

Supporting online material

Methods

Ancient DNA studies are extremely susceptible to contamination with extraneous DNA, and must demonstrate adequate experimental and authentication procedures (*S1*).

Consequently, all manipulation of ancient samples prior to PCR amplification were performed in dedicated ancient DNA laboratories at the Zoological Institute of University of Copenhagen and the Oxford University Museum of Natural History, in areas free from other molecular research. Appropriate negative extraction and amplification controls were used throughout (at a ratio of 1:5 and 1:1 for the extraction and amplification steps respectively), along with rigorous authentication procedures such as cloning to investigate contamination, jumping PCR products, and other damage-related artifacts (*S1-S6*).

Independently replicated samples and sequences are given in Tables 1-3. Real-time PCR was not possible due to varying levels of inhibition in the different samples.

Sample information

The permafrost samples (Table 1) were obtained from one pit-hole and six cores drilled along approximately 1200 km of the Arctic coast, in a tundra zone with continuous permafrost and a ground temperature between -9 to -12°C . The seasonal thawing zone was within the top 0.3-0.5m, and the thickness of the permafrost was calculated to be 800-1000m (*S7*), with no water infiltration or water-bearing horizons (*S8*). The latter is important to rule out the possibility of sample migration. The samples were fine dispersed sandy-loam and loamy lacustrine-alluvial sediments, representing a slightly reducing (average pH 6-7) and anaerobic (*Eh* +40 to -250)

environment. The hydrocarbon and fatty acid composition of the core samples indicates a primarily vegetative origin with a smaller portion of bacterial origin (S9). Epifluorescence microscopy revealed cells in the bacterial size range (about 10^7 cells/gram wet weight, average cell volume 0.03-0.05 μm^3 /cell).

The NZ cave samples were collected from the Clutha Valley, Otago, and consisted of colluvial/aeolian reworking of the weathered schist of the surrounding area, with a considerable organic macrofossil component. The coastal moa bone (MNZ S unregistered) was from an *in situ* late Holocene paleosol dune deposit, collected by T. Worthy in Jan, 2003. Sand was obtained from the exterior and interior of the bone, during the collection of a cortical bone sample. Gloves and breathing masks were used while collecting specimens, although they had been previously recovered by paleontologists. The outside of the samples was removed before analysis.

Standard ancient DNA techniques (S10, S11) were used to obtain control region sequences from sub-fossil bones of *Megalapteryx didinus* (Museum of New Zealand specimen DM400, tibiotarsus, Cromwell), *Euryapteryx curtus* (MONZ S25832, tibiotarsus, Tokerau Beach), and *Pachyornis elephantopus* (Cambridge University Museum 381A, tissue, Cromwell), and permafrost *Bison spp.* specimens (CRS-DY-42, metapodial, Duvanny Yar). Sedimentary moa sequences were obtained in Copenhagen to minimize the risk of cross-contamination. DNA sequences from contemporary musk ox and lemming were obtained in Copenhagen using established techniques (S12) once the ancient DNA work was completed, to avoid the risk of contamination. Reference parrot sequences had been previously obtained by AC at Victoria University, Wellington, using tissue samples and standard techniques (S10).

Stratigraphic information

Permafrost samples were obtained from syncryogenic horizons where thick ice veins within the late and middle Pleistocene icy complexes (*S13*) demonstrated continuous frozen conditions, except sample 2/01/4.8 from a layer epigenetically frozen after the Holocene optimum (Table 1). Strict protocols were used to avoid contamination during the drilling and subsequent handling of cores, including a rotation-column coring method to avoid drilling fluids, and the introduction of laboratory strains of *Serratia marcescens* bacteria during coring to monitor vertical contamination from the surface (*S7*, *S14*). The surface of cores was removed with a sterile scalpel prior to sampling, and samples were placed into sterile metal containers and stored in a borehole-refrigerator (-10°C) until the end of the field season. The samples were transported back to laboratories frozen, and not melted until analyzed. The late Pleistocene Siberian samples were AMS radiocarbon dated at the Oxford Radiocarbon Accelerator Unit at the University of Oxford. Small amounts of rootlets were initially extracted from the soil for dating using tweezers, but there were too few for analysis (<2 mg), so the humin fraction of the soil organic matter was dated. The soils were pretreated using the standard acid-base-acid method. They isolated a humic (base soluble) and a humin fraction (base insoluble) from each sample. One sample (1/93/4) yielded only a humic acid fraction. The samples were combusted and mass spectrometrically analysed using an Europa ANCA-MS 20/20 system operating under continuous flow mode. The CO₂ was cryogenically distilled and graphitised using the iron/hydrogen method (if large enough in size, ie: >1.7 mg C) or dated as directly-injected CO₂ on the Oxford gas ion source (*S15*, *S16*). The results were calibrated using the

INTCAL98 curve (S17) and the CALIB computer programme (S18). Calibration of 1/93/4.0 resulted in an age range spanning *c.* 10700—10110 cal BC (2 s.d. range; 0.885 prob.) while 2/01/4.8 yielded a calendrical age range of 21316—19900 BC (Table S1A). The middle Pleistocene samples (3/01/20.7 and 4/01/9.2) were drilled on a hill and neighboring slope some 0.5 km apart, and intersected the same geological section. Studies of pollen, diatoms, insects and fossil micromammals from the sampled layer suggest a dry shrubby tundra environment. Several methods of dating (mainly biostratigraphic analysis of collared lemming taxonomy) indicate it to be about 390 kyr (S19, S20), although conservative error margins (300-400 kyr) are used. The oldest samples (6/90/30.7 and 6/90/31.1) were obtained from a well-characterised layer (S21) which starts to accumulate 2.5 Ma ago (S22) and has been aged mostly on the basis of typical Olyor faunal assemblage (S23). Magnetic data (inclination $J = -77/-80$ and remnant magnetization $I_n = 1.1/1.3$) are characteristic of the Matuyama epoch (S24), with pollen spectra indicating tundra landscapes and stable permafrost presence (S25, S26). A feather towards the top of the New Zealand Clutha River cave sample was dated to 624 ± 50 yr B.P. (Table S1B). The Tokerau Beach samples were biostratigraphically dated to approximately 1-3 kyr, based on context and relationship to other biota.

