

## **Supplementary Information**

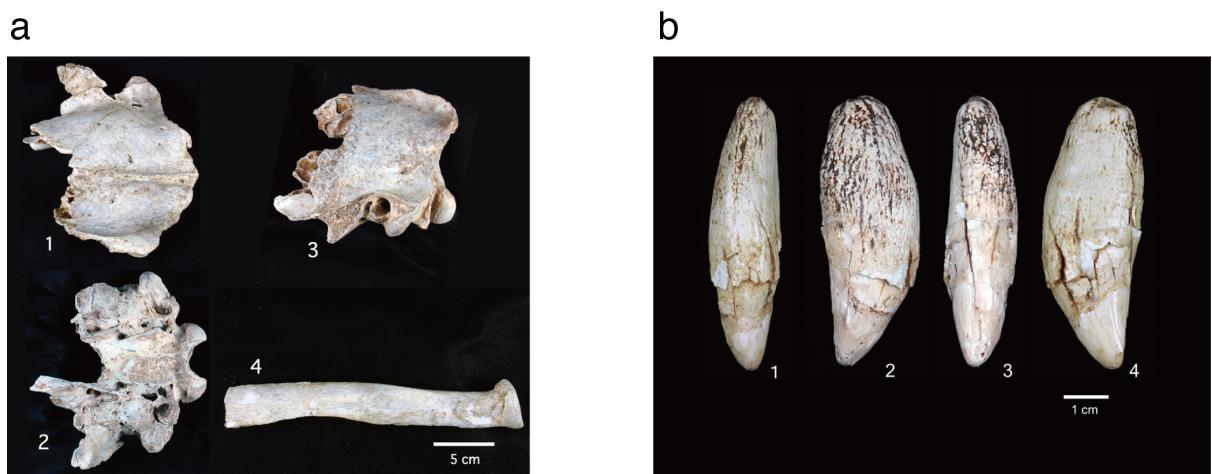
Ancient DNA reveals multiple origins and migration waves of extinct Japanese brown bear lineages

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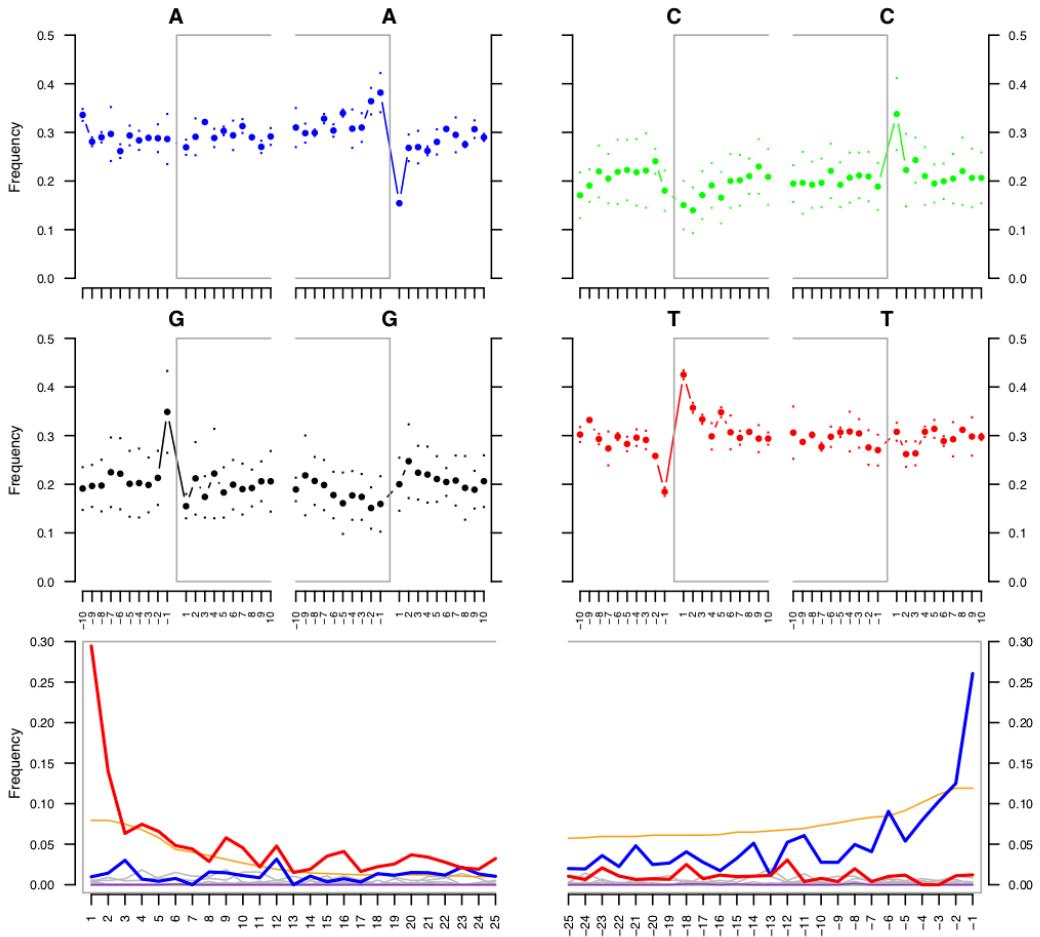
This PDF file includes:

