Supplementary Information

Bears in a forest of gene trees: Phylogenetic inference is complicated by incomplete lineage sorting and gene flow

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Phylogenetic analyses of concatenated data

We conducted phylogenetic analyses of concatenated autosomal data to highlight the extent of reduction in variation resulting from the concatenation procedure, and for comparison with the multi-locus species tree (fig. 2*A*) and phylogenetic trees from previous studies. Intraspecific and intra-individual polymorphisms were disregarded, because for concatenation, for each species all variation within and among individuals had to be collapsed into one single 50% majority-rule-consensus sequence. Unresolved sites with each variant occurring 50% were deleted from the alignments. Phylogenetic trees from concatenated nuclear data were calculated in MrBayes 3.2 (Ronquist et al. 2012) and in Treefinder version 2008 (Jobb et al. 2004). For Bayesian inferences in MrBayes we used one cold and three heated chains and ran the analyses for 10,000,000 Markov chain Monte Carlo generations sampling every 2,000th generation, with a burnin of 25%. We confirmed convergence in Tracer v1.5 (effective sampling size >200). Maximum likelihood analyses were performed in Treefinder with 10,000 bootstrap replicates. In Bayesian and maximum likelihood analyses of concatenated (1) Y-chromosomal, (2) autosomal, and (3) autosomal/Y-chromosomal markers combined, the American black bear was placed as sister-taxon to the brown and polar bear lineage with high statistical support, and the sun bear was sister-taxon to a clade including the sloth and Asian black bear (supplementary figure S2, Supplementary Material online). When analyzing the autosomal and Y-chromosomal data separately, support for the sun/sloth/Asian black bear clade was limited, but it was high in the combined analyses. Statistical support for Ursinae forming a monophyletic group and for the spectacled bear as sister-taxon to all ursines was high for all three datasets (Y-chromosomal, autosomal, autosomal/Y-chromosomal combined); the giant panda was the outgroup.

Supplementary tables

Supplementary table S1: Details of samples and sequences used in the study.

	Species name	Scientific name	Lab ID	Geographic origin	Sex	Accession numbers and/or source study		
						Autosomal markers	Y-chromosomal markers	Mitochondrial genomes
				14 autosomal introns and 5.9 kb Y-chromosomal sequence (present study)				
1	Giant panda	*Ailuropoda melanoleuca*	AmeC85	unknown	male	HG974607-HG974634	HG975027-HG975031	--
2	Giant panda	*Ailuropoda melanoleuca*	Amegenom	unknown	female	Giant panda genome (Li et al. 2010)	--	--
3	Spectacled bear	*Tremarctos ornatus*	TorCha	Zoo Basel, Switzerland; ISO Fdx 250229600006729	male	HG974803-HG974830	HG975052-HG975056; HG423284-HG423285 (Bidon et al. 2014)	--
4	Spectacled bear	*Tremarctos ornatus*	TorNob	Zoo Basel, Switzerland; ISO Fdx 968000002054943	male	HG974831-HG974858	HG975057-HG975061	--
5	Sloth bear	*Melursus ursinus*	MURL42	Sunset Zoo Manhattan, KS, USA; Studbook# 460	male	HG974719-HG974746	HG975042-HG975046	--
6	Sloth bear	*Melursus ursinus*	MURL43	Philadelphia Zoo, PA, USA	male	HG974747-HG974774	HG975047-HG975051	--
7	Sloth bear	*Melursus ursinus*	MURL44	India; Studbook# 442	female	HG974775-HG974802	--	--
8	Sun bear	*Helarctos malayanus*	HMAL45	Miami Metro Zoo, FL, USA; Studbook# 635	male	HG974635-HG974662	HG975032-HG975036	--
9	Sun bear	*Helarctos malayanus*	HMAL46	San Diego Zoo, CA, USA; Studbook# 617	male	HG974663-HG974690	HG975037-HG975041	--
10	Sun bear	*Helarctos malayanus*	HMAL47	St. Louis Zoo, MO, USA; Studbook# 644	female	HG974691-HG974718	--	--
11	Asian black bear	*Ursus thibethanus*	UTHL48	John Ball Zoo, MI, USA; Studbook# 401	male	HG974943-HG974970	HG975077-HG975081	--
12	Asian black bear	*Ursus thibethanus*	UTHL49	Southwick's Zoo, MA, USA	female	HG974971-HG974998	--	--
13	Asian black bear	*Ursus thibethanus*	UTHL50	Denver Zoo, CO, USA; Studbook# 585	female	HG974999-HG975026	--	--
14	American black bear	*Ursus americanus*	Uam1203	Yosemite NP, Mariposa, CA, USA	male	see Hailer et al. (2012)	--	--
15	American black bear	*Ursus americanus*	Uam13724	Wesley, Washington, ME, USA	female	see Hailer et al. (2012)	--	--
16	American black bear	*Ursus americanus*	Uam16103	Tanana Flats, AK, USA	female	see Hailer et al. (2012)	--	--