Ice content was determined by weight percentage, a common geological method. The ice content in frozen soils (as well as moisture in non-frozen soils) is calculated by $(P_w - P_d)/P_d \times 100\%$ which is the wet weight minus dry weight, divided by dry weight and multiplied by 100. In the case of high ice content, this can be more than 100 (e.g. if the weight of a 100g frozen sample is 45g when dried then $(100-45)/45 \times 100\% = 1.22 \times 100\% = 122\%$).

DNA extraction

The permafrost samples were divided with a microtome knife treated with 5% sodium hypochlorite in Copenhagen, and sub-samples were sent to Oxford for independent replication. Approximately 1-2 cm of the surface was removed and four separate samples of 0.25g of soil were transferred to MULTIMIX 2 tubes (BIO 101) for whole genomic DNA extraction. The soil samples were dissolved in 600 μ l lysis buffer (S27) 400 ug/ml proteinase K (Roche) and incubated at 65°C for at least 4 h under agitation, disrupted with four runs of a FASTprep 120 (BIO 101) at speed 6.5 for 45 sec, with 2 mins on ice between runs. The solution was adjusted with NaCl to 1.15M, treated with 1/2 vol. of chloroform/octanol (24:1), and slowly agitated overnight at room temperature, and the water phase isolated with centrifugation at 12,000g for 2 min and transferred to a separate microtube for incubation at 2-3°C for at least 1 hr. The precipitate was centrifuged at 12,000g for 2 min and the supernatant purified using silica spin columns and PB-buffer (Qiagen DNA purification kit II), followed by washes in 0.5 ml Salton wash 1 and 2 (BIO 101), and 0.5 ml AW 1 and 2 (Qiagen tissue kit). The DNA was eluted twice with 100 μ l EB buffer (Qiagen purification kit II) and stored at -20°C.

PCR/sequencing

PCR amplification was performed using the primers and conditions listed in Table S2, after (S3, S10), using 5 μ l of DNA extractions, 35-55 cycles of PCR (2-5 min initial denaturation at 94 C, 30-60 sec at 94 C, 30-60 sec at 45-60 C (Table S2), 1-2 min at 68-72 C and a final cycle of 8-10 min at 68-72 C). PCR products were cleaned using a

QIAquick PCR Purification Kit (QIAGEN). Amplification products from the four separate extracts of each sample were pooled, cloned, purified, and sequenced on both strands (S3). Sequences were aligned using ClustalW in BioEdit (S28) and possible recombination among the clone sequences investigated after (S3). Sequences were deposited on Genbank, with accession numbers AY262406-AY262685.

Phylogenetic analysis

Phylogenetic trees were estimated using the Neighbor-joining option in PAUP* (version 4B10), with uncorrected P distances, and base frequencies empirically set. Bootstrap values were based on 1000 replicates. It is important to note that the taxa identified in Copenhagen and Oxford (Table 1) had not been previously present in each laboratory respectively, with the exception of bison (Oxford) and reindeer (Copenhagen), removing the possibility of intra-laboratory contamination.

Bootstrap test

The similarity between sequences obtained from two samples was measured using a bootstrap test. The total number of sequences pooled from both samples (N_j), the proportion of the j th sequence in the pool (P_j) and the number of sequences that occur in both samples (K), were calculated. Two new samples were drawn probabilistically from the pooled sequences according to the proportions P_j , 1000 times. The number of sequences shared by the two simulated samples (K_j) was calculated for each of these 1000 parametric replicates. The proportion of calculated K_j 's that were smaller than the K in the original data set were determined. A 5%-significance level test, Bonferroni corrected, was applied to this one-tailed p-value. Eight out of 16 tests were accepted (Table S3).

Taxonomic diversity

Three families (Moraceae, Rhamnaceae and Flacourtiaceae), each represented by one clone sequence in the Pleistocene samples (Table 2), have not been reported from current Arctic environments. None of the taxa are obvious contaminants and have not previously been investigated in either the Copenhagen or Oxford laboratories. It is probable that this discrepancy stems from differences between genetic and morphological based systematic classifications. For example, the genus *Flacourtia* is listed as part of the family Flacourtiaceae in GenBank, but has recently been relocated to Salicaceae (a common family in the Arctic) based on genetic analyses (S29). Rhamnaceae is currently found in southern Alaska and temperate regions of North America, along with Moraceae (S30). Therefore, it seems quite possible that these families were part of the Pleistocene Beringian vegetation.

DNA damage

Damage artefacts in ancient DNA are commonly biased towards CG→TA transitions rather than AT→GC transitions, probably as a result of a higher rate of hydrolytic deamination of cytosine (and its homolog 5-methyl cytosine) to uracil and thymine, than the deamination of adenine to hypoxanthine (S4-S6, S31, S32). For the sediment DNA sequences it is difficult to determine whether substitutions in clone sequences might be caused by DNA from multiple individuals, or by miscoding lesions in the ancient DNA. The permafrost sequences do not show typical damage related biases (a total of 14 CG→TA and 42 AT→GC substitutions), while only 3 of 11 substitutions in the *cyt b* sequences causes amino acid changes. In contrast, the moa control region sequences

appear to show patterns consistent with ancient DNA damage (a total of 29 CG→TA and 24 AT→GC) (Tables S4). The moa 12S data contains many substitutions that are only observed in one clone sequence, and which have not been observed in moas or other ratites. Consequently, the data suggest the temperate moa sequences have undergone considerably more damage than the permafrost sequences. To minimise sequence variation caused by miscoding lesions in future studies, it may be appropriate to include treatment with Uracil-N-Glycosylase (*S5*, *S6*).

