

**Authors:** Hui-Ying Shang, Zhong-Hu Li, Miao Dong, Robert P. Adams, Georg Mieke, Lars Opgenoorth, Kang-Shan Mao

**Title:** Evolutionary origin and demographic history of an ancient conifer (*Juniperus microsperma*) in the Qinghai-Tibetan Plateau

**Supplementary Table S1.** Provenance for the sampled population of *Juniperus microsperma* and its five congeners.

Species	Pop	Location	Latitude	Longitude	Altitude (m)	n
<i>Juniperus microsperma</i> (n=60)	1	Bomi, XZ	29°39.006'	96° 12.907'	3180	10
	2	Bomi , XZ	29°41.577'	96° 12.181'	3181	10
	3	Bomi , XZ	29°37.244'	96° 18.214'	3254	10
	4	Bomi , XZ	29°39.267'	96°12.293'	3167	10
	5	Bomi , XZ	29°39.425'	96°12.475'	3184	8
	6	Bomi , XZ	29°37.003'	96°19.216'	3253	4
	7	Bomi , XZ	29°40.307'	96°12.645'	3191	8
<i>J. sabina</i> (n=12)	8	Fuyun, Xinjiang	47°13.67'	89°55.14'	1367	3
	9	Jimunai, Xinjiang	47°20.79'	86°11.59'	1250	3
	10	Chifeng, Neimenggu	43°31.96'	117°12.47'	1868	3
	11	Shandan, GS	38°26.625'	101°20.349'	3200	3
<i>J. semiglobosa</i> (n=11)	12	Aketao, Xinjiang	38°47'	75°31'	3450	1
	13	Aketao, Xinjiang	38°47'	75°23'	2700	1
	14	Aketao, Xinjiang	38°47'	75°18'	3060	1
	15	Gilgit-Baltistan, Pakistan	36°34'	73° 33'	3260	1
	16	Dushanbe, Tajikistan	37°50'	74°31'	3600	1
	17	Gilgit-Baltistan, Pakistan	36°30'	74°45'	3200	1
	18	Gilgit-Baltistan, Pakistan	36°51'	74°48'	4000	1
	19	Gilgit-Baltistan, Pakistan	36°33'	74°45'	3815	1
	20	Gilgit-Baltistan, Pakistan	36°35'	74°46'	3180	1
	21	Gilgit-Baltistan, Pakistan	35°42'	75°23'	3400	1
	22	Gilgit-Baltistan, Pakistan	36°49'	74°16'	3740	1
<i>J. convallium</i> (n=37)	23	Langxian, XZ	29°01.636'	093°03.469'	3150	8
	24	Sangri, XZ	29°18.246'	092°08.523'	4080	8
	25	Gongga, XZ	29°02.520'	090°05.033'	3900	8
	26	Mangkang, XZ	29°12.473'	098°39.796'	3720	1
	27	Nangqian, QH	32°45.113'	096°38.188'	4070	3
	28	Xiewu, QH	33°04.740'	097°17.335'	3670	2
	29	Jiangda, XZ	31°36.496'	098°30.340'	4140	7

<i>J. saltuaria</i> (n=39)	30	Linzhi, XZ	29°37.235'	094°37.619'	4160	7
	31	Mangkang, XZ	29°16.788'	098°40.824'	3870	8
	32	Baimaxueshan, YN	28°24.558'	098°58.953'	3780	10
	33	Songpan, SC	32°45.394'	103°56.989'	2600	1
	34	Hongyuan, SC	32°13.397'	102°30.898'	3600	2
	35	Bangda, XZ	30°18.197'	097°15.508'	4190	3
	36	Kangding, SC	29°28.483'	101°26.526'	3354	5
	37	Daofu, SC	31°04.524'	100°59.967'	3060	3
<i>J. tibetica</i> (n=80)	38	Langkazi, XZ	29°22.066'	090°53.136'	3660	3
	39	Jiacha, XZ	29°06.319'	092°29.916'	3650	7
	40	Sangri, XZ	29°18.246'	092°08.523'	4080	3
	41	Yadong, XZ	27°43.283'	088°58.092'	4000	4
	42	Langxian, XZ	29°01.636'	093°03.469'	3150	7
	43	Nangqian, QH	31°52.503'	096°17.552'	3720	4
	44	Nangqian, QH	31°56.511'	096°26.653'	4300	3
	45	Langlashan, XZ	30°18.197'	097°15.753'	4330	5
	46	Yelashan, XZ	30°07.385'	097°16.674'	4220	4
	47	Changdu, XZ	31°20.643'	097°12.869'	3320	5
	48	Jiangda, XZ	31°28.509'	098°11.627'	3590	6
	49	Jiangda, XZ	31°36.496'	098°30.340'	4140	1
	50	Dege, SC	31°55.592'	098°49.308'	3740	3
	51	Ranwu, XZ	29°30.249'	096°43.235'	3800	4
	52	Bomi, XZ	29°40.307'	096°12.643'	3200	2
	53	Yushu, QH	32°15.837'	096°55.470'	3660	6
	54	Nangqian, QH	32°30.054'	096°22.591'	3910	3
	55	Xinlong, SC	30°58.947'	100°18.710'	3070	4