Supplementary Figures S1-S7

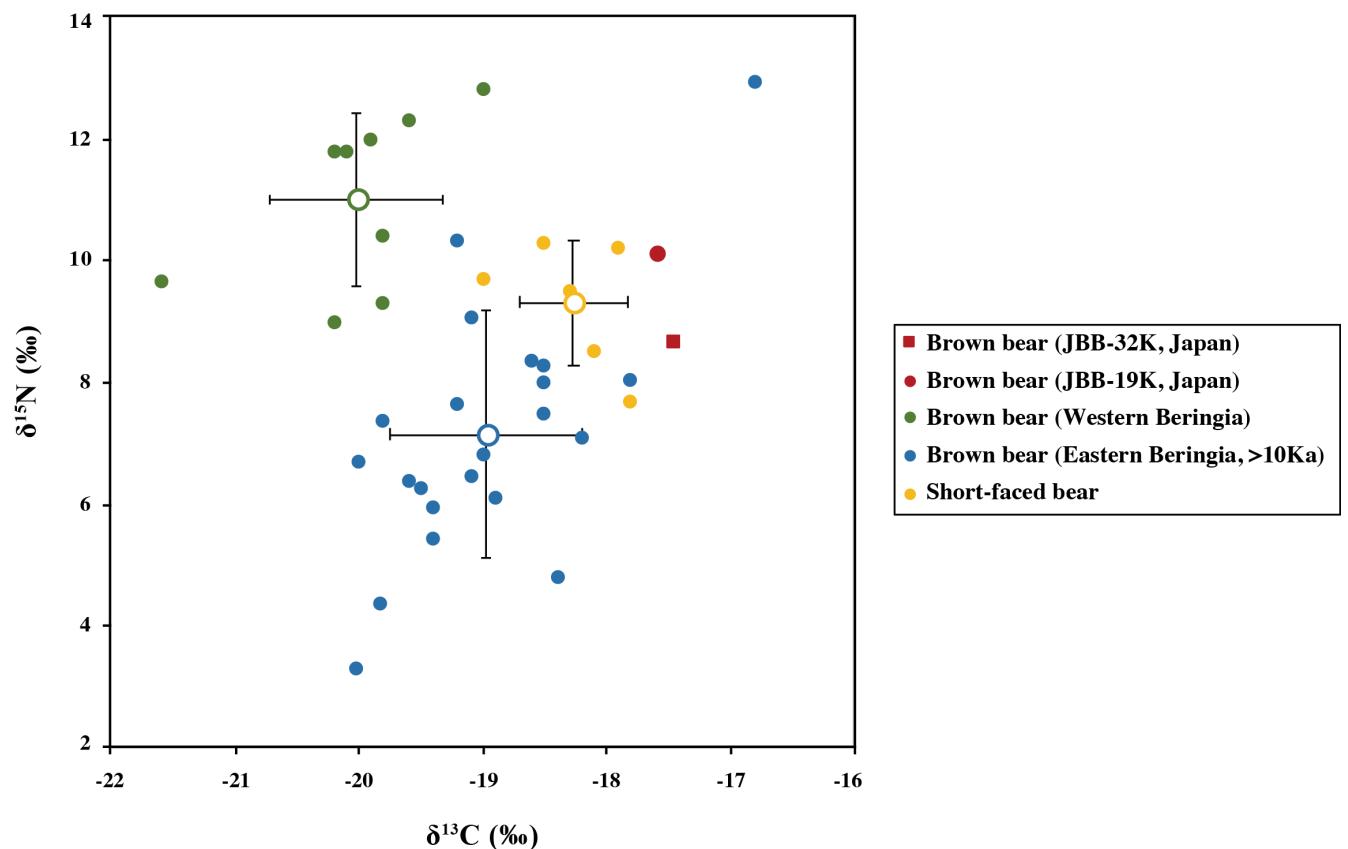
Supplementary Tables S1-S3



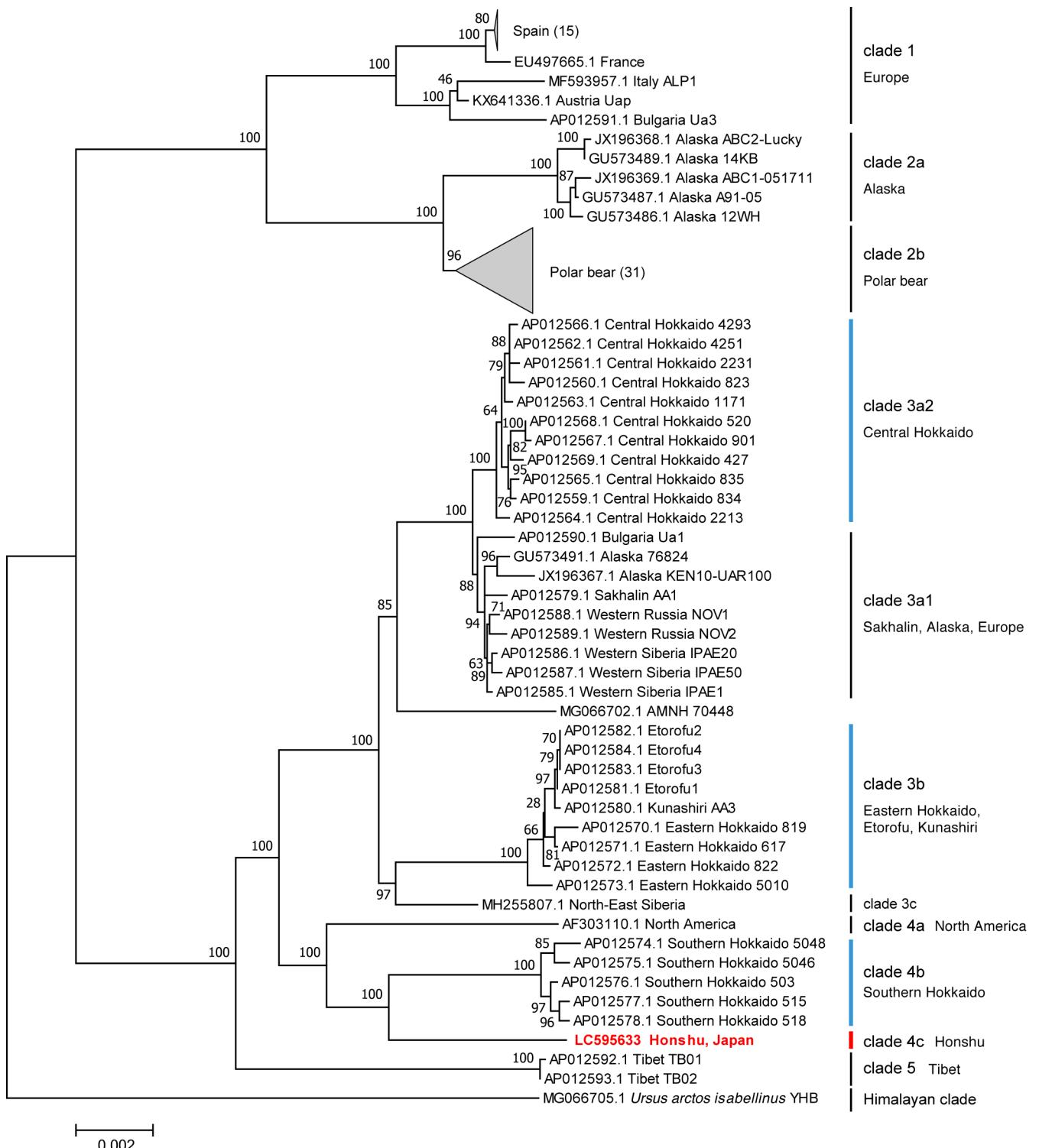
**Supplementary Fig. S1:** Fossils of the Pleistocene brown bears used in this study. **a** Braincase of JBB-32K from Gunma, central Honshu, Japan. 1, dorsal; 2, lateral; 3, ventral; 4, anterolateral view of right radius. **b** Left upper canine of JBB-19K from Saitama, central Honshu, Japan. 1, mesial; 2, lingual; 3, distal; 4, buccal views.