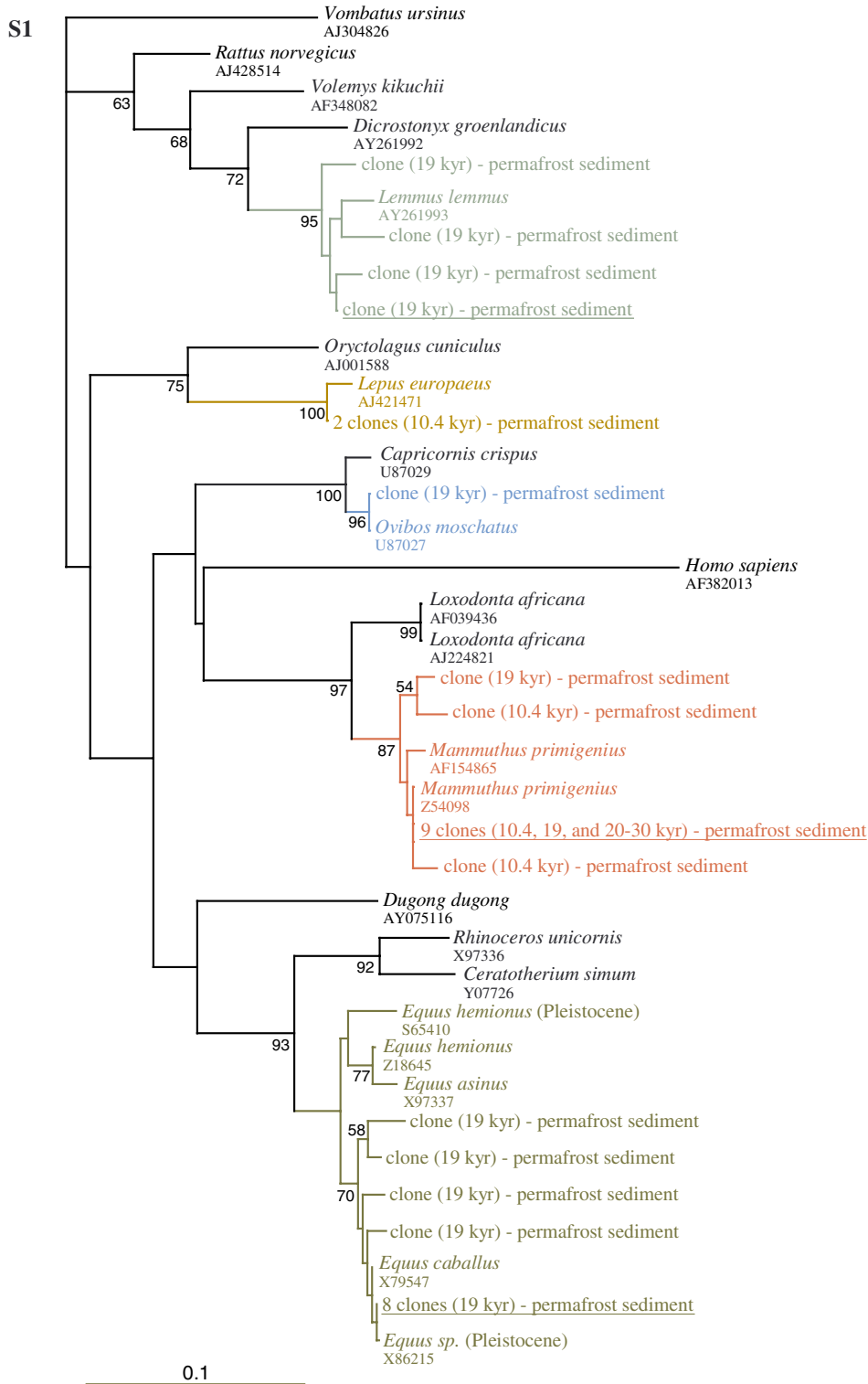


Fig. S1. Neighbor-joining phylogenetic tree based on mtDNA 16S sequences (88-95bp) from permafrost cores (clones, Access. #AY261956-AY261986) and Genbank sequences

(italics, with accession numbers). Coloured clades include clone sequences and their highest matches in GenBank BLAST searches. A marsupial outgroup (*Vombatus ursinus*) was used to root the tree. The age of the samples is indicated after clone or taxon names, and underlined sequences were independently obtained in Oxford. The number of identical clones is indicated, although only one sequence is represented in the analysis. Bootstrap values are based on 1000 replications.

