1. DNA Sequencing Replication

Independent replication of the ancient DNA sequences was done at the Australian Centre for Ancient DNA (ACAD) at the University of Adelaide. Ancient DNA was extracted from two Holocene samples using a Qiagen Blood & Tissue Kit (Valencia, California) and a modified protocol with added EDTA and Proteinase K [85]. PCR amplifications were carried out in 25 μL volumes, using 1× PCR buffer, 2.5 mM of $MgSO_4$, 1 mg/mL of rabbit serum albumin (RSA), 0.2 μM of each primer, 0.25 mM of dNTPs, 1 U of Platinum HiFiTaq (Invitrogen) and 2 μL of ancient DNA extract. The PCR was started with an initial denaturation at 94 °C for 2 min, followed by 50 cycles of denaturation at 94 °C for 15 s, annealing at 52 °C for 30 s, and extension at 68 °C for 30 s. The last step was a 10 min final extension period at 68 °C. PCR products were visualized under UV light on a 3.5% agarose gel stained with ethidium bromide. Successful amplifications were purified using Ampure (Agencourt) according to the manufacturer's instructions. Both DNA strands were sequenced directly using Big Dye chemistry and an ABI 3130XL Genetic Analyzer (Applied Biosystems). For both specimens, a total number of 488 bp of the cyt*b* gene, 374 bp of the 12s rRNA gene, and 469/ 470 bp of the D-loop were independently replicated at the Australian Centre for Ancient DNA (indicated by * in Table S1).

2. Authenticity of DNA Sequences

The following points support the authenticity of the newly obtained Holocene giant panda sequences: (a) DNA extractions and pre-PCR procedures were carried out in an isolated ancient DNA laboratory, PCR amplifications and post-PCR analyses were carried out in another physically separated laboratory; (b) both extraction and PCR blanks were conducted throughout the study and the results were consistently negative; thus there was no contamination from the reagents or environment; (c) fragments were amplified in several overlapping PCRs from multiple extractions, amplifications and multiple colonies were sequenced for each product. No mosaic haplotypes occurred in the two sequences, which suggests there was no cross contamination; (d) no modern giant panda samples have been studied in either of the both ancient DNA laboratories; (e) partial sequences have been successfully repeated at Australian Centre for Ancient DNA in Adelaide, Australia; (f) the two ancient haplotypes have never before been observed.

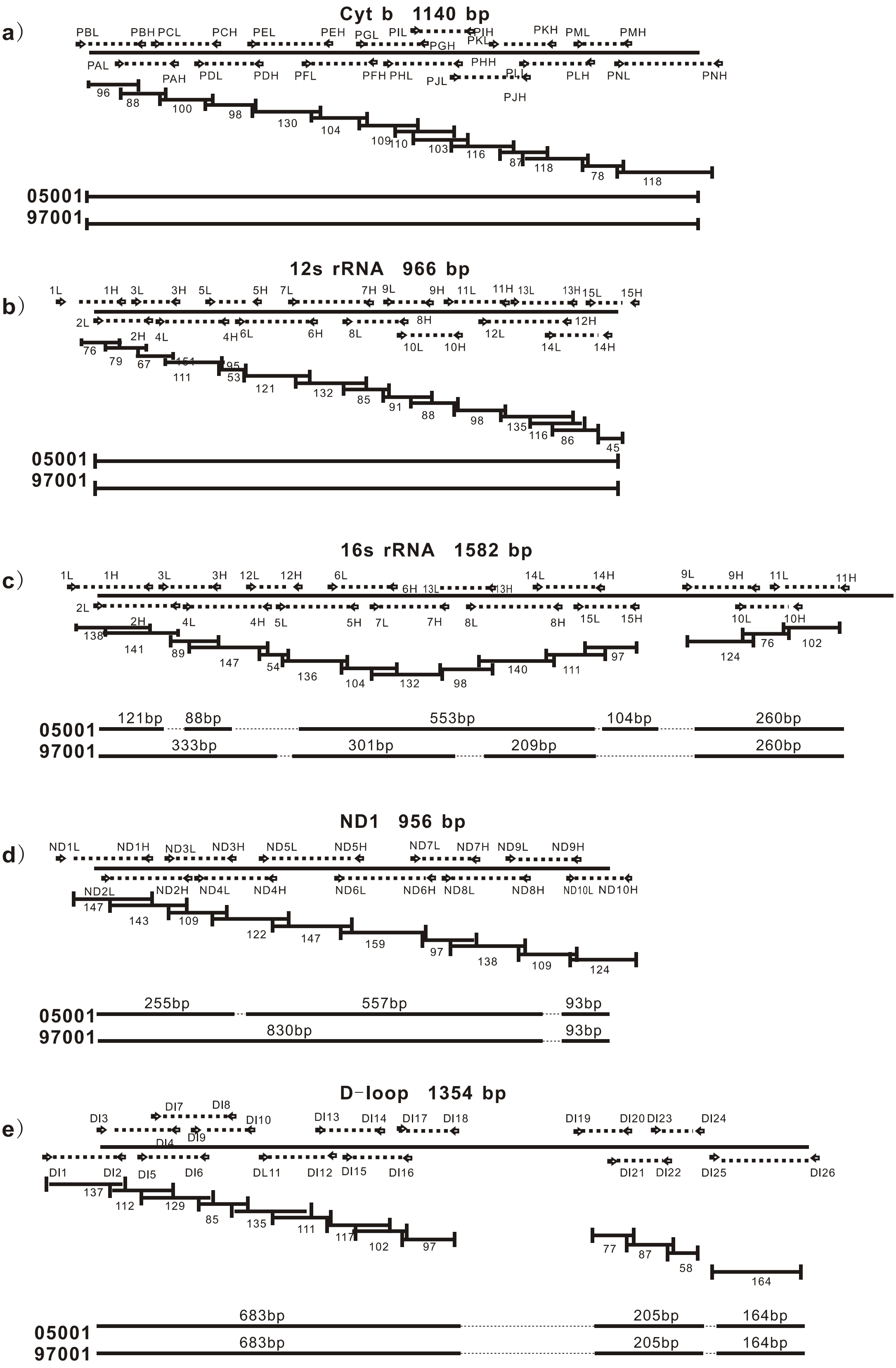

Figure S1. Schematic view of the complete or partial mitochondrial genes for the Holocene giant panda specimens using overlapping PCR fragments. (**a**) cyt*b*; (**b**) 12s rRNA; (**c**) 16s rRNA; (**d**) *ND1*; (**e**) D-loop. Numbers below fragments show length of the amplification products without primers.