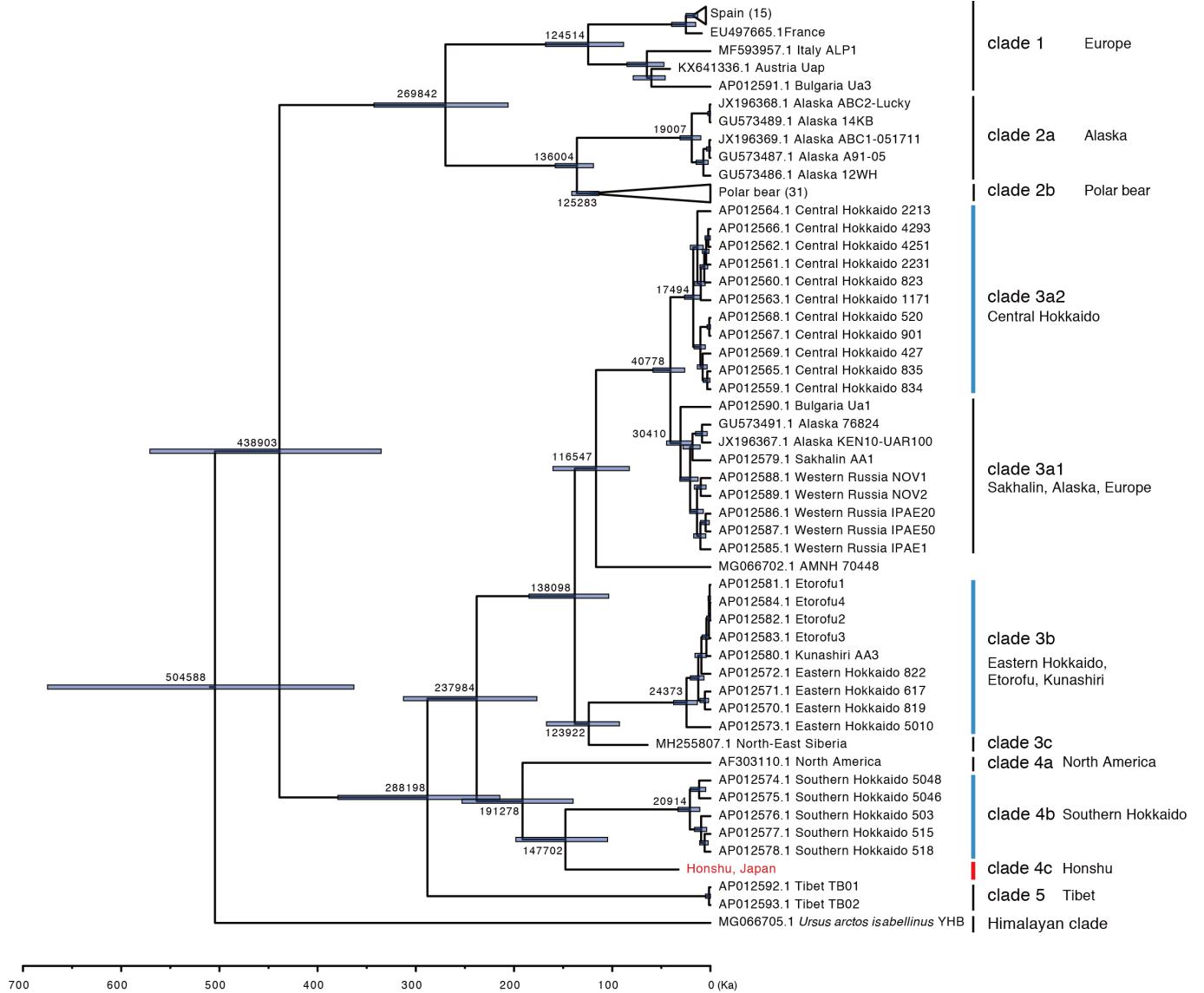


**Supplementary Fig. S2:** Patterns of fragmentation and nucleotide misincorporation in the mtDNA sequence reads. Shown are the base composition for each of the first and last 10 nucleotides of the mitochondrial reads as well as the upstream and downstream 10 nucleotides of the reconstructed mitochondrial genomes. Each colored point represents the average base frequency per nucleotide position. Frequencies for all base substitutions and indels calculated for the reads and the reconstructed mitochondrial genomes are shown as red (C to T), blue (G to A), and grey points and lines.