	Species name	Scientific name	Lab ID	Geographic origin	Sex	Accession numbers and/or source study		
						Autosomal markers	**Y-chromosomal markers**	**Mitochondrial genomes**
17	American black bear	*Ursus americanus*	Uam24064	Sixes, Curry, OR, USA	female	see Hailer et al. (2012)	--	--
18	American black bear	*Ursus americanus*	Uam6586	Garfield, CO, USA	female	see Hailer et al. (2012)	--	--
19	American black bear	*Ursus americanus*	Uam6616	Humboldt, CA, USA	female	see Hailer et al. (2012)	--	--
20	American black bear	*Ursus americanus*	UamC122	unknown	male	see Hailer et al. (2012)	--	--
21	American black bear	*Ursus americanus*	UamMTM33	MT, USA	male	--	HG975062-HG975066; HG423286-HG423287 (Bidon et al. 2014)	--
22	Brown bear	*Ursus arctos*	Uar001	Rumania	female	see Hailer et al. (2012)	--	--
23	Brown bear	*Ursus arctos*	UarKamK05	Kamchatka, Russia	male	HG974859-HG974886	--	--
24	Brown bear	*Ursus arctos*	Uar1254	Shoshone NF Park, WY, USA	female	see Hailer et al. (2012)	--	--
25	Brown bear	*Ursus arctos*	UarA9106	Admiralty Island, AK, USA	male	see Hailer et al. (2012)	--	--
26	Brown bear	*Ursus arctos*	UarBT1-8	Norway	male	see Hailer et al. (2012)	HG975067-HG975071; HG423290-HG423291 (Bidon et al. 2014)	--
27	Polar bear	*Ursus maritimus*	UmaB26	Turner Island, eastern Greenland	female	see Hailer et al. (2012)	--	--
28	Polar bear	*Ursus maritimus*	UmaB38	Savissivik, western Greenland	male	see Hailer et al. (2012)	--	--
29	Polar bear	*Ursus maritimus*	UmaAKL29	Chukchi Sea population, AK, USA	male	HG974887-HG974914	--	--
30	Polar bear	*Ursus maritimus*	UmaDSL57	Davis Strait population, Canada	male	HG974915-HG974942	--	--
31	Polar bear	*Ursus maritimus*	Uma009	Point Lay, AK, USA	male	see Hailer et al. (2012)	--	--
32	Polar bear	*Ursus maritimus*	UmaDSL51	Davis Strait population, Canada	male	--	HG975072-HG975076; HG423302-HG423303 (Bidon et al. 2014)	--

	Species name	Scientific name	Lab ID	Geographic origin	Sex	Accession numbers and/or source study		Mitochondrial genomes
						Autosomal markers	Y-chromosomal markers	
Autosomal and Y-chromosomal markers (previous studies)								
33	Giant panda	*Ailuropoda melanoleuca*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
34	Giant panda	*Ailuropoda melanoleuca*	--	unknown	unknown	--	see Nakagome et al. 2008	--
35	Spectacled bear	*Tremarctos ornatus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
36	Spectacled bear	*Tremarctos ornatus*	--	unknown	unknown	--	see Nakagome et al. 2008	--
37	Sloth bear	*Melursus ursinus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
38	Sloth bear	*Melursus ursinus*	--	unknown	unknown	--	see Nakagome et al. 2008	--
39	Sun bear	*Helarctos malayanus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
40	Sun bear	*Helarctos malayanus*	--	unknown	unknown	--	see Nakagome et al. 2008	--
41	Asian black bear	*Ursus thibethanus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
42	Asian black bear	*Ursus thibethanus*	--	unknown	unknown	--	see Nakagome et al. 2008	--
43	American black bear	*Ursus americanus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
44	American black bear	*Ursus americanus*	--	unknown	unknown	--	see Nakagome et al. 2008	--
45	Brown bear	*Ursus arctos*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
46	Brown bear	*Ursus arctos*	--	unknown	unknown	--	see Nakagome et al. 2008	--
47	Polar bear	*Ursus maritimus*	--	unknown	unknown	see Pagès et al. (2008)	see Pagès et al. (2008)	--
48	Polar bear	*Ursus maritimus*	--	unknown	unknown	--	see Nakagome et al. 2008	--

	Species name	Scientific name	Lab ID	Geographic origin	Sex	Accession numbers and/or source study		
						Autosomal markers	**Y-chromosomal markers**	**Mitochondrial genomes**
Mitochondrial genomes (previous studies)								
49	Giant panda	*Ailuropoda melanoleuca*	--	unknown	unknown	--	--	NC_009492 (Peng et al. 2007)
50	Spectacled bear	*Tremarctos ornatus*	--	unknown	unknown	--	--	NC_009969 (Yu et al. 2007)
51	Sloth bear	*Melursus ursinus*	--	unknown	unknown	--	--	NC_009970 (Yu et al. 2007)
52	Sun bear	*Helarctos malayanus*	--	unknown	unknown	--	--	NC_009968 (Yu et al. 2007)
53	Asian black bear	*Ursus thibethanus*	--	unknown	unknown	--	--	NC_009971 (Yu et al. 2007)
54	American black bear	*Ursus americanus*	--	unknown	unknown	--	--	NC_003426 (Delisle and Strobeck 2002)
55	Brown bear	*Ursus arctos*	--	unknown	unknown	--	--	NC_003427 (Delisle and Strobeck 2002)
56	Polar bear	*Ursus maritimus*	--	unknown	unknown	--	--	NC_003428 (Delisle and Strobeck 2002)

Supplementary table S2: Mean p-distances between species (number of differences/total length).

