

Supplementary Information

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

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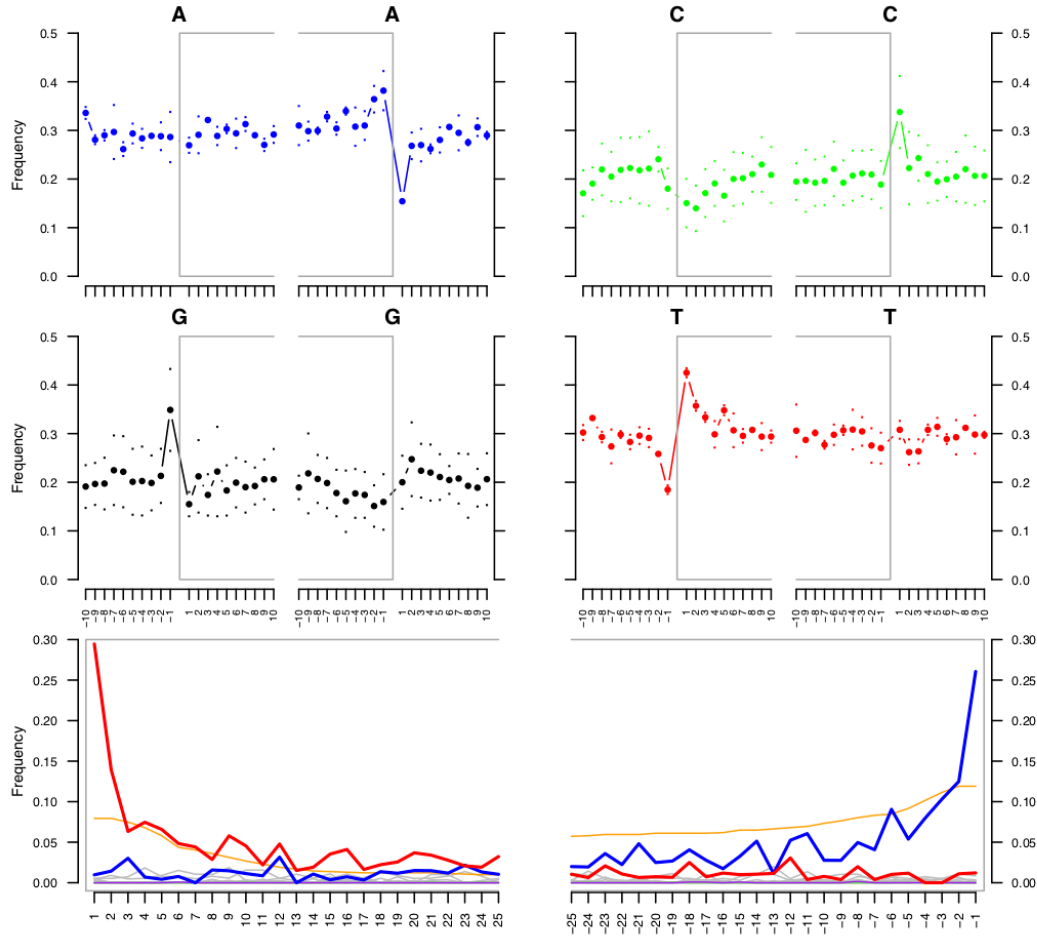
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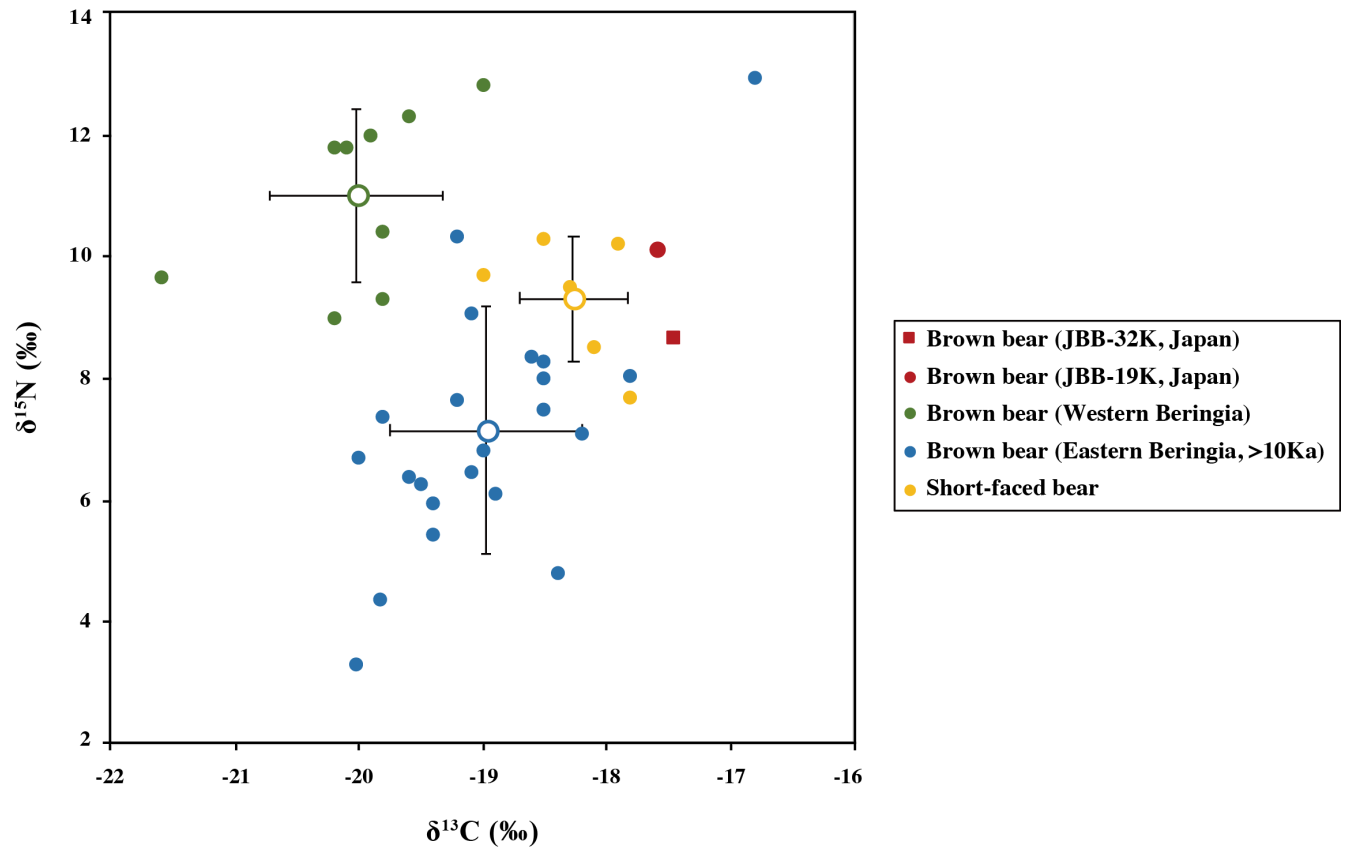