Abbreviations: XZ, Xizang; QH, Qinghai; SC, Sichuan; YN, Yunnan; GS, Gansu; *n*, number of trees sampled.

**Supplementary Table S2.** The variable sites among chloroplast haplotypes based on *trnT*-L and *trnL*-F sequences.

Chloroplast haplotypes	Nucleotide variable positions																
	<i>trnT-trnF</i>																
	34	39	55	60	85	152	174	277	339	350	367	381	406	534	632	767	833
H1	G	A	★	-	-	A	-	A	A	G	G	C	◆	G	C	C	C
H2	G	A	□	▲	-	A	-	A	A	G	G	C	◆	G	C	C	C
H3	G	A	★	◎	-	A	-	A	A	G	G	C	◆	G	C	C	C
H4	G	A	★	◎	◎	A	-	A	A	G	G	C	◆	G	C	C	C
H5	G	A	★	●	-	C	-	A	A	G	G	C	◆	G	C	C	C
H6	T	C	★	-	-	A	-	A	G	T	G	T	-	G	T	C	T
H7	T	C	-	-	-	A	-	A	G	T	G	T	-	A	C	C	T
H8	T	C	-	-	-	A	☆	A	G	T	G	T	-	A	C	C	T
H9	T	C	★	-	-	A	-	G	G	T	A	T	-	A	C	A	T
H10	T	C	★	-	-	A	-	A	G	T	A	T	-	A	C	A	T

Note, dashes indicate missing nucleotides; ★ = TAATA; □ = GAATA; ☆ = TTAATGGATTATTATAAAA; ▲ = AATTATAGCGAATCGAATTAGAATA; ◆ = GAATA; ◎ = AATTATAGCGAATCGAATTATAATA; ● = AATTCTAGCGAATCGAATTAGAATA.

**Supplementary Table S3.** Nucleotide variation, nucleotide diversity, haplotype diversity and neutrality tests at eight nuclear loci for *Juniperus microsperma* and its five congeners, *J. sabina*, *J. semiglobosa*, *J. tibetica*, *J. convallium*, and *J. saltuaria*.