```
                   10        20        30        40        50        60        70
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
FM177761   ACNTCTGCCGCCCGCTTTGCCTTCTTCAACTTCCTACGGCCAGCATAAGACCCTCCTAAGCACACTGAACT
05001      .T.........T......A...C...T.....T...T..........G............T...T......
97001      CT.CTC.TTA..TATC..A....TC...G.C...C...A.TGA......GTT.C.T...A.GT.T.....C
GP1        ...................T........................G..G....T.......T....N.N...
GP2        ...................................................................N...
GP3        ...................T........................G..G............T......N...
GP4        ................C..........G.T.....G..................T.C..........N...
GP5        ....................................T......A...G............T......N...
GP6        ................C..........G.T.....G..................T.C..........N...
GP7        ................C..........G.T.....G...T..............T.C........N.N...
GP8        ...................T........................G..G............T......N...
GP10       ....................................T......A...G............T.....NN...
GP12       ....................................T......A...G.........N..T......N...
GP13       ...................T........................G..G............T....N.N...
GP14       .................C.....T.....................C..A................CNN...
GP15       ......A..........C.....T.....................C..A................C.N...
GP16       ...................T........................G..G..........T.T......N...
GP17       ...................T........................G..G....T....N..T....N.N...
GP18       ...................T........................G..C............T......N...
GP19       ...................T........................G..C............T....N.N...
GP22       ...................................................................N...
GP23       .................C.....T.........T...........C..A................C.N...
GP24       ...................T........................G..G.........N..T....N.N...
GP25       ..A..............C.....T.....................C..A................C.N...
GP26       ...................T........................G..G............T..N...N...
GP27       .................C.....T.........T...........C..A................C.N...
GP28       .................C.....T.....................C..A................C.N...
GP29       ...................................................................N...
GP30       ...................T........................G..G............T......N...