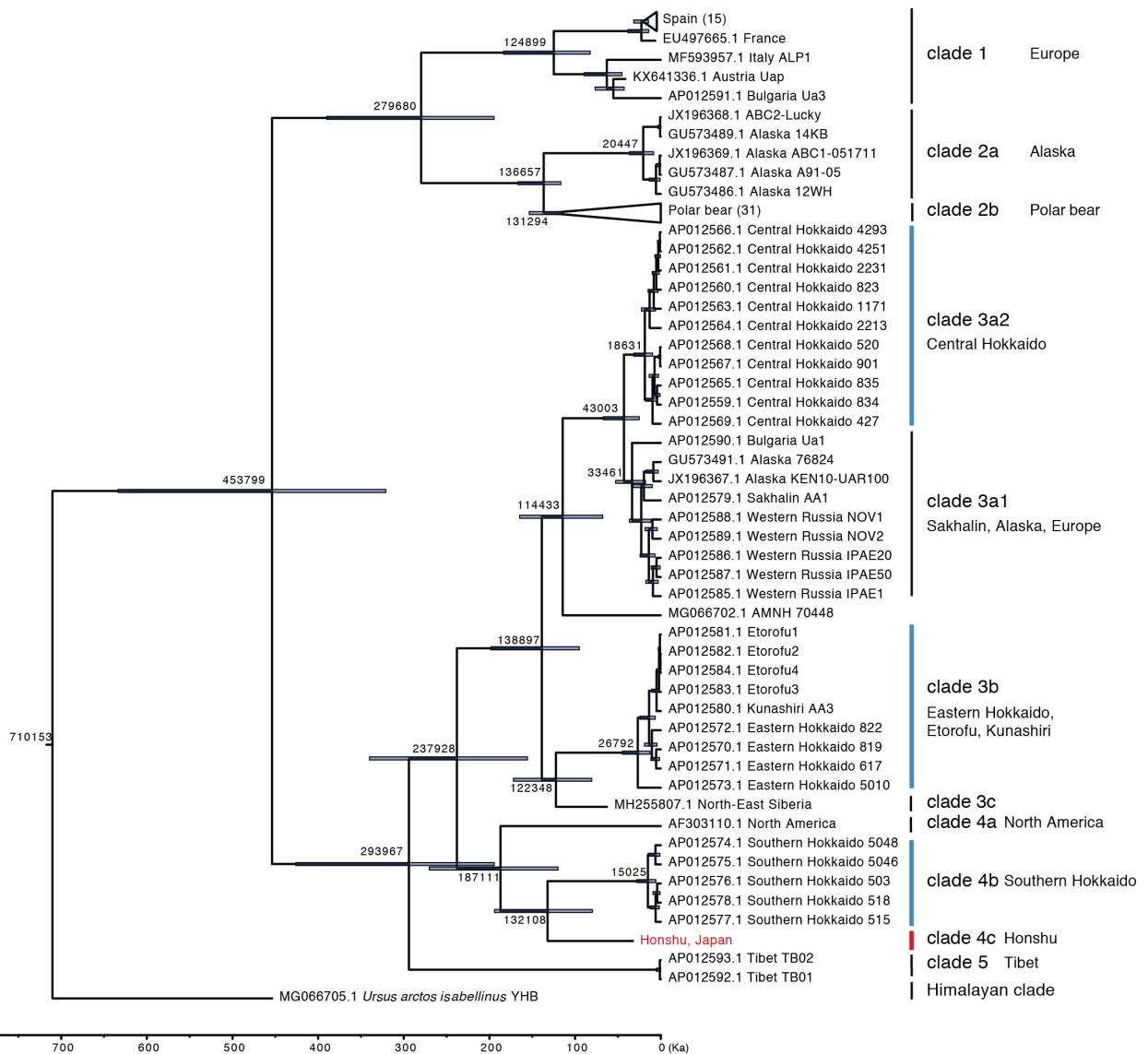


**Supplementary Fig. S3:** Plot of the stable nitrogen and carbon isotope values for the brown bears on Honshu Island (red), Western Beringia (green), Eastern Beringia (blue), and the Short-faced bear (yellow). Each open circle and bar represent the mean and standard deviation, respectively, for each population. All isotope data, except for the Honshu brown bear, were retrieved from Barnes et al. 2002 and Rey-Iglesia et al. 2019.