species	Loci	Total			Nonsynonymous			Silent sites		Diversity		Neutrality tests					
		N	L	S	$\theta_{wt}$	$\pi_t$	Rm	$\theta_{wa}$	$\pi_a$	$\theta_{sil}$	$\pi_{sil}$	Nh	He	D	H	D*	F*
<i>Juniperus microsperma</i>	<i>CC1147</i>	120	612	11	0.00335	0.00685	2	0.00231	0.0062	0.00727	0.0093	5	0.539	2.65064*	0.26246	1.41355	2.20923**
	<i>CC1333</i>	118	386	12	0.00582	0.00124	1	n.a.	0.00031	n.a.	0.00195	8	0.147	-2.04226*	-1.48573	-1.11961	-1.72993
	<i>CC2241</i>	118	351	4	0.00213	0.00209	2	0	0	0.00269	0.00263	7	0.397	-0.04152	-1.88556	0.92663	0.72154
	<i>CC2920</i>	116	685	7	0.00192	0.00201	2	0.00109	0.00101	0.00452	0.00515	6	0.598	0.11251	-2.5991	-0.60148	-0.42469
	<i>Hema</i>	118	909	2	0.00041	0.00009	0	0.00054	0.00012	0	0	3	0.083	-1.15799	0.0817	0.67279	0.12131
	<i>LHCA4</i>	126	733	6	0.00151	0.00122	2	n.a.	0.0015	n.a.	0.00104	5	0.207	-0.42625	-4.1219	1.10147	0.6946
	<i>Maldehy</i>	88	456	4	0.00174	0.00179	0	0.00208	0.00214	0.00166	0.00171	2	0.204	0.05834	0.71055	0.95186	0.78272
	<i>pgi</i>	118	372	5	0.00252	0.00428	0	0.00065	0.00111	0.00871	0.01484	3	0.33	1.45595	-2.45516	1.02291	1.37679
	Total	922	4504	51	0.0194	0.01957	9	0.00667	0.01239	0.02485	0.03662	39	2.505	0.00104	-11.49274	4.36812	1.54234
	Average	115.25	563	6.375	0.00243	0.00245	1.13	0.00111	0.00155	0.00414	0.00458	4.88	0.31313	0.00017	-1.43659	0.54601	0.22033
<i>J. sabina</i>	<i>CC1147</i>	28	612	10	0.0042	0.00522	2	0.00372	0.00353	0.00603	0.0116	10	0.799	0.78033	0.97884	1.40629*	1.42024
	<i>CC1333</i>	24	386	8	0.00555	0.00579	0	0.00165	0.00302	0.00851	0.00793	8	0.851	0.13958	0.21014	-0.4561	-0.32684
	<i>CC2241</i>	22	351	9	0.00703	0.00941	5	0	0	0.00887	0.01187	12	0.866	1.13242	1.74892	1.37569*	1.51473
	<i>CC2920</i>	24	685	8	0.00313	0.00375	1	0.00155	0.00255	0.00809	0.00752	8	0.609	0.63879	0.35507	0.14046	0.33332
	<i>Hema</i>	24	909	1	0.00029	0.00009	0	0	0	0.00124	0.00038	2	0.083	-1.15933	0.07971	-1.60583	-1.7042
	<i>LHCA4</i>	22	733	12	0.00487	0.0042	1	0.00288	0.00262	0.00614	0.00522	12	0.931	-0.48064	0.57143	0.29259	0.07463
	<i>Maldehy</i>	20	456	4	0.00309	0.00256	0	0.00592	0.0057	0.00236	0.00175	5	0.616	-0.52308	0.73684	0.38667	0.15284
	<i>pgi</i>	24	372	6	0.00432	0.00235	0	0.00281	0.00192	0.00931	0.00377	6	0.5	-1.38074	0.78986	-0.23775	-0.65959
	Total	188	4504	58	0.03248	0.03337	9	0.01853	0.01934	0.05055	0.05004	63	5.255	-0.85267	5.47081	-1.47996	0.80513
	Average	23.5	563	7.25	0.00406	0.00417	1.125	0.00232	0.00242	0.00632	0.00626	7.875	0.65688	-0.10658	0.68385	-0.2467	0.10064