S2

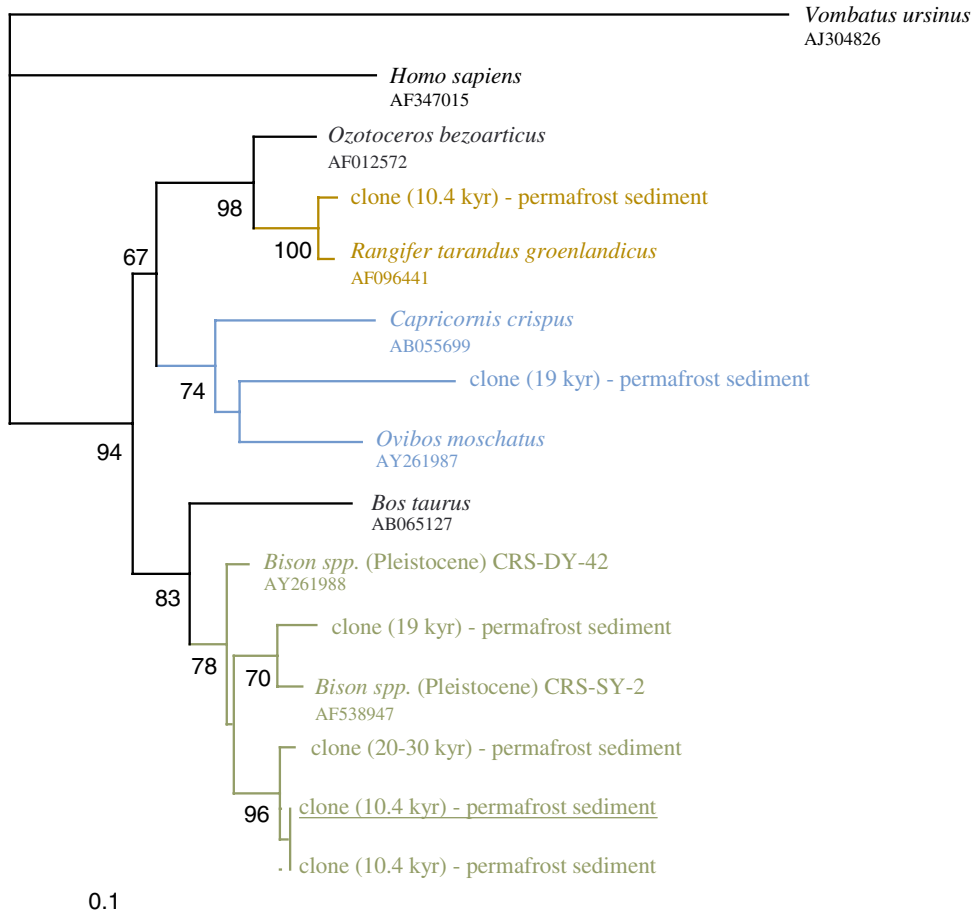
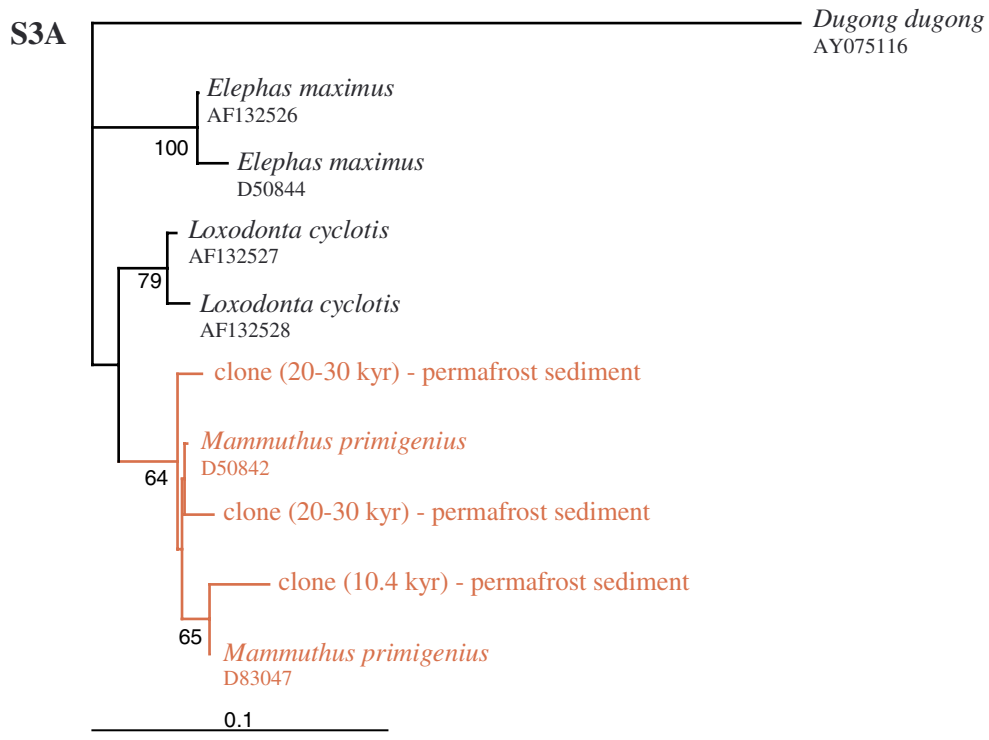


Fig. S2. Neighbor-joining phylogenetic tree based on control mtDNA region sequences (124-129bp, clones Access. # AY261950-AY261955), with description as for Figure S1.