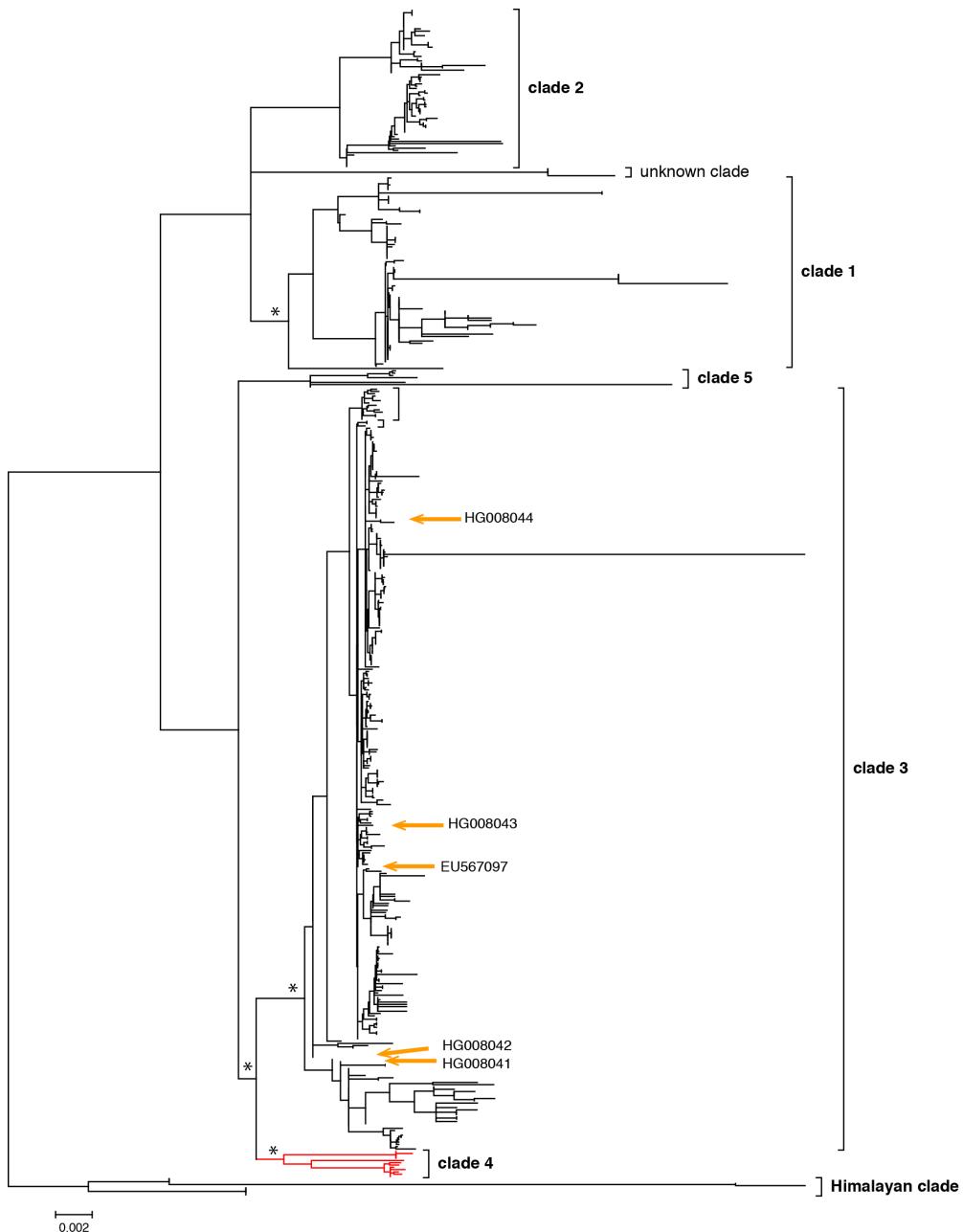




**Supplementary Fig. S5:** Tip-calibrated coalescence times for brown bears based on the 13 protein-coding mtDNA genes analyzed by BEAST ver. 1.10.4. Branch labels indicate the median estimated divergence time, and blue bars indicate the 95% highest posterior densities. Part of clade 1 as well as the whole clade 2b (polar bears) were collapsed.



**Supplementary Fig. S6:** Tip-calibrated coalescence times for brown bears based on the third codon positions of the 13 protein-coding mtDNA genes analyzed by BEAST ver. 1.10.4. Branch labels indicate the median estimated divergence time, and blue bars indicate the 95% highest posterior densities. Part of clade 1 as well as the whole of clade 2b (polar bears) were collapsed.