Species pairs	14 autosomal introns (present study)	14 autosomal introns – consensus (present study)	11 autosomal exon and intron markers (Pagès et al. 2008)
Giant Panda – Spectacled bear	0.033	0.032	0.022
Giant Panda – Sun bear	0.033	0.032	0.021
Giant Panda – Sloth bear	0.032	0.031	0.021
Giant Panda – Asian black bear	0.033	0.032	0.020
Giant Panda – American black bear	0.031	0.030	0.020
Giant Panda – Brown bear	0.032	0.031	0.021
Giant Panda – Polar bear	0.033	0.032	0.021
Spectacled – Sun bear	0.017	0.016	0.011
Spectacled – Sloth bear	0.017	0.016	0.012
Spectacled – Asian black bear	0.017	0.016	0.012
Spectacled – American black bear	0.016	0.015	0.011
Spectacled – Brown bear	0.017	0.015	0.013
Spectacled – Polar bear	0.017	0.016	0.013
Sun – Sloth bear	0.008	0.007	0.004
Sun – Asian black bear	0.009	0.007	0.004
Sun – American black bear	0.009	0.008	0.005
Sun – Brown bear	0.009	0.007	0.005
Sun – Polar bear	0.009	0.008	0.005
Sloth – Asian black bear	0.007	0.005	0.004
Sloth – American black bear	0.008	0.007	0.005
Sloth – Brown bear	0.009	0.007	0.005
Sloth – Polar bear	0.009	0.008	0.005
Asian black – American black bear	0.007	0.006	0.003
Asian black – Brown bear	0.008	0.006	0.003
Asian black – Polar bear	0.008	0.007	0.003
American black – Brown bear	0.007	0.006	0.003
American black – Polar bear	0.007	0.007	0.003
Brown – Polar bear	0.005	0.003	0.003

Calculations are based on (1) 14 autosomal introns (present study; 30 phased individuals), (2) 14 autosomal introns (present study; eight 50% majority-rule consensus individuals), and (3) 11 autosomal exon and intron markers from Pagès et al. (2008) (eight consensus individuals).

Supplementary table S3: Pairwise divergence statistics for 5.9 kb from the Y chromosome and at 14 autosomal introns.

Species pairs	Y chromosome	Autosomal introns					
	Mean distance	Mean distance	Fixed differences	Shared polymorphisms	Polymorphic in species 1, fixed in 2	Polymorphic in species 2, fixed in 1	Sum of polymorphic sites
Giant Panda – Spectacled bear	212	259.8	253	0	12	7	19
Giant Panda – Sun bear	215.5	266.5	253	0	12	24	36
Giant Panda – Sloth bear	207	258.5	251	0	12	10	22
Giant Panda – Asian black bear	205	262.8	235	0	12	56	68
Giant Panda – American black bear	208	250.4	232	0	12	34	46
Giant Panda – Brown bear	208	258	232	0	12	64	76
Giant Panda – Polar bear	209	262.7	255	0	12	13	25
Spectacled – Sun bear	115.5	136.6	124	0	7	24	31
Spectacled – Sloth bear	107	133.3	127	0	7	10	17
Spectacled – Asian black bear	108	134.1	108	0	7	56	63
Spectacled – American black bear	111	126	110	0	7	33	40
Spectacled – Brown bear	111	133.2	107	0	7	63	70
Spectacled – Polar bear	112	136.8	130	0	7	13	20
Sun – Sloth bear	34.5	62.3	49	0	24	10	34
Sun – Asian black bear	34.5	68.2	34	0	24	56	80
Sun – American black bear	40.5	73.6	52	0	24	34	58
Sun – Brown bear	40.5	69.6	36	1	23	63	87
Sun – Polar bear	41.5	70.1	57	1	23	12	36
Sloth – Asian black bear	24	55.1	32	1	9	55	65
Sloth – American black bear	32	63.3	47	0	10	34	44
Sloth – Brown bear	32	74.3	48	0	10	64	74
Sloth – Polar bear	33	73.5	66	0	10	13	23
Asian black – American black bear	30	58.1	23	4	52	30	86
Asian black – Brown bear	30	61.6	21	10	46	54	110
Asian black – Polar bear	31	65.7	38	0	56	13	69
American black – Brown bear	16	56.6	20	3	31	61	95
American black – Polar bear	17	59.6	42	0	34	13	47
Brown – Polar bear	13	38.9	14	1	63	12	76

Supplementary table S4: Haplotype sharing among bear species (fig. 1, supplementary figure S1).

	Giant panda	Spectacled bear	Sun bear	Sloth bear	Asian black bear	American black bear	Brown bear	Polar bear
Giant panda	---							
Spectacled bear	-	---						
Sun bear	-	-	---					
Sloth bear	-	-	2 loci, 2 haplotypes	---				
Asian black bear	-	-	2 loci, 2 haplotypes	-	---			
American black bear	-	-	-	-	2 loci, 2 haplotypes	---		
Brown bear	-	-	-	-	2 loci, 2 haplotypes	3 loci, 4 haplotypes	---	
Polar bear	-	-	2 loci, 2 haplotypes	-	-	1 locus, 1 haplotype	5 loci, 5 haplotypes	---

Supplementary table S5: Datasets analyzed in the present study.