S3B

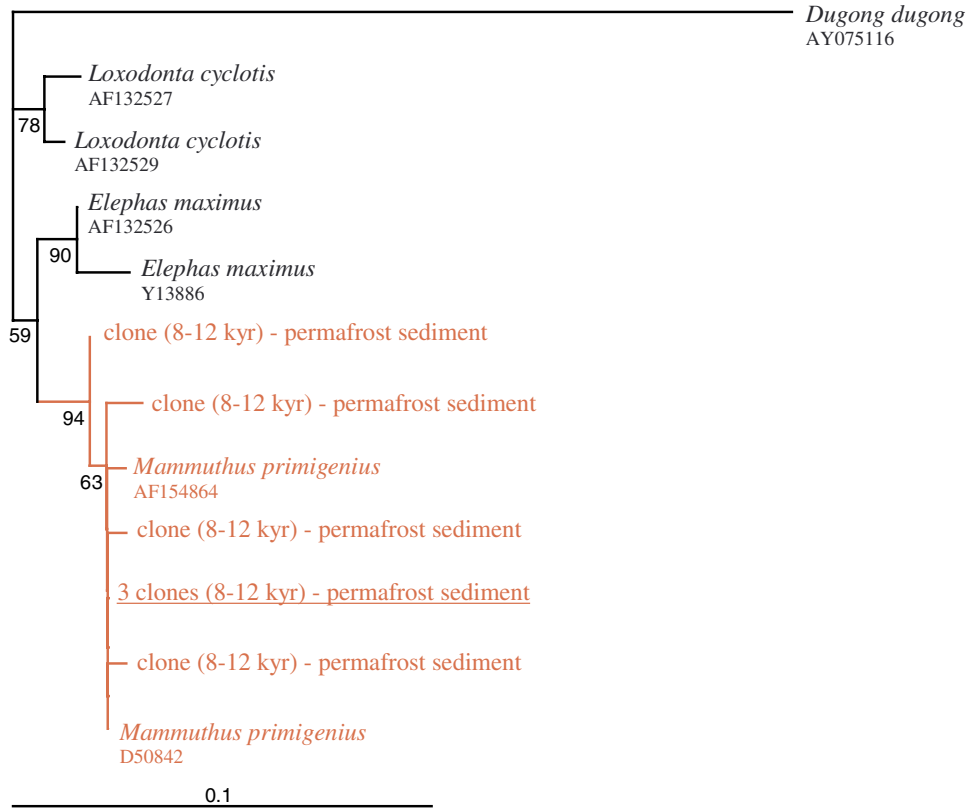


Fig S3. Neighbor-joining phylogenetic trees based on mtDNA *cyt b* sequences (A, 98bp, clones Access. # AY261900-AY261902 and B, 229bp, clones Access. # AY261903-AY261909), with description as for Figure S1. An outgroup (*Dugong dugong*) was used to root the tree.

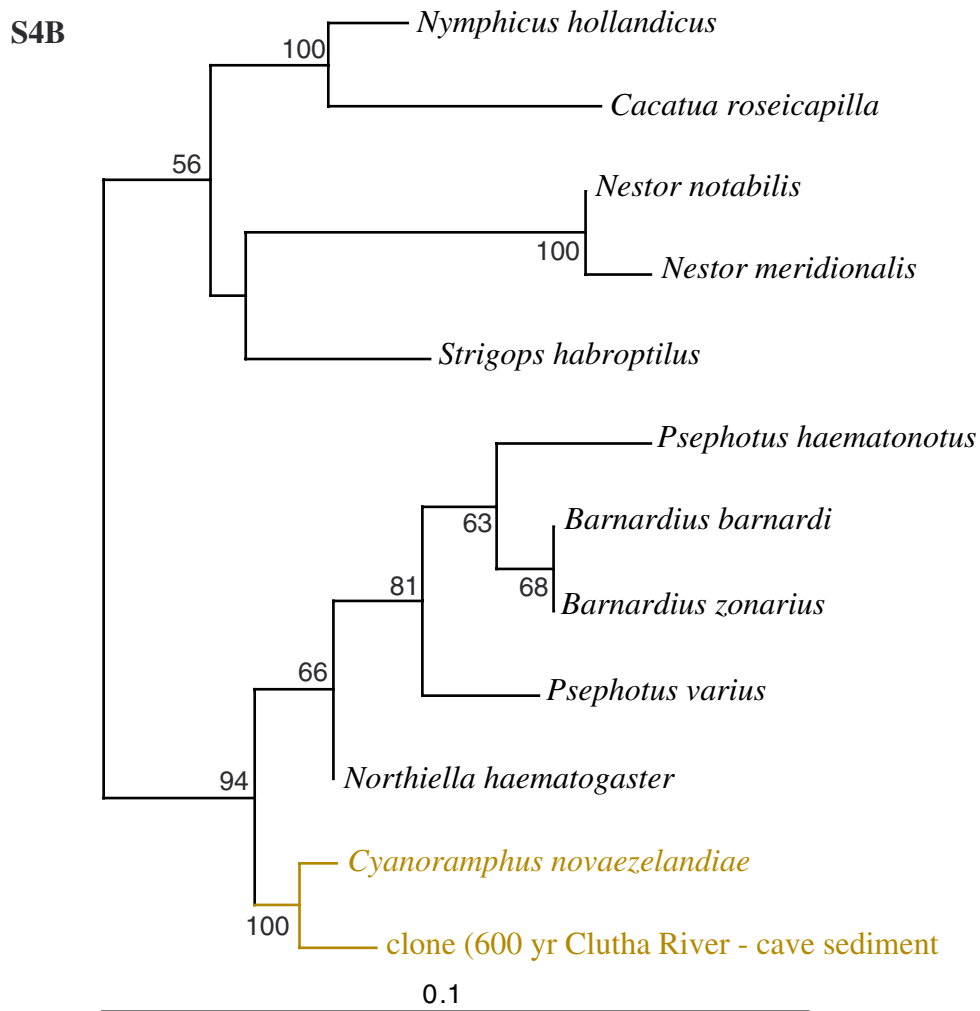


Fig S4. Neighbor-joining phylogenetic trees based on control mtDNA region sequences from New Zealand. (A, 202-203 bp and B, 234bp), with description as for Figure S1. (A) Moa clone sequences (Access. # AY261923-AY261949) obtained from sedimentary samples from Clutha River (cave sediment) and Tokerau Beach (inside moa bone) while (B) shows a parakeet clone sequence (Access. # AY261910) from Clutha River (cave sediment). The clone sequences are compared to (A) moa sequences obtained from bones and (B) sequences obtained from contemporary parrot sequences (S33).