**Supplementary Fig. S7:** Mitochondrial phylogeny of modern and ancient *Ursus arctos*. The ML tree was inferred from the supermatrix of complete mitochondrial genomes as well as complete or partial sequences of *cytochrome b* from 517 *U. arctos* individuals including the Pleistocene brown bear (JBB-32K) in Honshu, Japan. Asterisks indicate  $\geq 98\%$  bootstrap supports for the relationships among the major clades estimated from 1000 ultrafast bootstrap replicates using IQ-TREE (Nguyen et al. 2015). Clade 4, containing the Honshu brown bear, Southern Hokkaido brown bears, and North American brown bears, is shown in red. Taxa denoted by orange arrows are individuals from the Maritime Territory in Siberia.

**Supplementary Table S1: Sequence library information utilized in this study.**

Specimens	Parts	Experiment No.	Raw reads (only Forward)	Sequencer	DNA extraction	DNA repair method	PCR enzyme	Mitochondrial Reads	Accession number
JBB-19K	canine	Experiment 1	8,012,163	Miseq	Silica pellets	-	KAPA*	-	-
		Experiment 2	1,750,840	Miseq	Silica column	-	AmpliTaq gold	-	-
		Experiment 3	1,751,551	Miseq	Silica column	-	KAPA*	-	-
		Experiment 4	2,245,164	Miseq	Silica pellets	-	AmpliTaq gold	-	-
		Experiment 5	3,334,085	Miseq	Silica pellets	-	KAPA*	-	-
		Experiment 6	141,340,724	Hiseq	Silica pellets	-	AmpliTaq gold	2 reads (1 unique)	-
		Experiment 7	85,330,741	Hiseq	Silica pellets	FFPE DNA repair mix	AmpliTaq gold	-	-
		Experiment 8	79,304,417	Hiseq	Silica pellets	User Enzyme	AmpliTaq gold	-	-
		Experiment 9	400,694,570	Hiseq	Silica pellets	-	KAPA*	2 reads (1 unique)	-
JBB-32K	petrous radius	Experiment 10	243,394,094	Hiseq	Silica pellets	-	KAPA*	4,746 reads (1,280 unique)	LC595633
		Experiment 11	191,769,987	Hiseq	Silica pellets	-	KAPA*	42 reads (30 unique)	

\* KAPA HiFi HotStart Uracil+.

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**Supplementary Table S2: Results of the radiocarbon ( $^{14}\text{C}$ ) dating of the two brown bear specimens (JBB-32K and JBB-19K), including uncalibrated dates and parameters of collagen quality assessments.**

Sample name	Lab code	Collagen yield (Wt %)	$\delta^{13}\text{C}$ (Isoprime)	$^{14}\text{C}$ age (yrBP $\pm 1\sigma$ )	Calibrated age (yrBP $\pm 1\sigma$ )	C%	N%	C (mol)	N (mol)	C/N atomic ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
JBB-32K	YU-11664	19.04	-17.39 $\pm$ 0.31	28459 $\pm$ 86	32719 BP (68.2 %) 32209 BP	43.01	15.00	3.584	1.071	3.35	-17.45	8.68
JBB-19K	YU-4413	0.629	-18.65 $\pm$ 0.32	16010 $\pm$ 60	19434 BP (68.2 %) 19212 BP	42.05	12.76	3.504	0.911	3.84	-17.58	10.11

**Supplementary Table S3: Cytb data retrieved from NCBI and analyzed in this study.**

Clade	Accession No.	Sequence Name
clade 1	AM411406.1	Ursus arctos mitochondrial cytb for cytochrome b isolate AK01
clade 1	AM944505.1	Ursus arctos mitochondrial partial cytB gene for cytochrome b isolate C2
clade 1	AP012591.1	Ursus arctos mitochondrial DNA complete sequence isolate Ua3
clade 1	EU497665.1	Ursus arctos mitochondrion complete genome
clade 1	EU567090.1	Ursus arctos haplotype Gre cytochrome b cob gene complete cds mitochondrial
clade 1	EU567091.1	Ursus arctos haplotype Pol cytochrome b cob gene complete cds mitochondrial
clade 1	EU567092.1	Ursus arctos haplotype Ita cytochrome b cob gene complete cds mitochondrial
clade 1	EU567093.1	Ursus arctos haplotype Ro1 cytochrome b cob gene complete cds mitochondrial
clade 1	EU567094.1	Ursus arctos haplotype Ro2 cytochrome b cob gene complete cds mitochondrial
clade 1	EU567095.1	Ursus arctos haplotype Ro3 cytochrome b cob gene complete cds mitochondrial
clade 1	FN292993.1	Ursus arctos syriacus mitochondrial partial cytB gene for cytochrome b specimen voucher UAS11
clade 1	FN292994.1	Ursus arctos syriacus mitochondrial partial cytB gene for cytochrome b specimen voucher UAS12
clade 1	FN292995.1	Ursus arctos syriacus mitochondrial partial cytB gene for cytochrome b specimen voucher UAS13
clade 1	GU186047.1	Ursus arctos haplotype 2 cytochrome b CYTB gene partial cds tRNA-Thr and tRNA-Pro genes complete sequence and D-loop partial sequence mitochondrial
clade 1	GU186048.1	Ursus arctos haplotype 3 cytochrome b CYTB gene partial cds tRNA-Thr and tRNA-Pro genes complete sequence and D-loop partial sequence mitochondrial
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clade 3	HQ685917.1	Ursus arctos isolate 17 mitochondrion complete genome
clade 3	HQ685918.1	Ursus arctos isolate 18 mitochondrion complete genome
clade 3	HQ685919.1	Ursus arctos isolate 19 mitochondrion complete genome
clade 3	HQ685920.1	Ursus arctos isolate 20 mitochondrion complete genome
clade 3	HQ685921.1	Ursus arctos isolate 21 mitochondrion complete genome
clade 3	HQ685922.1	Ursus arctos isolate 22 mitochondrion complete genome
clade 3	HQ685923.1	Ursus arctos isolate 23 mitochondrion complete genome
clade 3	HQ685924.1	Ursus arctos isolate 24 mitochondrion complete genome
clade 3	HQ685925.1	Ursus arctos isolate 25 mitochondrion complete genome
clade 3	HQ685926.1	Ursus arctos isolate 26 mitochondrion complete genome
clade 3	HQ685927.1	Ursus arctos isolate 27 mitochondrion complete genome
clade 3	HQ685928.1	Ursus arctos isolate 28 mitochondrion complete genome
clade 3	HQ685929.1	Ursus arctos isolate 29 mitochondrion complete genome
clade 3	HQ685930.1	Ursus arctos isolate 30 mitochondrion complete genome
clade 3	HQ685931.1	Ursus arctos isolate 31 mitochondrion complete genome
clade 3	HQ685932.1	Ursus arctos isolate 32 mitochondrion complete genome
clade 3	HQ685933.1	Ursus arctos isolate 33 mitochondrion complete genome
clade 3	HQ685934.1	Ursus arctos isolate 34 mitochondrion complete genome