GP31       ...................T.................A......G..G............T......N...
GP33       ..A.......A......C.....T.....................C..A................CN....
GP35       ...................T...........C............G..G.........N..T..........
GP36       ...................T.....N..................G..G............T..........
GP37       ...................T........................G..G............T....N.N...
GP38       ...................T........................G..G............T......N...
GP39       ...................T.................A......G..G............T.....NN...
GP51       ...................T........................G..G.........N..T......N...
GP52       ...............................................G.........N........NN...
GP53       ..............................................GG.........N..T......N...
GP54       ...................T........................G..G.........N..T......N...
GP57       ..A..............C.....T.....................C..A................C..N..
GP58       .................C...A.T.....................C..A................C.N...
GP60       ...................T.................A......G..G............T......N...
GP61       .................................................................N.N...
GP64       ...................T........................G..G............T......N...
GP65       ...............................................G.................NNN...
GP66       ...................T........................G..G....T.......T....N.N...
GP67       ...............................................G.........N.........N...
GP68       ...................T........................G..G....T....N..T....N.N.N.
GP70       ................C..........G.T.....G..................T.C.........NN...
GP71       ...................T........................G..G....T....N..T....NNN...
GP72       ..................................................................NN...
AM711896   .................C..T..T.....................C..A................C.....
EF212882   ...................T........................G..G....T....G..T..........
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Figure S2. Variable nucleotide positions in the 4054 bp data set of 12s, 16s, cyt*b*, and *ND1* fragments from 54 giant panda individuals.

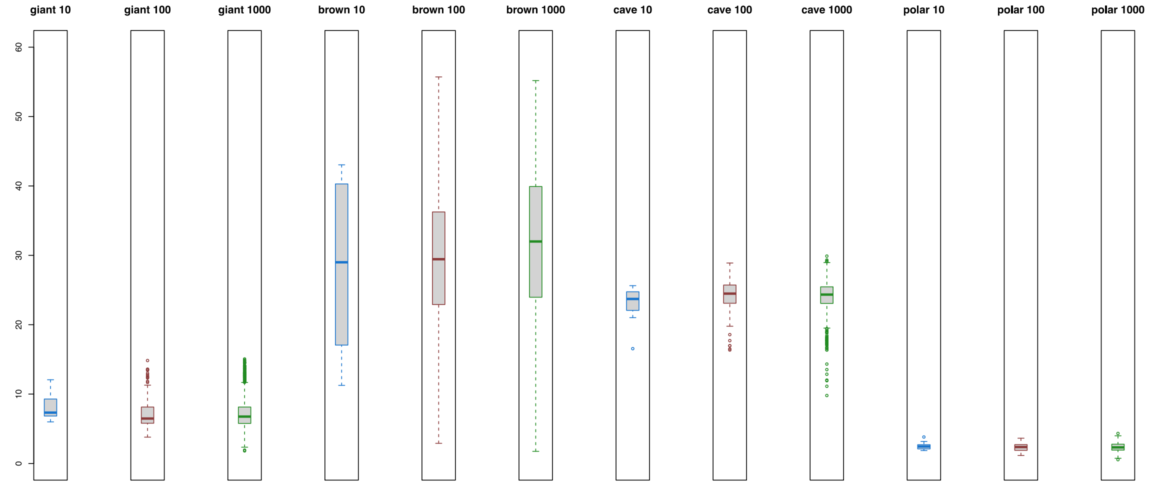

Figure S3. Genetic diversity distribution of the four subsampled species when random subsampling and pairwise identity computation have been performed 10, 100, and 1000 times, respectively.

Table S1. PCR primers for giant panda mitochondrial genes.