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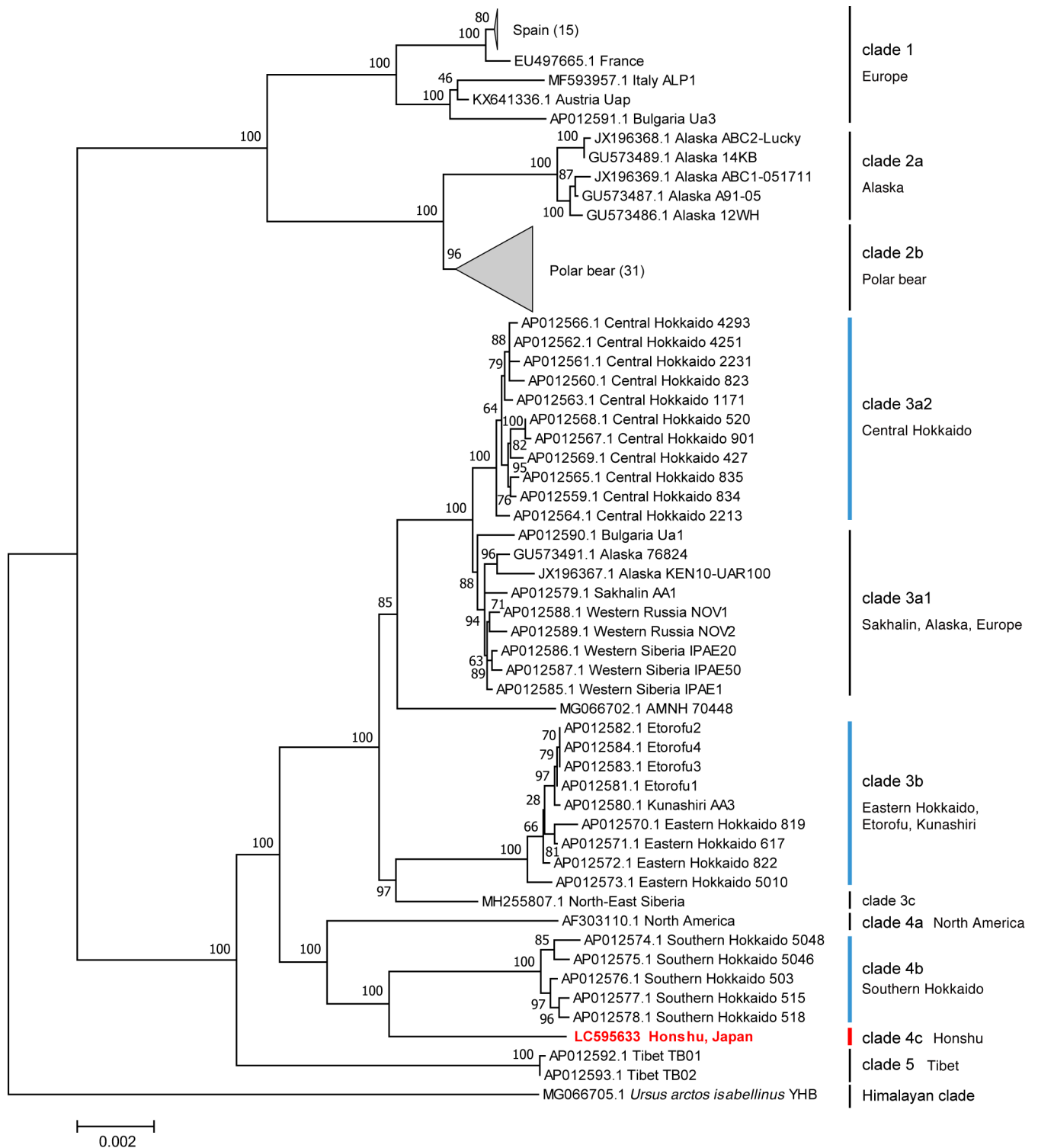
Supplementary Fig. S1: Fossils of the Pleistocene brown bears used in this study. **a** Braincase of JBB-32K from Gunma, central Honshu, Japan. 1, dorsal; 2, lateral; 3, ventral; 4, anterolateral view of right radius. **b** Left upper canine of JBB-19K from Saitama, central Honshu, Japan. 1, mesial;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