Table S1. Radiocarbon dates. **A.** AMS radiocarbon determinations for the permafrost samples. **B.** AMS radiocarbon determinations for a feather from the Zealand Clutha River cave sediment. Age, delta 14C, DELTA 14C and absolute percent Modern as defined by (S34).

A

Context	Radiocarbon age yr B.P.	Pretreated fraction	$\delta^{13}\text{C}$ (‰)	Calibrated age range cal BC (one s.d)	Calibrated age range cal BC (two s.d)
2/01/4.8	18980 ± 70	NaOH insoluble carbon (humin carbon)	-25.2	20929 – 20213 (1.00)	21316 – 19901 (1.00)
1/93/4.0	10425 ± 45	NaOH soluble fraction (humic acid)	-26.5	10681-10508 (0.571) 10473-10346 (0.255) 10280-10182 (0.172)	10858-10757 (0.071) 10732-10114 (0.885) 10078 – 10009 (0.044)

B

Context	Radiocarbon age yr B.P.	delta 13C	delta 14C	DELTA 14C	% modern	The calibrated age (smoothing parameter :1)	
						2 sigma	1 sigma
R28143/1a	624±50	-23.47	-77.7±5.8	-80.6±5.8	91.94	1282-1418 AD 668-532 BP (98.1% of area)	1293-1401 AD 657-549 BP (90.1% of area)

Table S2. PCR. Primer names and sequences are shown, along with references, annealing temperatures and approximate product length.

Name	Sequence	Reference	Annealing temp. (°C)	Product length (bp)
<i>rbcL</i> h1aF	5'-GGCAGCATTCCGAGTAACTCCTC	(S35)	45	130
<i>rbcL</i> h2aR	5'-CGTCCTTTGTAACGATCAAG			
16Smam1	5'-CGGTTGGGGTGACCTCGGA	(S36)	60	120
16Smam2	5'-GCTGTTATCCCTAGGGTAACT			
Cb-EHL	5'-TCAGTAGACAAAGCAACCTTAAAT	(S37)	53	140
Cb-EHH	5'-CTGAGTCTGAAGTGAGGCC			
Cb-EA	5'-CCATCCAACATATCAACATGATGAA		55	270
Cb-ED	5'-GGTATTTCAAGTTTCCGAGTAT			
BisCR-16633f	5'-GCCCCATGCATATAAGCAAG	B. Shapiro (unpub)	56	150
BisCR-16810r	5'-GCCTAGCGGGTTGCTGGTTTCACGC			
Moa262F	5'-GCGAAGACTGACTAGAAGC	(S10)	55	245
Moa441R	5'-CGCATACCGGGTCTGTTTATGC			
12SAFH1753	5'-CTGGGATTAGATACCCCACTAT		60	280
12SHRb1985	5'-CCTTGACCTGTCTTGTTAGC			

Table S3. Results of bootstrap tests for chloroplast clone sequences independently obtained in Copenhagen (Cph) and Oxford and for chloroplast clone sequences from samples covering the same or different ages. The H_0 is that the two samples are identical. At a significance level of 0.05, the Bonferroni corrected level is 0.003125. The H_0 is rejected (-) if the probability is less than the corrected level.

Comparisons	Permafrost Sample	Rank	Prob.	Bonf acc/rej
Modern vers. 19 kyr	Borepit 1/02/0.5 vers. 2/01/4.8	1	0.0000	-
Modern vers. 10.4 kyr	Borepit 1/02/0.5 vers. 1/93/4.0	2	0.0000	-
Modern vers. 20-30 kyr	Borepit 1/02/0.5 vers. 7/90/1.6	3	0.0000	-
Modern vers. 300-400 kyr	Borepit 1/02/0.5 vers. (3/01/20.7 and 4/01/9.2)	4	0.0000	-
19 kyr vers. 300-400 kyr	2/01/4.8 vers. (3/01/20.7 and 4/01/9.2)	5	0.0000	-
10.4 kyr vers. 300-400 kyr	1/93/4.0 vers. (3/01/20.7 and 4/01/9.2)	6	0.0000	-
20-30 kyr vers. 300-400 kyr	7/90/1.6 vers. (3/01/20.7 and 4/01/9.2)	7	0.0000	-
19 kyr vers. 10.4 kyr	2/01/4.8 vers. 1/93/4.0	8	0.0020	-
Cph vers. Oxford repl.300-400 kyr	3/01/20.7	9	0.0210	+
10.4 kyr vers. 20-30 kyr	1/93/4.0 vers. 7/90/1.6	10	0.0400	+
300-400 kyr vers. 300-400 kyr	4/01/9.2 vers. 3/01/20.7	11	0.3330	+
Cph vers. Oxford repl. 300-400 kyr	4/01/9.2	12	0.4927	+
19 kyr vers. 20-30 kyr	2/01/4.8 vers. 7/90/1.6	13	0.7131	+
Cph vers. Oxford repl. 10.4 kyr	1/93/4.0	14	0.7401	+
Cph vers. Oxford repl. 20-30 kyr	7/90/1.6	15	0.7921	+
Cph vers. Oxford repl. 19 kyr	2/01/4.8	16	0.8497	+

Tables S4. Sequence differences from the highest match in GenBank BLAST searches.

Sample descriptions are given in Table 1.