<i>J. semiglobosa</i>	<i>CC1147</i>	16	612	9	0.00443	0.00613	0	0.00436	0.00646	0.0047	0.00487	3	0.575	1.40516	1.63333	0.42855	0.80053
	<i>CC1333</i>	36	386	6	0.00375	0.00127	0	0.00298	0.00069	0.00438	0.00172	5	0.213	-1.80614*	-6.9619	-1.21901	-1.63271
	<i>CC2241</i>	40	351	10	0.00737	0.00571	0	0	0	0.00929	0.0072	10	0.812	-0.68167	-2.38974	-0.18881	-0.40872
	<i>CC2920</i>	12	685	1	0.00048	0.00024	0	0.00064	0.00032	0	0	2	0.167	-1.14053	-1.66667	-1.32974	-1.44334
	<i>HemA</i>	8	909	2	0.00085	0.0009	0	0.00056	0.00036	0.00178	0.00264	3	0.679	0.24178	0.21429	-0.14931	-0.06487
	<i>LHCA4</i>	16	733	2	0.00082	0.00072	0	0	0	0.00135	0.00118	3	0.425	-0.3301	-0.68333	-0.50381	-0.52297
	<i>Maldehy</i>	10	456	9	0.00698	0.00819	0	0.00371	0.00374	0.0079	0.00944	5	0.889	0.75971	2.13333	1.43508*	1.42579
	<i>pgi</i>	36	372	4	0.00259	0.0006	0	0.00169	0.00039	0.00556	0.00129	2	0.056	-1.88514*	-7.55556	-3.14683*	-3.22414*
	Total	174	4504	43	0.02727	0.02376	0	0.01394	0.01196	0.03496	0.02834	33	3.816	0.25435	-15.27625	-2.96213	-1.84629
	Average	21.75	563	5.375	0.00341	0.00297	0	0.00174	0.0015	0.00437	0.00354	4.125	0.477	0.04239	-1.90953	-0.49369	-0.26376
<i>J. convallium</i>	<i>CC1147</i>	56	612	5	0.00178	0.00114	0	0.0009	0.001	0.00509	0.00164	6	0.545	-0.85346	0.51948	1.08675	0.5581
	<i>CC1333</i>	70	386	5	0.00269	0.00332	0	0.00257	0.00378	0.00283	0.00304	4	0.343	0.53389	-4.1027	1.06726	1.05281
	<i>CC2241</i>	62	351	2	0.00121	0.00256	0	0.00305	0.00557	0.00077	0.00182	3	0.626	1.87657	-0.7636	0.7263	1.24621
	<i>CC2920</i>	70	685	5	0.00151	0.00085	0	0.0016	0.0009	0.00127	0.00067	5	0.31	-1.00067	0.52174	1.06726	0.47607
	<i>HemA</i>	66	909	4	0.00092	0.00071	0	0.0003	0.00009	0.00291	0.00269	4	0.274	-0.50698	-2.2863	0.97791	0.60001
	<i>LHCA4</i>	60	733	4	0.00117	0.00135	1	0	0	0.0019	0.0022	6	0.716	0.33606	-0.3254	0.98685	0.91743
	<i>Maldehy</i>	72	456	4	0.00181	0.00092	0	0	0	0.00231	0.00117	4	0.341	-1.03543	0.38811	0.96989	0.39549
	<i>pgi</i>	72	372	11	0.0061	0.0029	0	0.00431	0.0028	0.01214	0.00322	5	0.516	-1.43819	-13.336	1.43322	0.52958
	Total	528	4504	40	0.01719	0.01375	1	0.01273	0.01414	0.02922	0.01645	37	3.671	-2.08821	-19.385	8.31544	5.7757
	Average	66	563	5	0.00215	0.00172	0.125	0.00160	0.00177	0.00365	0.00206	4.625	0.45888	-0.26103	-2.4231	1.03943	0.72197
<i>J. saltuaria</i>	<i>CC1147</i>	26	612	5	0.00214	0.00298	0	0.00163	0.00244	0.00409	0.00499	5	0.8	1.10571	0.44308	1.15807	1.32578
	<i>CC1333</i>	42	386	7	0.00421	0.0051	1	0.00287	0.00574	0.00528	0.00473	9	0.785	0.57785	-1.62137	1.25279	1.21994
	<i>CC2241</i>	54	351	8	0.005	0.00357	1	0.0063	0.00209	0.00473	0.00398	9	0.769	-0.77108	0.84416	1.2963	0.73433
	<i>CC2920</i>	60	685	9	0.00282	0.00203	0	0.00124	0.00158	0.00786	0.00349	9	0.694	-0.75499	1.02147	1.34474	0.76469
	<i>HemA</i>	72	909	5	0.00113	0.00117	0	0	0	0.00476	0.00489	6	0.587	0.06097	0.82316	1.06482	0.87262