clade 3	KY419670.1	Ursus arctos isolate Perm71 mitochondrion partial genome
clade 3	KY419671.1	Ursus arctos isolate Perm73 mitochondrion partial genome
clade 3	KY419672.1	Ursus arctos isolate Perm74 mitochondrion partial genome
clade 3	KY419673.1	Ursus arctos isolate Perm75 mitochondrion partial genome
clade 3	KY419674.1	Ursus arctos isolate Perm76 mitochondrion partial genome
clade 3	KY419675.1	Ursus arctos isolate Perm77 mitochondrion partial genome
clade 3	KY419676.1	Ursus arctos isolate Perm78 mitochondrion partial genome
clade 3	KY419677.1	Ursus arctos isolate Perm79 mitochondrion partial genome
clade 3	KY419678.1	Ursus arctos isolate Perm80 mitochondrion partial genome
clade 3	KY419679.1	Ursus arctos isolate Perm81 mitochondrion partial genome
clade 3	KY419680.1	Ursus arctos isolate Perm82 mitochondrion partial genome
clade 3	KY419681.1	Ursus arctos isolate Perm83 mitochondrion partial genome
clade 3	KY419682.1	UNVERIFIED Ursus arctos isolate Prim350 mitochondrion partial genome
clade 3	KY419683.1	Ursus arctos isolate Ro16ok mitochondrion partial genome
clade 3	KY419684.1	Ursus arctos isolate Rom10 mitochondrion partial genome
clade 3	KY419685.1	Ursus arctos isolate Rom11 mitochondrion partial genome
clade 3	KY419686.1	Ursus arctos isolate Sver205 mitochondrion partial genome
clade 3	KY419687.1	Ursus arctos isolate Sver206 mitochondrion partial genome
clade 3	KY419688.1	Ursus arctos isolate Sver208 mitochondrion partial genome
clade 3	KY419689.1	Ursus arctos isolate Sver209 mitochondrion partial genome
clade 3	KY419690.1	Ursus arctos isolate Sver213 mitochondrion partial genome
clade 3	KY419691.1	Ursus arctos isolate Sver215 mitochondrion partial genome
clade 3	KY419692.1	Ursus arctos isolate Sver216 mitochondrion partial genome
clade 3	KY419693.1	Ursus arctos isolate Sver217 mitochondrion partial genome
clade 3	KY419694.1	Ursus arctos isolate Sver219 mitochondrion partial genome
clade 3	KY419695.1	Ursus arctos isolate SWE10E mitochondrion partial genome
clade 3	KY419696.1	Ursus arctos isolate SWE11E mitochondrion partial genome
clade 3	KY419698.1	Ursus arctos isolate SWE21E mitochondrion partial genome
clade 3	KY419699.1	Ursus arctos isolate SWE27E mitochondrion partial genome
clade 3	KY419700.1	Ursus arctos isolate Tver201 mitochondrion partial genome
clade 3	KY419701.1	Ursus arctos isolate Tver202 mitochondrion partial genome
clade 3	KY419702.1	Ursus arctos isolate Tver203 mitochondrion partial genome
clade 3	MF593959.1	Ursus arctos isolate SLK5 mitochondrion partial genome
clade 3	MF593960.1	Ursus arctos isolate SLK2 mitochondrion partial genome
clade 3	MF593961.1	Ursus arctos isolate SLK1 mitochondrion partial genome
clade 3	MF593962.1	Ursus arctos isolate SLK4 mitochondrion partial genome
clade 3	MF593963.1	Ursus arctos isolate SLK3 mitochondrion partial genome
clade 3	MG066702.1	Ursus arctos isolate 70448 mitochondrion complete genome
clade 3	MG254039.1	Ursus arctos haplotype H1 cytochrome b CYTB gene partial cds tRNA-Thr gene complete sequence and tRNA-Pro gene partial sequence mitochondrial
clade 3	MG254040.1	Ursus arctos haplotype H2 cytochrome b CYTB gene partial cds tRNA-Thr gene complete sequence and tRNA-Pro gene partial sequence mitochondrial
clade 3	MG254041.1	Ursus arctos haplotype H3 cytochrome b CYTB gene partial cds tRNA-Thr gene complete sequence and tRNA-Pro gene partial sequence mitochondrial
clade 3	MG254042.1	Ursus arctos haplotype H4 cytochrome b CYTB gene partial cds tRNA-Thr gene complete sequence and tRNA-Pro gene partial sequence mitochondrial
clade 3	U18875.1	Ursus arctos GB06 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18876.1	Ursus arctos GB07 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18877.1	Ursus arctos GB08 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18878.1	Ursus arctos GB09 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18879.1	Ursus arctos GB10 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18880.1	Ursus arctos GB11 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18881.1	Ursus arctos GB12 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18882.1	Ursus arctos GB13 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18883.1	Ursus arctos GB14 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18884.1	Ursus arctos GB15 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18885.1	Ursus arctos GB16 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18886.1	Ursus arctos GB17 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18887.1	Ursus arctos GB18 mitochondrion cytochrome b cyt b gene complete cds