Mammuthus primigenius. Kolyma lowland and Laptev Sea (Siberia), permafrost, 16S rRNA, 13 clones, 92-93 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events. Substitution summary: 4AT→GC, 1AT→TA. Positions are given according to GenBank sequence Z54098.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			4579
			3770
<i>M. primigenius</i> 2/01/4.8	9.7 kyr BP, Siberia 19 kyr	Access. # Z54098 3	TTAT
		1
1/93/4.0	10.4 kyr	6	...C
		1
		1	C.TC
		1	.C..
7/90/1.6	20-30 kyr	1

Mammuthus primigenius. Laptev Sea (Siberia), permafrost, Cytochrome *b*, 7 clones, 229 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above. The underlined G indicates a sequence substitution at the first position of a codon,

resulting in an amino acid change (N→D). The remaining substitutions are silent (third codon position). Substitution summary: 4AT→GC, 1CG→TA.

Positions are given according to GenBank sequence D50842.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			22222
			22359
			28737
<i>M. primigenius</i> 2/01/4.8	Pleistocene	Access. # D50842	CTTAA
	19 kyr	3
	19 kyr	1	. <u>C</u> . . .
	19 kyr	1	. . <u>C</u> . .
	19 kyr	1	. . . <u>G</u> .
	19 kyr	1	T . . . <u>G</u>

Mammuthus primigenius. Kolyma lowland (Siberia), permafrost, Cytochrome *b*, 3 clones, 98 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above. The underlined C and G indicate sequence substitutions at the first position of a codon, resulting in amino acid changes (F→L and M→V). The remaining substitutions are silent (third codon position). Substitution summary: 4AT→GC (positions that are variable in GenBank sequences are excluded. Positions are given according to GenBank sequence D50842.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			5555
			3568
			2855
<i>M. primigenius</i>	Pleistocene	Access. # D50842	TAAA
<i>M. primigenius</i>	Pleistocene	Access. # D83047

1/93/4.0	10.4 kyr	1	CG..
7/90/1.6	20-30 kyr	1	...G
7/90/1.6	20-30 kyr	1	..G.

Equus spp. Laptev Sea (Siberia), permafrost, 16S rRNA, 12 clones, 90 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and - represents sequence missing data. Substitution summary: 3AT→GC, 1AT→TA, 1AT→CG.

Positions are given according to GenBank sequence X79547.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			22222222
			22222333
			89999223
			90126698
<i>E. caballus</i>	Modern	Access. # X79547	GAACAAAA
<i>Equus sp.</i>	Pleistocene	Access. # X86215	----....
2/01/4.8	19 kyr	8
		1CG..
		1T..
		1G
		1G.

Rangifer tarandus. Kolyma lowland (Siberia), permafrost, d-loop, 1 clone and 124 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above. Substitution summary: 1AT→GC

(positions that are variable in GenBank sequences are excluded). Positions are given according to GenBank sequence AF096441.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			34 54 58
<i>Rangifer tarandus groenlandicus</i> 1/93/4.0	Modern 10.4 kyr	Access. # AF096441 1	GT AC

Bison spp. Laptev Sea and Kolyma lowland (Siberia), permafrost, d-loop, 4 clones and 125 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and - represents missing data. Substitution summary: 10AT→GC, 3CG→TA (positions that are variable in GenBank sequences are excluded). Positions are given according to *Bos taurus* GenBank sequence NC_001567.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			111111111111 666666666666 00000001111 45566781177 90126690315
<i>Bison spp.</i> CRS-DY-42	Pleistocene	Access. # AY261988	TCTAAACCTT-
<i>Bison spp.</i> CRS-SY-2	>55600	Access. # AF538947	.TC....TC.G
2/01/4.8	19 kyr	1	.TCGGG.T...
1/93/4.0	10.4 kyr	1	C....GTT..G
1/93/4.0	10.4 kyr	1	C....GTT..G
7/90/1.6	20-30 kyr	1	C....GTT.CG

Lemmus spp. Laptev Sea (Siberia), permafrost, 16S rRNA, 4 clones and 88-90 bp.

Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events.

Substitution summary: 3AT→GC, 3CG→TA, 3AT→TA, 1AT→CG, 4AT→~.

Positions are given according to *Volemys kikuchii* GenBank sequence AF348082.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			2222222
			2223333
			6681112
			2881489
<i>Lemmus lemmus</i>	Modern	Access. # AY261993	AAAGAAT
2/01/4.8	19 kyr	1	. . GA . . ~
2/01/4.8	19 kyr	1	. G . . GT .
2/01/4.8	19 kyr	1	~C . A . T~
2/01/4.8	19 kyr	1	. . . A . T~

Lepus spp. Kolyma lowland (Siberia), permafrost, 16S rRNA, 1 clones and 95

bp. Only sequence positions differing from the closest Genbank match are

shown. Dots represent identity to the sequence given above and ~

insertion/deletion events. Substitution summary: 1CG→TA, 1GC→~, 1~→CG,

1AT→~. Positions are given according to GenBank sequence AJ421471.

Samples (Core, Year & Depth) / Taxa	Age	# of identical clones/sample	Sequence positions
			2222
			2233
			8913
			6768
<i>Lepus europaeus</i>	Modern	Access. # AJ421471	TC~C
1/93/4.0	10.4 kyr	1	~TG~

Unidentified bovid. Laptev Sea (Siberia), permafrost, d-loop, 1 clones and 129 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events. Substitution summary: 7CG→TA, 13AT→GC, 1AT→CG, 1CG→GC, 1GC→~. Positions are given according to *Capricornis crispus* GenBank sequence AB055699.