Gene Name	Primer Name	Primer Sequence (5′-3′)	Amplicon Size	Multiplex Set
Cyt*b*	PAL PAH	ATTGACCTTCCAACACCATC GTGTGCGACTGATGAAAAGGCT	150 bp	1
	PBL PBH	TCAACTATAAGAACCCCA CCTAACAGGGACCCAAAG	132 bp	2
	PCL PCH	TCCCTGTTAGGAGTGTGTCT ATCGGATAAATCACCCATA	139 bp	1
	PDL * PDH *	GCTACAGCCTTTTCATCAGTC GATCCATAGTATAAGCCTCGC	140 bp	
	PEL * PEH *	TATCTGCCTATTTATACACG AATGACGGTTGCTCCT	166 bp	2
	PFL * PFH *	GGGTATGTACTACCTTGAG CGGTTAGTGTTGCTTTATCT	143 bp	1
	PGL * PGH *	GGGGTTTCTCCGTAGATAAAGC ATCTGGGTCAGATGGAATTCCG	152 bp	2
	PHL * PHH *	CTCAGCACTAGCAATAGTCC AGGCGAGGACAAGAAATAGG	149 bp	1
	PIL PIH	ATAACCCCTCCGGAATTCCATC TCAGGGTCTCCTAACAGGTCT	145 bp	2
	PJL PJH	AACCCTGGCTTTATTCTCAC GCACCCCTCCTAGTTTATTA	155 bp	1
	PKL PKH	GCCTACGCTATCCTGCGATCT AGGTCGGAATATCATGCTTCGT	129 bp	2
	PLL PLH	GGGGTGCTAGCTCTAATCTTC CTGGCTGTCCTCCAATTCATGT	160 bp	1
	PML PMH	TCCGACCTCTAAGTCAATGC GAGGCCAATTGCCCAATA	139 bp	2
	PNL PNH	GAATTGGAGGACAGCCAGTA TGTTGCTCCTTCCTTGAGTC	219 bp	1
12s rRNA	12s-1L 12s-1H	AGGTACTGAAAATGCCTAGATGG GCATTTTCACTGGAGCGTGGAGA	120 bp	2

	12s-2L *	GGTTTGGTCCTAGCCTTCCT	118 bp	1
	12s-2H *	GTGCTTGATACCCGCTCCTT		
	12s-3L *	ATGTAAGTCTCCACGCTCCA	103 bp	2
	12s-3H *	AGCAAGGCGTTGTGAGC		
	12s-4L *	AGGAGCGGGTATCAAGCACA	151 bp	1
	12s-4H *	GTGGCTGGCACGAGATTTAC		
	12s-5L *	CGACTAAGCTATGTTAATACTAGGG	95 bp	1
	12s-5H *	CGCTTTACGCCGTAGGC		
	12s-6L *	CCGCGGTCATACGATTAACTCGAGT	171 bp	2
	12s-6H *	GGCCCTAGCTATCGTGTAATCAGAA		
	12s-7L	GGCGTAAAGCGTGTAAAAGA	172 bp	1
	12s-7H	TTAGAGCTAGGCATAGTGGG		
	12s-8L	TCTGATTACACGATAGCTAGGGCCC	135 bp	2
	12s-8H	CCAAGTCCTTTGAGTTTTAAGCTGT		
	12s-9L	ACTAAACAAAACTATTCGCCAGAG	135 bp	1
	12s-9H	TTAGCAAGGGATGGTGAGGT		
	12s-10L	AACAGCTTAAAACTCAAAGGACTTG	136 bp	2
	12s-10H	AAGGGTTTGCTGAAGATGGCGGT		
	12s-11L	GATAGACCTCACCATCCCTTGC	139 bp	1
	12s-11H	CCATTTCTTCCCAGCCCATA		
	12s-12L	CATAAAAAAGTTAGGTCAAGGTGTA	185 bp	2
	12s-12H	GGGTGACGGGCGGTGTGTGCGTGCT		
	12s-13L	TGGGCTGGGAAGAAATGGGCTAC	139 bp	1
	12s-13H	AGGAGGGTGACGGGCGGTGT		
	12s-14L	TTGAACAGGGCAATGAAGCACG	127 bp	2
	12s-14H	CCAGTATGCTTACCTTGTTA		
	12s-15L	GCAAAGCATAAGAGGAGACAAGTCG	95 bp	1
	12s15H	AAGCCAGACGCTTTGTTTAAGCTAC		
16s rRNA	16s-1L	GGCTTACATCCAGAAGATTTCATTA	185 bp	2
	16s-1H	CTTTCATCATTCCCTTGCGGTA		
	16s-2L	CAAAGCTAGCCCAAGCAACAATGAC	189 bp	1
	16s-2H	ATACAAAAGGTAGAAGGGGCAATC		
	16s-3L	CTATAGAGAGAGTACCGCAAGGGAA	136 bp	2
	16s-3H	TCGGGGGACTTAGCTTAAGTTCT		