clade 3	U18888.1	Ursus arctos GB19 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18889.1	Ursus arctos GB20 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18890.1	Ursus arctos GB21 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18891.1	Ursus arctos GB22 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18892.1	Ursus arctos GB23 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18893.1	Ursus arctos GB24 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18894.1	Ursus arctos GB25 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18895.1	Ursus arctos GB26 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18896.1	Ursus arctos GB27 mitochondrion cytochrome b cyt b gene complete cds
clade 3	U18897.1	Ursus arctos GB28 mitochondrion cytochrome b cyt b gene complete cds
clade 3	X82308.1	U.arctos mitochondrial cytochrome b gene
clade 4	AB020908.1	Ursus arctos mitochondrial gene for cytochrome b complete cds
clade 4	AB020909.1	Ursus arctos mitochondrial gene for cytochrome b complete cds
clade 4	AF303110.1	Ursus arctos mitochondrion complete genome
clade 4	AP012574.1	Ursus arctos mitochondrial DNA complete sequence isolate 5048
clade 4	AP012575.1	Ursus arctos mitochondrial DNA complete sequence isolate 5046
clade 4	AP012576.1	Ursus arctos mitochondrial DNA complete sequence isolate 503
clade 4	AP012577.1	Ursus arctos mitochondrial DNA complete sequence isolate 515
clade 4	AP012578.1	Ursus arctos mitochondrial DNA complete sequence isolate 518
clade 4	KF184274.1	Ursus arctos isolate 31 cytochrome b cytB gene partial cds mitochondrial
clade 4	KF184275.1	Ursus arctos isolate 32 cytochrome b cytB gene partial cds mitochondrial
clade 4	KF184276.1	Ursus arctos isolate 33 cytochrome b cytB gene partial cds mitochondrial
clade 5	AP012592.1	Ursus arctos mitochondrial DNA complete sequence isolate TB01
clade 5	AP012593.1	Ursus arctos mitochondrial DNA complete sequence isolate TB02
clade 5	MG066703.1	Ursus arctos pruinosus isolate LZHR mitochondrion complete genome
clade 5	MG131870.1	Ursus arctos pruinosus isolate 1938 hair cytochrome b cytB gene partial cds and tRNA-Thr cytB gene partial sequence mitochondrial
clade 5	MG131871.1	Ursus arctos pruinosus isolate 1996 paw cytochrome b cytB gene partial cds and tRNA-Thr cytB gene partial sequence mitochondrial
clade 5	MG131872.1	Ursus arctos pruinosus isolate Tashi cytochrome b cytB gene partial cds and tRNA-Thr cytB gene partial sequence mitochondrial
clade 5	MG131874.1	Ursus arctos pruinosus isolate Chemo cytochrome b cytB gene partial cds and tRNA-Thr cytB gene partial sequence mitochondrial
clade 5	MG131876.1	Ursus arctos pruinosus isolate Chemo clone 2 cytochrome b cytB gene partial cds mitochondrial
Himalayan	AM411404.1	Ursus arctos mitochondrial cytB for cytochrome b isolate TAK4
clade		
Himalayan	AM411405.1	Ursus arctos mitochondrial cytB for cytochrome b isolate ELK1
clade		
Himalayan	MG066705.1	Ursus arctos isabellinus isolate YHB mitochondrion complete genome
clade		
Himalayan	MG131873.1	Ursus arctos isabellinus isolate ISB cytochrome b cytB gene partial cds and tRNA-Thr cytB gene partial sequence mitochondrial
clade		
Himalayan	MG131877.1	Ursus arctos isabellinus isolate TF0017 cytochrome b cytB gene partial cds mitochondrial
clade		
Himalayan	MG131878.1	Ursus arctos isabellinus isolate TF0063 cytochrome b cytB gene partial cds mitochondrial
clade		
Himalayan	MG131879.1	Ursus arctos isabellinus isolate TF0101 cytochrome b cytB gene partial cds mitochondrial
clade		
Himalayan	MG131880.1	Ursus arctos isabellinus isolate TF0103 cytochrome b cytB gene partial cds mitochondrial
clade		

## Supplementary references

1. Barnes, I., Matheus, P., Shapiro, B., Jensen, D., Cooper, A. Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears. *Science* **295**, 2267-2270 (2002).
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