Samples/ Taxa	Age	# of identical clones/sample	Sequence positions
			444444444444444444445555555 333344444456777790022234 23461456884034913403729
<i>Ovibos moschatus</i> 2/01/4.8	Modern 19 kyr	Access. # AY261987 1	CATTTGTATTTTACGCGCGTTCT TGGCCCCGCCCCGTATAT~CCTC

Megalapteryx didinus. Cave sediment (New Zealand), 15 clones, d-loop, 202-203 bp.

Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events.

Substitution summary: 13CG→TA, 11AT→GC, 1CG→GC, 1AT→CG, 1~→AT.

Positions are given according to *Emeus crassus* GenBank sequence AY016015.

Clone / Taxa	Age	# of identical clones/sample	Sequence positions
			44444444445555566 5555677991256701 2679023079816406
DM400 Cromwell	>10 kyr	Access. # AY261991	CAACCC~TTATCCTCT
Clutha river	1-3 kyr	6
Clutha river	1-3 kyr	1	T.GT.....CTTC..

Clutha river	1-3 kyr	1	T...T.....TTC..
Clutha river	1-3 kyr	1	.G.....
Clutha river	1-3 kyr	1T.....TCT.
Clutha river	1-3 kyr	1T.....C.C
Clutha river	1-3 kyr	1GAC.....
Clutha river	1-3 kyr	1C.....
Clutha river	1-3 kyr	1C.....
Clutha river	1-3 kyr	1T.C..

Pachyornis elephantopus. Cave sediment (New Zealand), 2 clones, d-loop, 204 bp.

Only sequence positions differing from the closest Genbank match are shown. Dots

represent identity to the sequence given above. Substitution summary: 2CG→TA,

1AT→GC (positions that are variable in GenBank sequences are excluded). Positions

are given according to *Emeus crassus* GenBank sequence AY016015.

Clone / Taxa	Age	# of identical clones/sample	Sequence positions
			56
			70
			71
381A, Cromwell	>10 kyr	Access. # AY261990	CT
Clutha river	1-3 kyr	1	T.
Clutha river	1-3 kyr	1	TC

Euryapteryx curtus. Tokerau beach (New Zealand), Coastal sand inside bone, 10 clones, d-loop, 202-

203 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent

identity to the sequence given above and ~ insertion/deletion events. Substitution summary:

14CG→TA, 12AT→GC, 2AT→TA, 4AT→CG, 1CG→AT, 1AT→~, 4~→CG, 1~→AT. Positions are given according to *Emeus crassus* GenBank sequence AY016015.

Clone / Taxa	Age	# of identical clones/sample	Sequence positions
			4444444444445555555556666666 566677778880112334560034455 068947893678595466562958956
MONZ S25832	>10 kyr	Access. # AY261989	~CTACCCCT~CTATTATCTAAAAT~
Tokerau beach	1-3 kyr	1A.A.....C...
Tokerau beach	1-3 kyr	1	...TT..TT..C.....G....C..C.
Tokerau beach	1-3 kyr	1	.T.....C.....T.G.C~.C
Tokerau beach	1-3 kyr	1TTT.G.....A.....C.
Tokerau beach	1-3 kyr	1G.C.....
Tokerau beach	1-3 kyr	1	C.C.....C.....C.
Tokerau beach	1-3 kyr	1T..TT.....C.....
Tokerau beach	1-3 kyr	1	C.....
Tokerau beach	1-3 kyr	1TTT.....
Tokerau beach	1-3 kyr	1C.....

Megalapteryx didinus. Cave sediment (New Zealand), 11 clones, 12S, 228-230 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events. Substitution summary: 11CG→TA, 15AT→GC, 2AT→CG, 12AT→TA, 2CG→GC, 2GC→~, 24~→AT, 15~→CG. Positions are given according to *Emeus crassus* GenBank sequence AY016015.

Clone / Taxa	Age	# of identical clones/sample	Sequence positions
			00000000000000000000000000000000 22222222222222222222222222222222 000001111111111111111222222222 3556900112566666888922333444 2461566470901669456517222236
<i>Megalapteryx didinus</i>	3.5 kyr	Access. # X67634	ACCAT~GC~CC~C~TCT~CCCA~~~CAA
Clutha river	1-3 kyr	1	G.T..A....T...A.C.....ACA...
Clutha river	1-3 kyr	1	.T.....GT.T..A.CC....ACA...

Clutha river	1-3 kyr	1	..T.....~....A.C.....ACAT..
Clutha river	1-3 kyr	1	...C.GA.....TGA.C.....ACA...
Clutha river	1-3 kyr	1	...G...G.....A.C..T..ACA.GT
Clutha river	1-3 kyr	1G.....A.C.....ACA...
Clutha river	1-3 kyr	1T.....A.C.....ACA...
Clutha river	1-3 kyr	1A.C.....GACA...
Clutha river	1-3 kyr	1ATC.....ACA...
Clutha river	1-3 kyr	1A.C.G.~.ACA...
Clutha river	1-3 kyr	1A.C.....ACA...

Cyanoramphus spp. Cave sediment (New Zealand), 2 clones, 12S, 234 bp. Only sequence positions differing from the closest Genbank match are shown. Dots represent identity to the sequence given above and ~ insertion/deletion events. Last 17 bp of the clone sequences are not shown do to lack of reference sequence for those positions. Substitution summary: 1AT→GC, 1CG→AT, 4~→AT, 4~→GC. Positions are given according to *Northellia haematogaster* GenBank sequence U88014.

Clone / Taxa	Age	# of identical clones/sample	Sequence positions
			001111
			223899
			781578
<i>Cyanoramphus novaezeelandiae</i>	Modern	Oxford unpublished data	~~C~A~
Clutha river	1-3 kyr	1	TGAC.T
Clutha river	1-3 kyr	1	CA.CGT

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