	16s-4L	ATTGCCCCTTCTACCTTT	181 bp	1
	16s-4H	CACCAGGCTCGTTAGGC		
	16s-12L	AGTGAGAAGATCCATAGGTAGAGGT	103 bp	2
	16s-12H	AGTTTTGTAGGCAGGTTTAAAGTCG		
	16s-5L	CGAGCCTGGTGATAGCTGGTTGCC	182 bp	1
	16s-5H	GCCTCTAGGCCTACTATGGCTTATA		
	16s-6L	TACAGCTTTTTAGAGTTAAGGATAC	153 bp	2
	16s-6H	CATTAGGAGTTGATTAAATATTCTT		
	16s-7L	GGCCTAGAGGCAGCCATCAATTAAG	179 bp	1
	16s-7H	TGCTGTTATAAGCTTATGCATGGGA		
	16s-13L	CTCCTAATGTATTACTGGGTCAATC	146 bp	2
	16s-13H	CATTAGGTTATTTCTATGTTGTTGT		
	16s-8L	CAGCAACGGATAACCACTGATAG	187 bp	1
	16s-8H	AATACTGGAAATGCTGGAGGTGATG		
	16s-14L	GGCAAATACAAACCCCGCCTGT	157 bp	2
	16s-14H	TCATACAAGTCCTTATTTAGAGAAC		
	16s-15L	TATTCTGACCGTGCAAAGGTAGCAT	146 bp	1

	16s-15H	TCGTCTTATTGTCTTATCCCCGCCT		
ND1	ND1L	GTGTCCAGAGGTTCAATTCC	187 bp	2
	ND1H	GCATCTGCGATTGGTTGTAG		
	ND2L	CAGCTTCGAAAAGGCCCAAA	183 bp	1
	ND2H	CGGTATTGGTAAGGGGACTCA		
	ND3L	GCCCCTATTCTAGCCTTAACAC	150 bp	2
	ND3H	TGAGGCTCATCCAGATCAGA		
	ND4L	ACCATGTGAGTCCCCTTACCAA	175 bp	1
	ND4H	ATGAGATCGTTTGAGCTACGGC		
	ND5L	GATGAGCCTCAAACTCAAAA	187 bp	2
	ND5H	CTAGTGGTCATGCAGGGAAG		
	ND6L	CGCTATCCACGCTAATCA	194 bp	1
	ND6H	TAGGAAGAACAGGGCAAA		
	ND7L	CTCTGGGTTTAATGTCGA	132 bp	2
	ND7H	GGTATGTAGGGGCTGTGA		
	ND8L	TAGCAGAATATGCCAACATC	175 bp	1
	ND8H	TCGTATCGGAATCGAGGA		
	ND9L	TTTCTATGAATCCGAGCA	146 bp	2
	ND9H	GTTTGAGGTGGAATGCTTGC		
	ND10L	CGACCAACTAATACACCTA	161 bp	1
	ND10H	CCCCTATGGCTTACTCTA		
D-loop	DL-1	GGAAGGAGCAACAACCCCACTACCA	138 bp	2
	DL-2	GAAAACATACTATGATGGCACAGAG		
	DL-3	TCCACCTCTCATTTTATTCACTTCA	160 bp	1
	DL-4	GGGGCACGCCATTAATGCACGATA		
	DL-5	GTATGTTTTCATACATCCTCCCT	176 bp	2
	DL-6	CGCTTGAAATAAGTTCTTGTAATG		
	DL-7 *	TGTGCTTGGCTTTACATGAGG	126 bp	1
	DL-8 *	CTCGCAAGGATTGCTGGTTT		
	DL-9 *	TATTTCAAGCGATAGTCTATGAGC	177 bp	2
	DL-10 *	TGGCCCTGAGGTAAGAAC		
	DL-11 *	TGCGAGTACGTGTACCTCTTC	153 bp	1
	DL-12 *	ATTAGTCCATCGAGATGTCCC		
	DL-13 *	CAACTCAATCCTACTAACCCTTCA	161 bp	2
	DL-14 *	ACTGCGACGAGACCTTTACG		
	DL-15 *	TTTTAGGGGGGGAACTTGCTATGAC	150 bp	1
	DL-16 *	TAGTGTTATGTCCTGTGACCATT		
	DL-17 *	GTCTCGTCGCAGTCAAATCA	137 bp	2
	DL-18 *	CGTACACGTAACGCCAGTCC		
	DL-19	TAAGTTAGCTTAGACAAACCCCCC	126 bp	1
	DL-20	GGTGTAAGTGACTTCATGTTTGCGC		
	DL-21	TGGATGTCCTGCCAAACC	126 bp	2
	DL-22	AGTCCCATGTCCGTAGTTGTT		
	DL-23	ATAATTAAGCTAACCCCCCAGCCAA	107 bp	1
	DL-24	CAATGCTGGGGAGACACAGTAAAA		
	DL-25	TGTGTCTCCCCAGCATTGATTTTTT	150 bp	2
	DL-26	GGTAGCGTGACCCATCTAGGCATTT		

* Primer pairs have been replicated at ACAD.