Dataset	Sequence alignments	Alignment length [bp]
A. Concatenated alignments reconstructed for traditional phylogenetic analyses		
14 autosomal introns	14 autosomal introns (present study)	7,991
5.9 kb Y-chromosomal sequence	9 Y-chromosomal markers (present study)	5,907
15 nuclear markers	14 autosomal introns (present study) + 9 Y-chromosomal markers (present study)	13,898
9.7 kb Y-chromosomal sequence (total evidence)	9 Y-chromosomal markers (present study) + 2 Y-chromosomal markers from Nakagome et al. (2008) + 3 Y-chromosomal markers from Pagès et al. (2008)	9,794
Mitochondrial genomes	Protein-coding regions from the mitochondrial genomes (excluding ND6) (Jameson et al. 2003)	10,807
B. Alignments included in multi-locus species tree analyses (*BEAST) and population genetic analyses under the isolation-with-migration model (IMa2)		
14 autosomal introns	14 autosomal introns (present study)	7,991
14 autosomal introns (non-recombining)	Largest non-recombining sections from 14 autosomal introns (present study) as reconstructed in IMgc (Woerner et al. 2007)	5,127
15 nuclear markers	14 autosomal introns (present study) + 5.9 kb Y-chromosomal sequence (present study)	13,898
30 nuclear markers (total evidence)	14 autosomal introns (present study) + 9.7 kb Y-chromosomal sequence (total evidence) + 4 X chromosomal markers from Nakagome et al. (2008) + 11 autosomal markers from Pagès et al. (2008)	28,681

A. Concatenated alignments reconstructed for traditional phylogenetic analyses and for topology tests. For concatenation, one 50%-majority-rule-consensus individual was reconstructed per species from sequence data generated in the present study. B. Alignments included in multi-locus species tree analyses (*BEAST) and population genetic analyses under the isolation-with-migration model (IMa2).

Supplementary table S6: Results from approximately unbiased (AU) topology tests.

A. Mitochondrial genomes (protein-coding regions, excl. ND6).

Topologies	p-value AU test	LogL	ΔLogL
Top 5 topologies, ranked according to p-value			
(((((Uma,Uar),Mur),((Uam,Uth),Hma)),Tor,Ame);	0.75	-36097.13	0.00
(((Uma,Uar),(((Uam,Uth),Hma),Mur)),Tor,Ame);	0.65	-36097.02	-0.11
((((Uma,Uar),((Uam,Uth),Hma)),Mur),Tor,Ame); (Krause et al. 2008)	0.44	-36096.99	-0.14
(((((Uma,Uar),Mur),((Uam,Hma),Uth)),Tor,Ame);	0.26	-36101.45	4.32
(((((Uma,Uar),Mur),((Uam,Hma),Uth)),Tor,Ame);	0.21	-36101.57	4.44
Nuclear DNA topologies			
(((((Uma,Uar),Uam),(Uth,(Hma,Mur))),Tor,Ame); (fig. 2*A*)	0.00	-36131.84	34.71
(((((Uma,Uar),Uam),((Uth,Mur),Hma)),Tor,Ame); (fig. 2*B*, suppl. fig. S2)	0.00	-36131.86	34.73
((((((Uma,Uar),Uam),Uth),(Hma,Mur)),Tor,Ame); (Nakagome et al. 2008; Pagès et al. 2008)	0.00	-36131.79	34.66
(((((((Uma,Uar),Uam),Uth),Mur),Hma),Tor,Ame); (Pagès et al. 2008)	0.00	-36131.53	34.40
(((((((Uma,Uar),Uam),Uth),Hma),Mur),Tor,Ame); (Pagès et al. 2008)	0.00	-36128.41	31.28

B. 5.9 kb Y-chromosomal sequence (present study).

Topologies	p-value AU test	LogL	ΔLogL
Top 5 topologies, ranked according to p-value			
(((((Uma,Uar),Uam),((Uth,Mur),Hma)),Tor,Ame); (fig. 2*B*, suppl. fig. S2)	0.89	-10141.62	0.00
(((((Uma,Uar),Uam),(Uth,(Hma,Mur))),Tor,Ame); (fig. 2*A*)	0.71	-10142.80	1.18
((((((Uma,Uar),Uam),Uth),Hma),Mur),Tor,Ame); (Pagès et al. 2008)	0.44	-10144.61	2.99
((((((Uma,Uar),Uam),Uth),(Hma,Mur)),Tor,Ame); (Nakagome et al. 2008; Pagès et al. 2008)	0.34	-10144.77	3.15
((((((Uma,Uar),Uam),Hma),(Uth,Mur)),Tor,Ame);	0.30	-10143.67	2.05
Additional nuclear topologies			
((((((Uma,Uar),Uam),Uth),Mur),Hma),Tor,Ame); (Pagès et al. 2008)	0.00	-10144.6	2.98
mtDNA topologies			
(((((Uma,Uar),Mur),((Uam,Uth),Hma)),Tor,Ame);	0.00	-10214.81	73.19
(((Uma,Uar),(((Uam,Uth),Hma),Mur)),Tor,Ame);	0.00	-10213.06	71.44
((((Uma,Uar),((Uam,Uth),Hma)),Mur),Tor,Ame); (Krause et al. 2008)	0.00	-10214.85	73.23

C. 9.7 kb of Y-chromosomal sequence (total evidence): 5.9 kb Y-chromosomal sequence (present study) concatenated with five Y-linked markers from Pagès et al. (2008) and Nakagome et al. (2008).