	<i>LHCA4</i>	58	733	4	0.00118	0.00092	0	0.00076	0.00024	0.00144	0.00134	4	0.515	-0.48692	0.50333	0.99007	0.62147
	<i>Maldehy</i>	66	456	9	0.00415	0.00214	0	0.00221	0.00121	0.0047	0.00241	8	0.649	-1.28792	-0.75338	1.34047	0.54425
	<i>pgi</i>	68	372	15	0.00842	0.00781	0	0.00728	0.00693	0.01223	0.01077	9	0.771	-0.21075	-7.50132	1.58187*	1.13075
	Total	446	4504	62	0.02905	0.02572	2	0.02229	0.02023	0.04509	0.0366	59	5.57	-1.76713	-6.24087	8.44726	7.21383
	Average	55.75	563	7.75	0.00363	0.00321	0.25	0.00279	0.00253	0.00564	0.00458	7.375	0.69625	-0.22090	-0.78011	1.20675	0.90173
<i>J. tibetica</i>	<i>CC1147</i>	98	612	3	0.00095	0.00112	1	0.0008	0.00111	0.00151	0.00118	5	0.575	0.33028	0.55207	0.82851	0.78754
	<i>CC1333</i>	112	386	6	0.00294	0.0041	2	0.00234	0.00483	0.00343	0.00363	9	0.728	0.87027	-2.78378	1.11091	1.2182
	<i>CC2241</i>	92	351	7	0.00392	0.0026	0	0.00845	0.00404	0.00282	0.00227	7	0.668	-0.79801	0.46249	1.19959	0.62548
	<i>CC2920</i>	112	685	6	0.00193	0.00148	1	0.00145	0.00143	0.00346	0.00166	9	0.631	-0.53434	0.80824	1.18496	0.71171
	<i>HemA</i>	138	909	5	0.001	0.00132	0	0.00053	0.00012	0.00252	0.00514	6	0.64	0.64601	0.15022	1.01	1.05133
	<i>LHCA4</i>	126	733	10	0.00252	0.0032	2	0.00065	0.00011	0.00369	0.00513	12	0.769	0.66132	0.87721	1.35975	1.32355
	<i>Maldehy</i>	156	456	7	0.00273	0.00135	0	0.00187	0.0015	0.00298	0.00132	6	0.496	-1.1116	-1.40281	1.15961	0.4455
	<i>pgi</i>	156	372	3	0.00143	0.00167	0	0.00186	0.00217	0	0	4	0.55	0.27839	0.47113	0.79108	0.73804
	Total	990	4504	47	0.01742	0.01684	6	0.01795	0.01531	0.02041	0.02033	58	5.057	0.34232	-0.86523	8.64441	6.90135
	Average	123.75	563	5.875	0.00218	0.00211	0.75	0.00224	0.00191	0.00255	0.00254	7.25	0.63215	0.04279	-0.10815	1.08055	0.86267

Abbreviations: N, sample size; L, length in base pairs; S, number of segregating sites;  $\pi$ , nucleotide diversity (Nei and Li, 1979);  $\theta$ , Watterson's parameter (Watterson, 1975); Rm, the minimum number of recombinant events; Nh, number of haplotypes; He, Nei's haplotypic diversity; D, Tajima's D statistic (Tajima, 1989); H, Fay and Wu's H (Fay and Wu, 2000); D\*, F\*, Fu and Li's D\*, F\* test (Fu and Li, 1993); NE, not informative; NA, failed to be computed due to insufficient variation. Significance level: \*0.01  $\leq$  P < 0.05; \*\* 0.001  $\leq$  P < 0.01; \*\*\*P < 0.001.

**Supplementary Table S4.** Genetic divergence at eight nuclear loci between *Juniperus microsperma* and each of its five congeners based on pairwise comparisons.

Group	JM vs. JSA		JM vs. JSE		JM vs. JCON		JM vs. JSAL		JM vs. JTIB		JSA vs. JSE	
	<i>FST</i>	Da	<i>FST</i>	Da	<i>FST</i>	Da	<i>FST</i>	Da	<i>FST</i>	Da	<i>FST</i>	Da
<i>CC1147</i>	0.31552***	0.00304	0.05811	0.00044	0.69387***	0.01146	0.5937***	0.00908	0.72757***	0.01143	0.21579***	0.00151
<i>CC1333</i>	0.7149***	0.00491	0.85111***	0.00714	0.82662***	0.00959	0.80684***	0.00934	0.77959***	0.00931	0.22052***	0.00083
<i>CC2241</i>	0.78491***	0.01155	0.81251***	0.01294	0.90008***	0.02024	0.88781***	0.02016	0.89689***	0.0201	0.30017***	0.00297
<i>CC2920</i>	0.71686***	0.0058	0.81884***	0.00847	0.95322***	0.0321	0.94085***	0.03212	0.94783***	0.03185	0.41843***	0.30017
<i>HemA</i>	0.00684	0	0.67338***	0.00024	0.8961***	0.00268	0.71101***	0.00122	0.68871***	0.00167	0.44439***	0.00019
<i>LHCA4</i>	0.7057***	0.00389	0.90402***	0.01096	0.90299***	0.01172	0.91232***	0.01168	0.80543***	0.00913	0.74511***	0.00806
<i>Maldehy</i>	0.16192**	0.00036	0.54634***	0.00259	0.62432***	0.00232	0.54839***	0.00235	0.60986***	0.00235	0.35681***	0.00227
<i>PGI</i>	0.68656***	0.00872	0.76078***	0.01096	0.87865***	0.02722	0.79947***	0.02219	0.90643***	0.02708	0.60238***	0.00194