Table S2. Sequence information used in this study.

No. in This Study	Species	GenBank Accession No.	Length	Reference
97001	*Ailuropoda melanoleuca*	KP306773 (cyt*b*)	1140 bp	This study
		KP306768 (12s rRNA)	966 bp	This study
		KP306766 (16s rRNA)	1103 bp	This study
		KP306770 (ND1)	923 bp	This study
		KF386262 (D-loop)	1052 bp	This study
05001	*Ailuropoda melanoleuca*	KP306772 (cyt*b*)	1140 bp	This study
		KP306769 (12s rRNA)	966 bp	This study
		KP306767 (16s rRNA)	1126 bp	This study
		KP306771 (ND1)	905 bp	This study
		KF386263 (D-loop)	1052 bp	This study
AM711896	*Ailuropoda melanoleuca*	AM711896	16,846 bp	[47]
EF212882	*Ailuropoda melanoleuca*	EF212882	16,805 bp	[38]
FM177761	*Ailuropoda melanoleuca*	FM177761	16,796 bp	[48]
NC009492	*Ailuropoda melanoleuca*	NC_009492	16,805 bp	[38]
EF100819-57	*Ailuropoda melanoleuca*	EF100819	655 bp	[13]
SRA053353	*Ailuropoda melanoleuca*	SRA053353	2.25 Gb	[26]
KX641289-337	*Ursus spelaeus/Ursus arctos*	KX641289-337	~16,300 bp	[50]
AP012559-97	*Ursus arctos/Ursus maritimus*	AP012559-97	~16,900 bp	[86]
NC_003426-28	*Ursus americanus Ursus arctos Ursus/maritimus*	NC_003426	~16,840 bp	[48]
FM177762-65	*Ursus thibetanus/Melursus ursinus/Tremarctos ornatus*	FM177762-65	~16,800 bp	[48]

Table S3. Haplotypes identification of 54 giant panda individuals used for phylogenetic analyses in Figure 3.

Haplotype Nos.	Sample Nos. In Figure 3(b)
Hap_1	05001
Hap_2	97001
Hap_3	GP1, GP17, GP66, GP68, GP71, EF212882
Hap_4	GP2, GP22, GP29, GP61, GP72, FM177761
Hap_5	GP3, GP8, GP13, GP24, GP30, GP36, GP37, GP38, GP51, GP54, GP64
Hap_6	GP4, GP6, GP70
Hap_7	GP5, GP10, GP12
Hap_8	GP7
Hap_9	GP14, GP25, GP28, GP57
Hap_10	GP15
Hap_11	GP16
Hap_12	GP18, GP19
Hap_13	GP23, GP27
Hap_14	GP31, GP39, GP60
Hap_15	GP33
Hap_16	GP35
Hap_17	GP52, GP65, GP67
Hap_18	GP53
Hap_19	GP58
Hap_20	AM711896

Table S4. Optimal data partitions and substitution models selected by partitionfinder for the BEAST analyses.