Topologies	p-value AU test	LogL	ΔLogL
Top 5 topologies, ranked according to p-value			
(((((Uma,Uar),Uam),((Uth,Mur),Hma)),Tor,Ame); (fig. 2*B*, suppl. fig. S2)	0.92	-16672.99	0.00
((((((Uma,Uar),Uam),(Uth,Mur)),Hma),Tor,Ame);	0.75	-16674.60	1.61
(((((Uma,Uar),Uam),(Uth,(Hma,Mur))),Tor,Ame); (fig. 2*A*)	0.62	-16674.33	1.34
(((((((Uma,Uar),Uam),Uth),Mur),Hma),Tor,Ame); (Pagès et al. 2008)	0.32	-16675.57	2.58
(((((((Uma,Uar),Uam),Uth),Hma),Mur),Tor,Ame); (Pagès et al. 2008)	0.19	-16675.57	2.58
Additional nuclear topologies			
((((((Uma,Uar),Uam),Uth),(Hma,Mur)),Tor,Ame); (Nakagome et al. 2008; Pagès et al. 2008)	0.14	-16676.03	3.04
mtDNA topologies			
(((((Uma,Uar),Mur),((Uam,Uth),Hma)),Tor,Ame);	0.00	-16795.51	122.52
(((Uma,Uar),(((Uam,Uth),Hma),Mur)),Tor,Ame);	0.00	-16794.17	121.18
((((Uma,Uar),((Uam,Uth),Hma)),Mur),Tor,Ame); (Krause et al. 2008)	0.00	-16795.70	122.71

Ame: giant panda, Tor: spectacled bear, Hma: sun bear, Mur: sloth bear, Uth: Asian black bear,

Uam: American black bear, Uar: brown bear, Uma: polar bear.

Supplementary table S7: Genetic diversity within bear species based on 14 autosomal introns and 5.9 kb from the Y chromosome.

Species	n (total)	Autosomes			Y chromosome		
		n	S	π (x 10⁻³)	n	S	π (x 10⁻³)
Giant panda	2	2	12	1.4 ± 1.0	1	-	-
Spectacled bear	2	2	7	0.6 ± 0.5	2	0	0.0 ± 0.0
Sun bear	3	3	24	2.0 ± 1.2	2	1	0.2 ± 0.2
Sloth bear	3	3	10	1.1 ± 0.7	2	0	0.0 ± 0.0
Asian black bear	3	3	55	5.1 ± 3.0	1	-	-
American black bear	8	7	34	2.1 ± 1.1	1	-	-
Brown bear	5	5	64	4.4 ± 2.4	1	-	-
Polar bear	6	5	13	0.8 ± 0.5	1	-	-

n = number of analyzed individuals; S = number of segregating sites; π = Tamura-Nei-corrected nucleotide diversity (π ± S.D.). Note that for American black bears and polar bears, different individuals were sequenced for autosomal and Y-chromosomal markers (see supplementary table S1).

Supplementary table S8: Primers (in 5' to 3' orientation) and amplification conditions of 14 autosomal introns and nine Y-linked markers.

Marker	Forward primer	Reverse primer	gene (intron#)	size [bp]	T [°C]
1247	TATTGGTGGAGGCTTCACAG	AGACATCCAACAAAGGGCTG	*AZIN1*(6)	805	65-58[d]
2331	CCAGGATATTTTGAYGCAATC	CTCAGCTTTYGGTAGGCAAC	*LRGUK*(14)	674	63-56[d]
3471	AKACTGAGTCCCAGCAGCAG	CCRTTCTGGGAAACTTGCTC	*SPTBN1*(31)	712	67-60[d]
4464	TCCTTTCCCAGAGCAARAAG	TGGTCCTTGGCGAAGTTTAC	*ABCA1*(49)	738	58
4779	TTTGCAAATCTRAGAGCAGAG	CAGTTGCTGCTTAACTTGTTCC	*CCDC90B*(4)	708	63-56[d]
7545	GGGAAAGTTCCGGTTTTTG	TTTCTCAGACACCCTGTCCC	*GGA3*(3)	726	58
9072	TTTCATCGGTGTCATCATCG	TGTCATGAAGATGTCCTGGC	*SCN5A*(24)	694	65-58[d]
9205	CYAAATGTCGGAGTGCRGGG	CTTGGCAATAGCTTTGGCTG	*ATP12A*(12)	817	65-58[d]
11080	AAGGGCAAGCTGTTGTAAGR	TCAGCTTTRGTTCCATTTCC	*PREX2*(29)	753	67-60[d]
13102	ACACYGTGGGKTTATGGAGC	TCCACACAGATAGCCCAAGG	*TRAPPC10*(8)	690	65-58[d]
15923 [a]	CTGAGCCCAAGTTCGAGAAG [a]	GTGTAGTCCTCCAGGGAGATATAG [a]	*SPTA1*(51)	739	68-58[d]
17701 [b]	CTCAGTGGTAGCCAAGGACC	GCTGGAGTTGGAGGAATCAG	*IGSF22*(15)	692	67-60[d]
22245	TTCYTGGAAATTGACCCAAC	GGCTGAAGGACTCCTCRCTG	*SEL1L3*(20)	714	58
OSTA-5	TGMWGGYCATGGTGGAAGGCTTTG	AGATGCCRTCRGGGAYGAGRAACA	*OSTA*(5)	724	67-60[d]
318.2C_Ame [c]	AAGAACTGTATTCCATCTRTCCC	AGKAAATGTGAAAGTACTGGTTTAC	Y chr.[g]	979	69-62[d]
318.3C	CGACCTTGACCAACAAGAGG	GAGATGGTCTCTGCAAGATGG	Y chr.	1216	66-61[e]
318.3C_Ame [c]	CCCTRTGCCATCATAAATCCC	TTAAGGCTGTGTTTGAGTGCC	Y chr.	724	69-62[d]
318.7C	TCTTCGTCTTCATGCTGTGG	CCAGCTCCTTATATGCTGAACC	Y chr.	1095	68-58[f]
318.7C_Ame [c]	TTGGAGGAGTCAGCTGATGAG	TGTTGGTGTTTCAGTTGTATGTTG	Y chr.	766	69-62[d]
579.3C	TTAACTGCTCTGACCTTCATCG	GTGCACAGGCAAGTGTTAGG	Y chr.	1157	68-58[f]
579.3C_Ame [c]	AATGAACTGCTTGACCTTCG	TGATGGAGGAAATTGAGTGC	Y chr.	1174	68-61[d]
318.10B	TGCACAGTTCAATGGCTACAG	TCAGCAGACATTTTCTTGGAAC	Y chr.	529	66-61[b]
318.11C	GATGATGCATAAGCAATCCTTG	TGCAACCATAACTTGTTTACTTCC	Y chr.	1012	69-64[e]
389	ACCCACTGCTGTTCTGTATCC	CCAACAGTGTAGTGGTTGTGC	Y chr.	679	68-61[d]
322	GAGTAGAGCTGGTGCTTGTGAG	GAAGCAGAGCTCAAGTCTGAAG	Y chr.	821	70-63[d]
403	CACTCAGGAGAGCACAGGTC	TGTGTGTCGTAAGCAGAGGTC	Y chr.	796	69-62[d]

