P*-value | | | | |
|---|---|---|---|---|---|---|
| | | Hokkaido | Northern Europe | Siberia | Russian Far East (mainland) | Kamchatka |
| $F_{ST}$ | Hokkaido | – | 0.00 ± 0.00 | 0.00 ± 0.000 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Northern Europe | 0.496 | – | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Siberia | 0.208 | 0.149 | – | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Russian Far East (mainland) | 0.266 | 0.164 | 0.057 | – | 0.00 ± 0.00 |
| | Kamchatka | 0.341 | 0.378 | 0.29 | 0.401 | – |

Values below the diagonal are the population pairwise $F_{ST}$ and those above the diagonal are $F_{ST}$ *P*-values.

Most of the modern samples from northern Eurasia belong to clade 3a1, and our data on Siberian and Far Eastern samples is concordant with widespread dispersion of this clade. This clade corresponds to the latest wave of brown bear migration (Anijalg *et al.*, 2018). Two samples from Siberia belonged to clade 3b, which was previously found in Hokkaido and the Kuril islands, based on complete mitochondrial DNA analysis (Hirata *et al.*, 2013), and additional samples from Northern Caucasus, Siberia and the Russian Far East were attributed to clade 3b based on mitochondrial sequences of *cytB*, CR and amplified product length polymorphism analysis (Gus'kov *et al.*, 2013; Hirata *et al.*, 2014; Salomashkina *et al.*, 2014; Tumendemberel *et al.*, 2019). Finding modern brown bears from South Siberia (Irk) from both clades 3b1 and 3b2 further confirms the continental origin of clade 3 proposed by Hirata *et al.* (2013).

Clade 4 includes modern brown bears from Hokkaido (Hirata *et al.*, 2013) and North America (Barnes *et al.*, 2002; Delisle & Strobeck, 2002). Based on the presence of three genetic lineages in Hokkaido, Matsuhashi *et al.* (2001) proposed that the island was invaded by bears in three distinct waves similar to those in North America. Descendants from the most recent wave inhabit the northern and central parts of the island, whereas descendants from the first wave inhabit the south-western part (Hirata *et al.*, 2013). Similar phylogeographical structures in Hokkaido and North America indicate their simultaneous migration from Eurasia through Beringia to Alaska and through Sakhalin to Japan during glacial periods of lowered sea levels. This model of migration to Japan through Sakhalin was also described for other species, such as the Siberian flying squirrel, *Pteromys volans* (Oshida *et al.*, 2005), Siberian chipmunk, *Tamias sibiricus* (Lee *et al.*, 2008), and sable, *Martes zibellina* (Kinoshita *et al.*, 2015).

Phylogenetic analysis revealed that the analysed ancient samples are very diverse and contain haplotypes characteristic for different migration waves. For example, the youngest ancient bear, U16 Kol (~4.5 kyr old), from the foothills of the Altai belongs to the widespread Eurasian haplogroup 3a1, being highly similar to modern bears from the European part of Russia and Estonia. A sample (U2 Chu) from the Chumysh River (~40 kyr old) takes a basal position within the entire clade 3a, which includes the Eurasian population and the population of central Hokkaido. Clade 3a is the most widespread modern clade, and it is believed that it spread widely after the Last Glacial Maximum. The ancient bear U17 DC from Denisova Cave (~5.5 kyr old) belongs to subclade 3b, which includes modern bears from Hokkaido and the Kuril islands, Siberia, Northern Caucasus and the continental Russian Far East. The phylogenetic analysis suggests that the representatives of clade 3b from Japan and Siberia had a most recent common ancestor ~82 kya (Fig. 2), which roughly corresponds to estimates by Salis *et al.* (2021). Sample U3 Chi from Chik river (~30 kyr old) is basal to the entire clade 3, with a divergence time estimated as 216 kya. This result might be somewhat confounded by the fact that this genome has the lowest completeness (91%, as opposed to > 96% in other ancient samples). However, similar observations of a basal position of ancient brown bears from Siberia relative to modern Eurasian populations were reported recently (Rey-Iglesia *et al.*, 2019).

In this study, we have shown that most of modern brown bears from Siberia and the Russian Far East belong to the main Eurasian haplogroup 3. High genetic diversity among ancient brown bears indicates the shift that occurred in haplotype diversity and the presence of a common ancestral population for modern bears of the continental Russian Far East and Siberia. The isolation of the West European group might have resulted from human activities, which limited the free movement of surviving populations. Siberia and the Russian Far East are less susceptible to the impact of anthropogenic factors, and the habitat remains intact, which could have contributed to the wide distribution of clade 3a1. The data obtained on the representation of haplogroups, in combination with reconstruction of possible waves of migration of brown bears through Eurasia, highlight the importance of a southern Siberian refugium for brown bear populations not only during the Last Glacial Maximum, but also in earlier periods for at least the last 200 kyr.

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### AUTHOR CONTRIBUTIONS

A.S.G., N.V.V. and U.S. conceived the original idea; all authors collected the data; A.S.M., A.I.M., I.G.K. and

N.V.V. analysed the data; A.S.M. and A.I.M. wrote the first draft of the manuscript; and all authors contributed to writing the final version.

## DATA AVAILABILITY

Mitochondrial genomes generated in this study are available at NCBI GenBank under accession numbers MW991379–MW991401.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Description of modern samples of brown bears, for which complete sequences of mitochondrial genomes were obtained. Colours represent regions as follows: blue, Siberia; purple, Russian Far East.
**Table S2.** Primers for *Ursus arctos* full mitochondrial DNA probe (length of fragments ~4 kb, primer melting temperature ($T_m$) ~68 °C).
**Table S3.** Samples used for phylogenetic analysis; a total of 259 mitochondrial sequences of modern bears. Colours represent geographical regions as follows: green, Hokkaido and Kuril islands; yellow, Europe; blue, Siberia; purple, Russian Far East (mainland); red, Kamchatka.
**Table S4.** Within-population nucleotide diversity calculated for samples indicated by colour in the Supporting Information (Tables1 and S3; Western and Southern European samples are excluded from the analysis).
**Figure S1.** Phylogeny for *COXII* and mitochondrial control region (CR) sequences for Central Asian brown bears from the study by Tumendemberel *et al.* (2019) and complete mitochondrial genomes from this study.