Partition	Ursidae Model	Skyline Model Selected	Skyline Model Used *	Composition
p1	TrN + G	TrN	TrN	12s, 16s
p2	K80 + G	HKY	HKY	ND1_CP1, cytb_CP1
p3	HKY + I	K80+I	K80	ND1_CP2, cytb_CP2
p4	TrN + I	HKY	HKY	ND1_CP3, cytb_CP3
p5	-	HKY+I+G	HKY+G	D-loop

* For some partitions, individual parameters of the partitionfinder selected models failed to converge satisfactorily, and so the number of free parameters were reduced in order to achieve convergence.

Table S5. Optimal data partitions selected by partitionfinder for maximum likelihood phylogenetic analysis.

Partition	Model	Composition
p1	GTR + G	12s, 16s, ND1_CP1
p2	GTR + G	ND1_CP2, cytb_CP2
p3	GTR + G	ND1_CP3, cytb_CP1
p4	GTR + G	cytb_CP3

Table S6. Variable sites in the aligned 655 bp D-loop data set.

	42	**87**	**88**	**110**	**136**	**137**	**143**	**205**	**226**	**274**	**278**	**370**	**371**	**402**	**472**	**530**	**617**	**628**
GH01	C	C	C	A	-	T	C	C	A	T	G	T	A	C	T	-	G	G
GH02	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	T	A	A
GH03	T	C	C	G	C	T	C	C	A	T	A	T	A	T	T	T	A	A
GH04	T	C	C	A	T	C	-	T	A	T	A	T	A	C	T	-	A	A
GH05	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH06	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH07	C	C	C	A	C	T	C	C	A	T	A	T	A	C	T	-	A	G
GH08	C	C	C	A	-	T	C	C	A	T	A	T	A	C	T	-	G	G
GH09	C	C	C	A	-	T	-	C	A	T	G	T	A	C	T	-	A	G
GH10	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH11	T	T	C	A	C	T	C	T	A	T	G	T	A	C	T	-	A	A
GH12	C	C	C	A	C	C	-	C	A	T	A	T	A	C	T	-	A	G
GH13	C	C	C	A	C	T	C	C	A	T	A	T	A	C	T	-	G	G
GH14	C	C	C	A	C	T	-	C	A	T	A	T	A	C	T	-	A	G
GH15	C	C	C	A	-	T	C	C	A	T	A	T	A	C	T	-	A	G
GH16	C	T	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH17	T	C	T	A	T	C	-	C	A	T	A	T	G	C	T	-	A	A
GH18	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH19	T	C	C	G	-	T	C	C	A	T	A	T	A	T	T	T	A	A
GH20	C	C	C	A	-	-	C	C	A	T	A	T	A	C	T	-	G	G
GH21	T	T	C	A	C	T	C	C	A	T	G	T	A	T	T	-	A	A
GH22	C	C	C	A	C	T	-	C	A	T	A	T	A	C	T	-	A	G
GH23	T	T	C	A	T	T	C	C	A	T	G	T	A	T	T	-	A	A
GH24	T	C	C	A	T	T	C	C	A	T	G	T	A	T	T	-	A	G
GH25	T	C	C	A	C	T	C	C	A	T	A	T	A	C	T	-	A	A
GH26	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	A
GH27	C	C	C	A	T	C	-	C	A	T	G	T	A	C	T	-	A	G
GH28	T	T	C	A	C	T	C	C	A	T	G	T	A	C	T	-	A	A
GH29	T	T	C	A	-	T	C	C	A	T	A	T	A	C	T	-	A	A
GH30	T	C	C	A	C	T	C	C	A	T	A	T	A	C	T	T	A	A
GH31	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	T	A	A

GH32	C	C	C	A	-	-	C	C	A	T	A	T	A	C	T	-	A	G
GH33	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH34	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH35	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	A
GH36	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	A
GH37	C	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	G
GH38	C	C	C	A	T	C	-	C	A	C	A	T	A	C	T	-	A	G
GH39	C	C	C	A	T	C	-	C	A	C	A	T	A	C	A	-	A	G
GH40	T	C	T	A	C	T	C	C	A	T	A	T	G	C	T	-	A	A
GH41	T	C	C	A	T	C	-	C	A	T	A	T	A	C	T	-	A	A
GH42	T	C	C	A	C	T	C	C	G	T	G	C	A	C	T	T	A	A

* Numbers in the first line indicate the nucleotide positon in 655 bp sequences.