Abbreviations: JM, *J. microsperma*; JSA, *J. sabina*; JSE, *J. semiglobosa*; JCON, *J. convallium*; JSAL, *J. saltuaria*; JTIB, *J. tibetica*. Significance level: \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

**Supplementary Table S5.** Distribution of segregating sites at eight nuclear loci in pairwise comparisons between *Juniperus microsperma* and each of its five congeners. In each pairwise comparison, S1, S2 are the number of polymorphic sites unique to species 1 and 2, respectively; Ss is the number of sites with shared alleles; and Sf is the number of sites with fixed alleles in either species;  $\Phi_{ST}$  values over all nuclear loci. Abbreviations: JM, *J. microsperma*; JSA, *J. sabina*; JSE, *J. semiglobosa*; JCON, *J. convallium*; JSAL, *J. saltuaria*; JTIB, *J. tibetica*.

Species	Gene	N1	N2	S1	S2	Ss	Sf	$\Phi_{ST}$
JM vs. JSA	<i>CC1147</i>	120	28	11	10	16	0	0.52257***
	<i>CC1333</i>	118	24	12	8	20	0	
	<i>CC2241</i>	118	22	4	9	13	0	
	<i>CC2920</i>	116	24	7	8	16	1	
	<i>HemA</i>	118	24	2	1	3	0	
	<i>LHCA4</i>	126	22	6	12	18	0	
	<i>Maldehy</i>	88	20	4	4	8	0	
	<i>PGI</i>	118	24	5	6	11	0	
	Total	922	188	51	58	105	1	
	Average	115	24	6	7	23	0	
JM vs. JSE	<i>CC1147</i>	120	16	11	9	13	0	0.78665***
	<i>CC1333</i>	118	36	12	6	18	0	
	<i>CC2241</i>	118	40	4	10	14	0	
	<i>CC2920</i>	116	12	7	1	11	3	
	<i>HemA</i>	118	8	2	2	4	0	
	<i>LHCA4</i>	126	16	6	2	13	5	
	<i>Maldehy</i>	88	10	4	9	13	0	
	<i>PGI</i>	118	36	5	4	7	0	
	Total	922	174	51	43	93	8	
	Average	115	22	6	5	12	1	
JM vs. JCON	<i>CC1147</i>	120	56	11	5	21	5	0.83332***
	<i>CC1333</i>	118	70	12	5	19	2	
	<i>CC2241</i>	118	62	4	2	11	6	
	<i>CC2920</i>	116	70	7	5	32	20	
	<i>HemA</i>	118	66	2	4	7	1	
	<i>LHCA4</i>	126	60	6	4	16	6	
	<i>Maldehy</i>	88	72	4	4	9	1	
	<i>PGI</i>	118	72	5	11	17	2	
	Total	922	528	51	40	132	43	
	Average	115	66	6	5	17	5	
JM vs. JSAL	<i>CC1147</i>	120	26	11	5	19	3	0.89475***
	<i>CC1333</i>	118	42	12	7	21	2	
	<i>CC2241</i>	118	54	4	8	17	6	
	<i>CC2920</i>	116	60	7	9	34	18	



	<i>HemA</i>	118	72	2	5	8	1	
	<i>LHCA4</i>	126	58	6	4	16	6	
	<i>Maldehy</i>	88	66	4	9	14	1	
	<i>PGI</i>	118	68	5	15	21	2	
	Total	922	446	51	62	150	39	
	Average	115	56	6	8	19	5	
JM vs. JTIB	<i>CC1147</i>	120	98	11	3	19	5	0.83588***
	<i>CC1333</i>	118	112	12	6	20	2	
	<i>CC2241</i>	118	92	4	7	16	6	
	<i>CC2920</i>	116	112	7	6	31	18	
	<i>HemA</i>	118	138	2	5	8	1	
	<i>LHCA4</i>	126	126	6	10	19	3	
	<i>Maldehy</i>	88	156	4	7	12	1	
	<i>PGI</i>	118	156	5	3	16	8	
	Total	922	990	51	47	141	44	
	Average	115	124	6	6	18	6	

**Supplementary Table S6.** An HKA test for *Juniperus microsperma* and five of its congeners. Another congener, *J. communis*, was assigned as the outgroup.