Markers are named consecutively based on our list of aligned giant panda and dog sequences, with the numbering reflecting their relative position along the dog chromosomes. Gene and intron numbers in the giant panda are given; size for nuclear markers denotes expected amplicon size in base pairs in the giant panda; and T is the annealing temperature used in PCR. Y-specific markers are indicated in the column "gene" and named based on the scaffold they were obtained from.

[a] The primers for this locus were newly designed compared to Hailer et al. (2012), to improve specificity.

[b] These sequences correct an error in the primer sequences given in Hailer et al. (2012).

[c] Panda-specific primers were designed, in case no PCR product was obtained in any of giant panda, spectacled bear, Asian black bear, sun bear or sloth bear.

[d] Touchdown PCR, during which the annealing temperature was lowered by 0.5°C in each of 14 cycles, followed by 26 normal cycles.

[e] Touchdown PCR, during which the annealing temperature was lowered by 0.5°C in each of 10 cycles, followed by 30 normal cycles.

[f] Touchdown PCR, during which the annealing temperature was lowered by 1.0°C in each of 10 cycles, followed by 30 normal cycles.

[g] This marker includes exon 4 of *Usp9Y*.

Supplementary figures
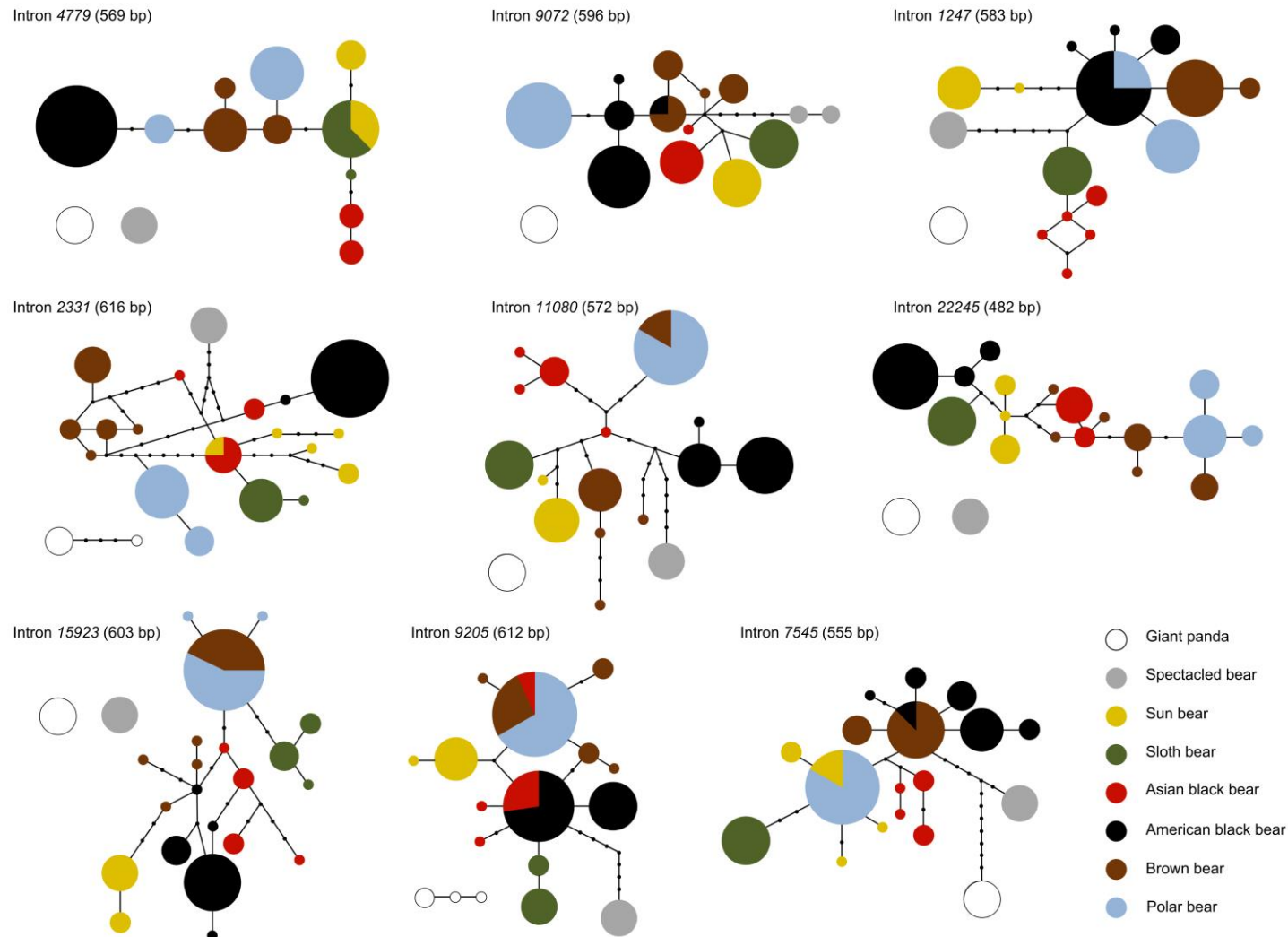
Supplementary figure S1: Statistical parsimony networks for nine autosomal intron markers in bears. Circle areas are proportional to haplotype frequencies and inferred intermediate states are shown as black dots. For some loci, spectacled bear and giant panda haplotypes were too divergent to be connected at the 95% credibility limit.
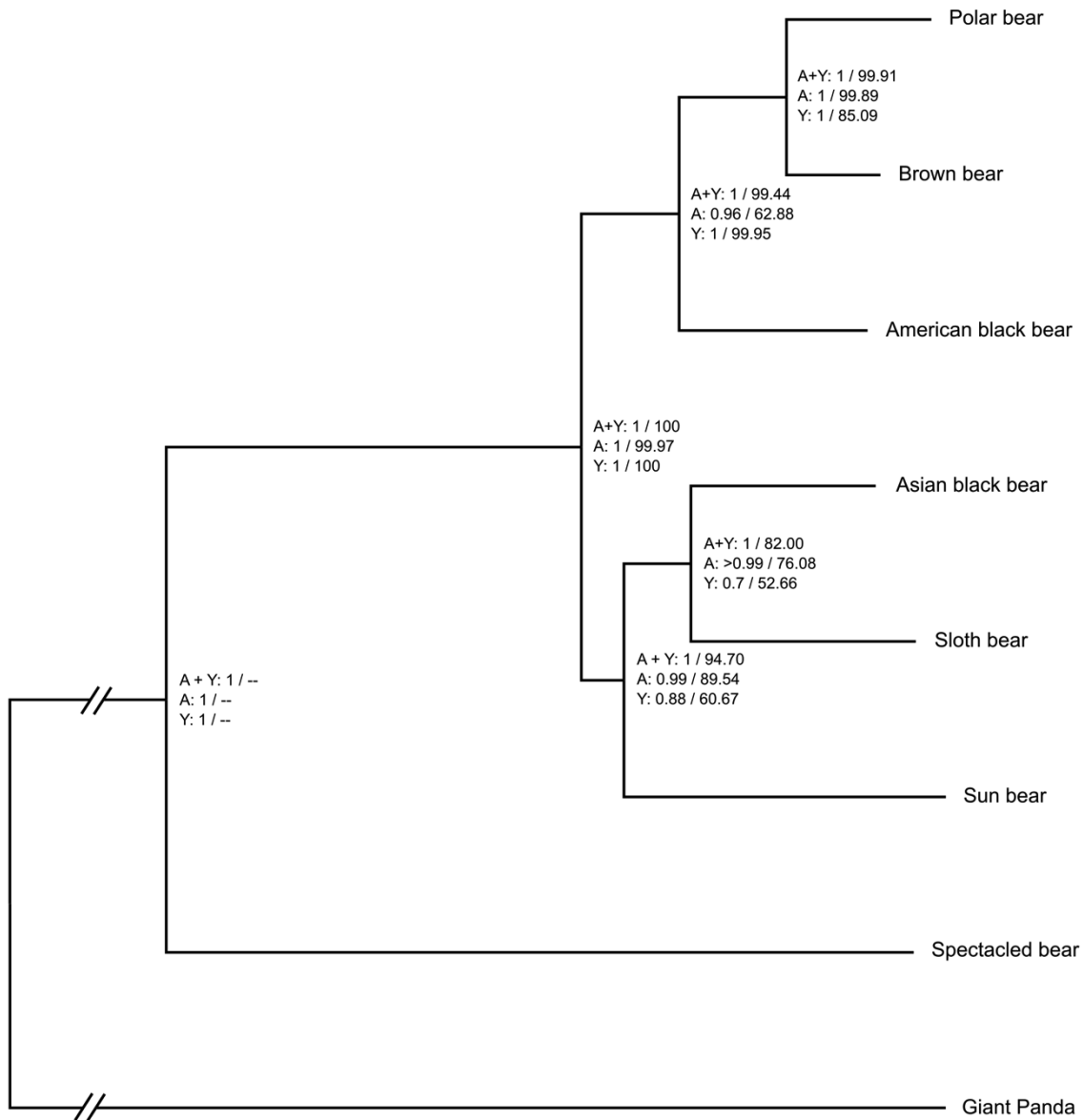