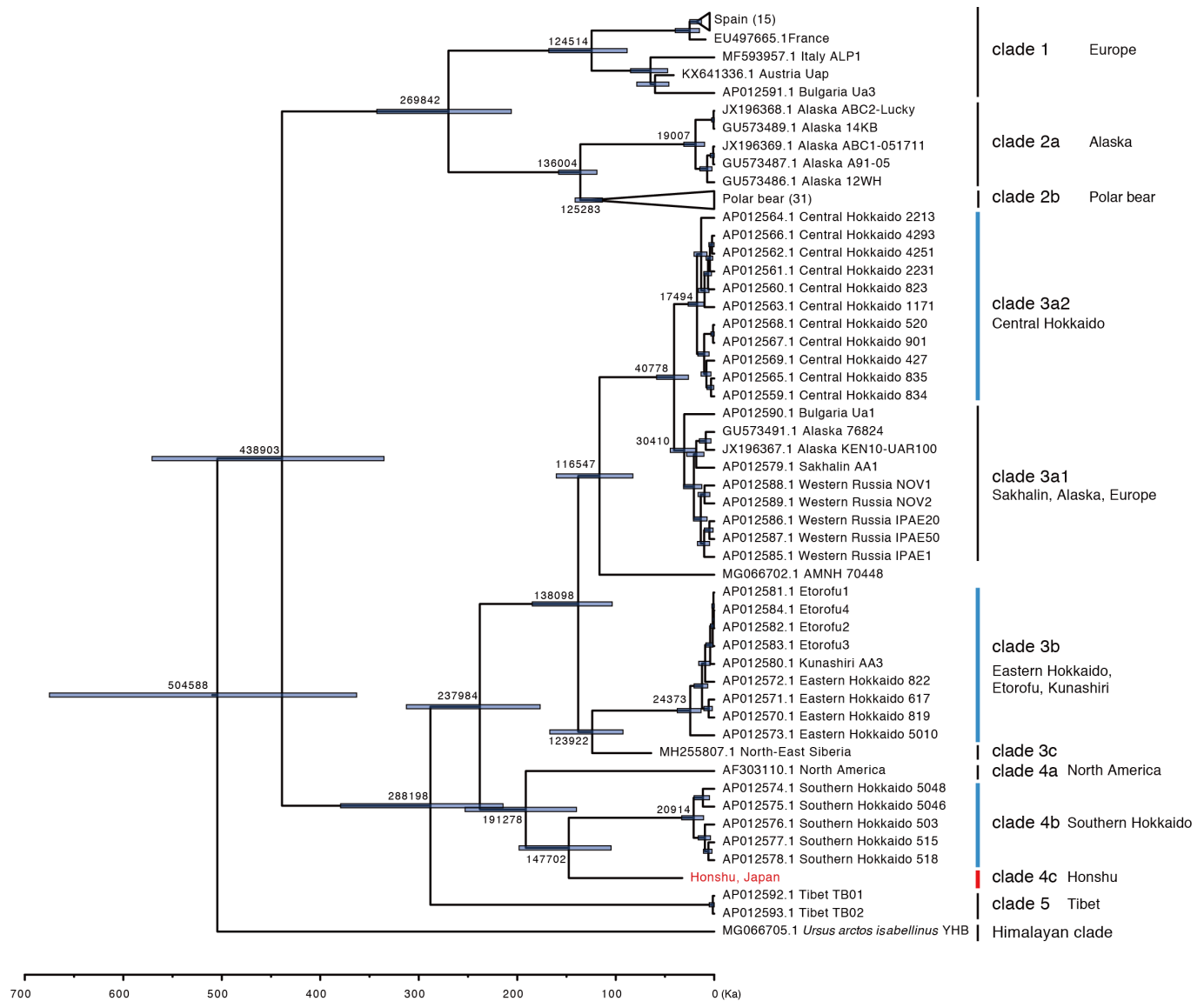
Supplementary Fig. S2: Patterns of fragmentation and nucleotide misincorporation in the mtDNA sequence reads. Shown are the base composition for each of the first and last 10 nucleotides of the mitochondrial reads as well as the upstream and downstream 10 nucleotides of the reconstructed mitochondrial 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.