Supplementary figure S2: Phylogenetic tree of 14 concatenated autosomal introns and 5.9 kb Y-chromosomal sequence obtained from MrBayes. Numbers next to nodes denote branching support (first number: posterior probability values from MrBayes, second number: bootstrap values from maximum likelihood analyses in Treefinder), for three different datasets: (1) A+Y: 14 concatenated autosomal introns concatenated with nine Y-chromosomal markers, (2) A: 14 concatenated autosomal introns, and (3) Y: 5.9 kb Y-chromosomal sequence.
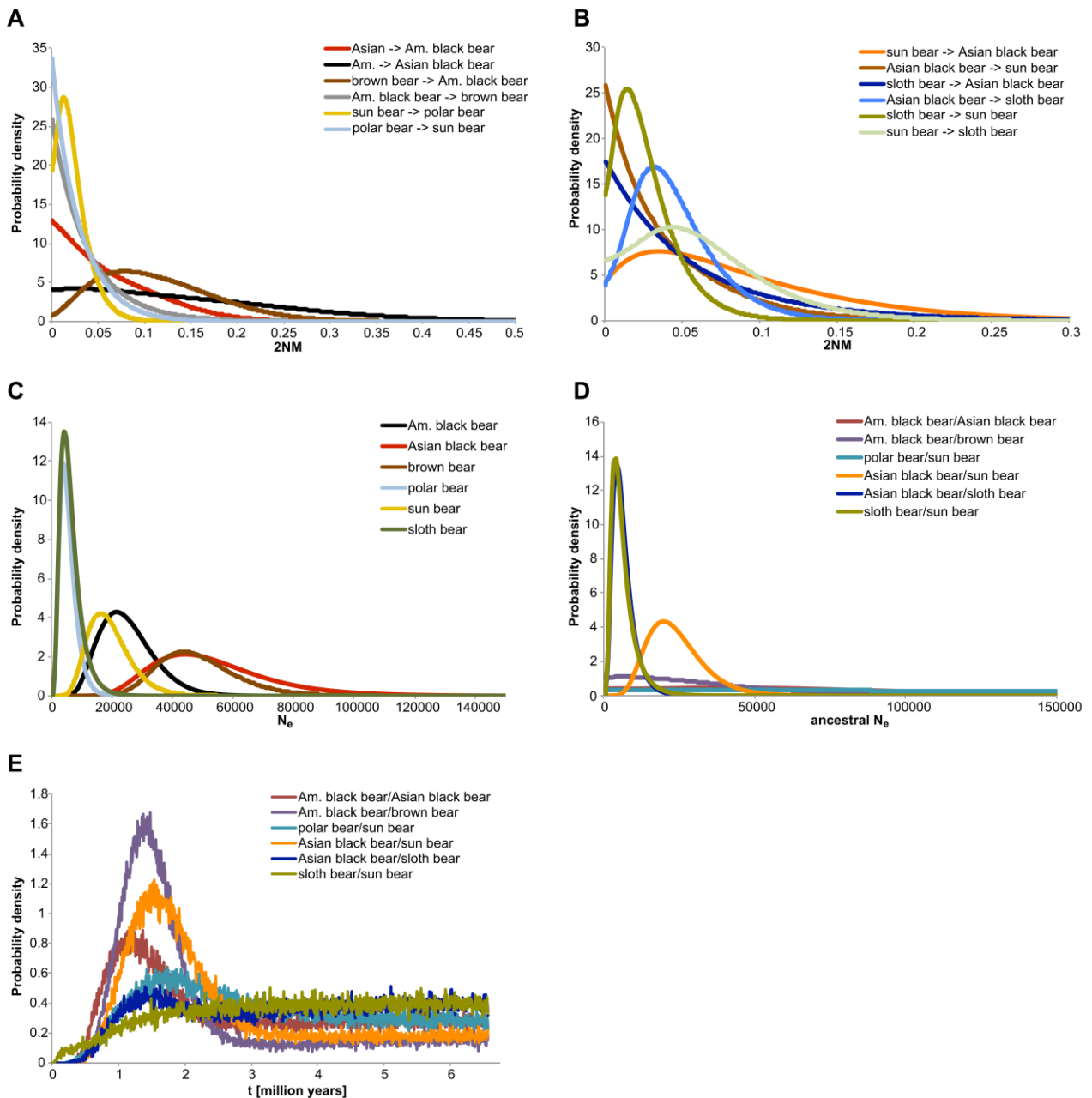
Supplementary figure S3: Posterior probability distributions for parameters in IMa2 pairwise comparison analyses. Curves are shown for (*A*) and (*B*) estimated population migration rates (2NM) between species; (*C*) effective population sizes (N_e) of the analyzed species; (*D*) effective population sizes of ancestral populations of the analyzed species pairs; (*E*) splitting time estimates (in million years).

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