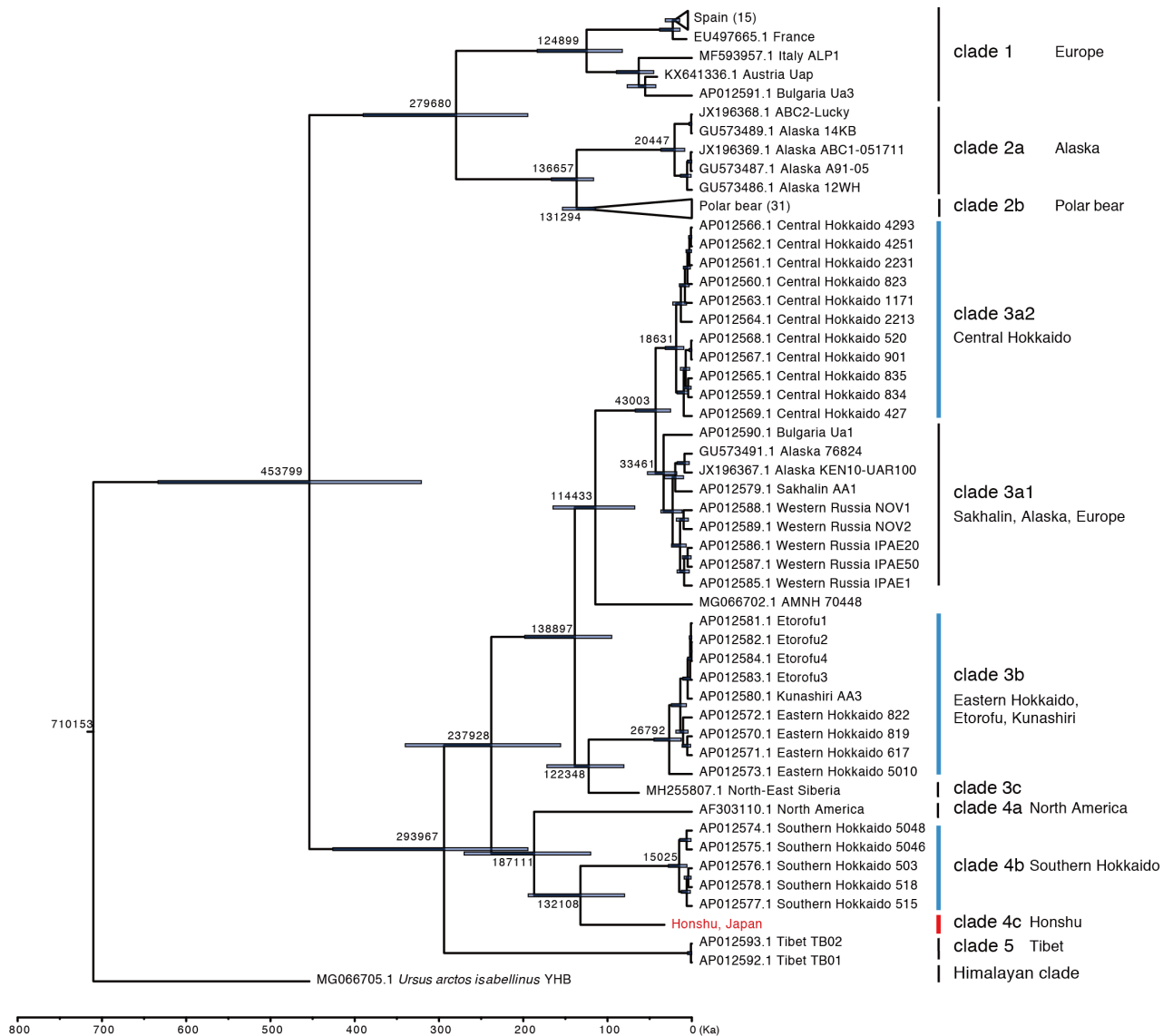
Supplementary Fig. S3: Plot of the stable nitrogen and carbon isotope values for the brown bears on Honshu Island (red), Western Beringia (green), Eastern Beringia (blue), and the Short-faced bear (yellow). Each open circle and bar represent the mean and standard deviation, respectively, for each population.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



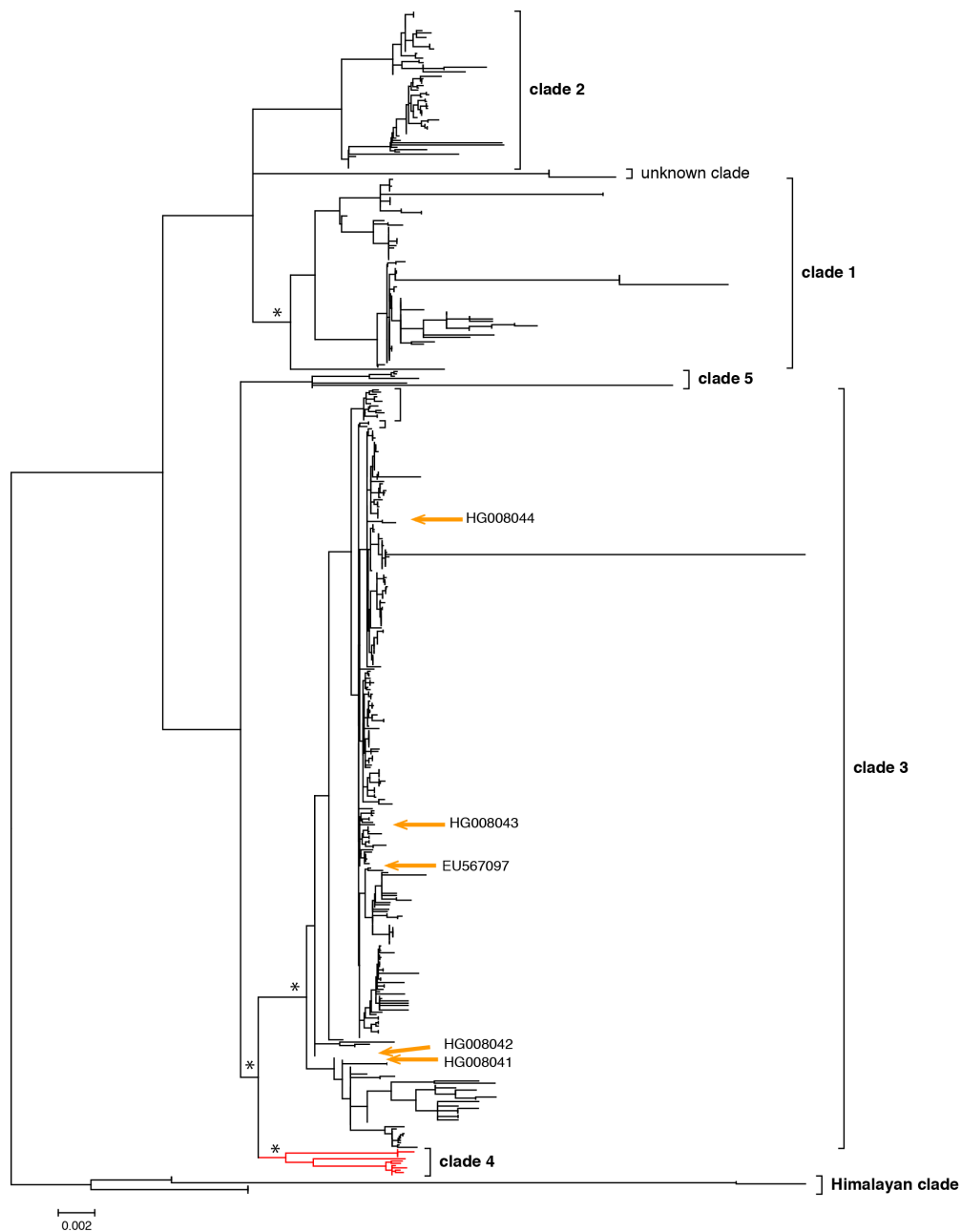
Supplementary Fig. S4: Mitochondrial phylogeny of 96 brown bears including the Pleistocene individual from Honshu (red). Node supports are bootstrap probabilities calculated from 1000 replications. The clades denoted by light-blue and red lines contain extant and extinct individuals in the Japanese Archipelago.



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. Nodal support values were estimated based on 1000 standard 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. Node support values were estimated based on 1000 ultrafast bootstrap replicates using IQ-TREE.

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	2reads (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*	2reads (1 unique)	-
JBB-32K	petrous	Experiment 10	243,394,094	Hiseq	Silica pellets	-	KAPA*	4,746 reads (1,280 unique)	LC595633
	radius	Experiment 11	191,769,987	Hiseq	Silica pellets	-	KAPA*	42 reads (30 unique)	

* KAPA HiFi HotStart Uracil+.

Supplementary Table S2: Results of the radiocarbon (^{14}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}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 ± 0.31	28459 ± 86	32719BP (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 ± 0.32	16010 ± 60	19434 BP (68.2 %) 19212 BP	42.05	12.76	3.504	0.911	3.84	-17.58	10.11

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).
2. Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution*, **32**(1), 268-274.
3. Rey-Iglesia, A. *et al.* Evolutionary history and palaeoecology of brown bear in North-East Siberia re-examined using ancient DNA and stable isotopes from skeletal remains. *Sci. Rep.* **9**, 4462 (2019).