Species	$\chi^2$	P-value
<i>Juniperus microsperma</i> vs. <i>J. communis</i>	8.6225	0.28091
<i>Juniperus sabina</i> vs. <i>J. communis</i>	4.9341	0.66801
<i>Juniperus semiglobosa</i> vs. <i>J. communis</i>	14.3698*	0.04498
<i>Juniperus convallium</i> vs. <i>J. communis</i>	8.1044	0.88382
<i>Juniperus saltuaria</i> vs. <i>J. communis</i>	2.9173	0.89254
<i>Juniperus tibetica</i> vs. <i>J. communis</i>	6.5182	0.4807

Significance level: \*  $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

**Supplementary Table S7.** Maximum-likelihood estimates (MLE) and the 95% highest posterior density (HPD) intervals of demographic parameters from pairwise IMA2 multi-loci analyses.

Pairwise comparison		$\theta_1$	$\theta_2$	$\theta_A$	m1	m2	t	N1	N2	NA	T(year)	2N1m1	2N2m2
<i>J. microsperma</i> vs. <i>J. convallium</i>	HiPt	0.708	0.756	3.804	0.00015	0.00015	1.209	4.09E+04	4.37E+04	2.20E+05	1.40E+07	0.0001065	0.0001251
	HPD95Lo	0.468	0.476	1.644	0	0	0.5238	2.71E+04	2.75E+04	9.50E+04	6.06E+06		
	HPD95Hi	1.028	1.116	7.732	0.1225	0.1345	2.321	5.94E+04	6.45E+04	4.47E+05	2.68E+07		
<i>J. microsperma</i> vs. <i>J. saltuaria</i>	HiPt	0.772	1.54	3.02	0.0001	0.0001	1.754	4.23E+04	8.44E+04	1.65E+05	1.92E+07	0.0000822	0.000145
	HPD95Lo	0.524	1.1	0.852	0	0	1.006	2.87E+04	6.03E+04	4.67E+04	1.10E+07		
	HPD95Hi	1.092	2.116	7.276	0.1159	0.0775	3.31	5.98E+04	1.16E+05	3.99E+05	3.63E+07		
<i>J. microsperma</i> vs. <i>J. tibetica</i>	HiPt	0.6885	0.9225	2.696	0.0001	0.0001	1.619	4.59E+04	6.15E+04	1.80E+05	2.16E+07	0.00007492	0.00009383
	HPD95Lo	0.4545	0.6255	0.5085	0	0	0.8745	3.03E+04	3.39E+04	3.39E+04	1.17E+07		
	HPD95Hi	0.9945	1.292	7.601	0.1261	0.0905	2.804	6.63E+04	8.61E+04	5.07E+05	3.74E+07		
<i>J. microsperma</i> vs. <i>J. sabina</i>	HiPt	0.482	1.646	0.814	0.3305	0.0225	1.234	2.69E+04	9.19E+04	4.54E+04	1.38E+07	0.09371	0.02146
	HPD95Lo	0.286	1.062	0.01	0	0	0.2835	1.60E+04	5.93E+04	5.58E+02	3.16E+06		
	HPD95Hi	0.782	2.502	3.322	0.8435	0.3195	2.749	4.36E+04	1.40E+05	1.85E+05	3.07E+07		
<i>J. microsperma</i> vs. <i>J. semiglobosa</i>	HiPt	0.5805	0.7065	3.757	0.0005	0.0005	0.3985	3.24E+04	3.94E+04	2.10E+05	4.45E+06	0.0002171	0.0004241
	HPD95Lo	0.3285	0.3825	1.831	0	0	0.1785	1.83E+04	2.13E+04	1.02E+05	1.99E+06		
	HPD95Hi	0.9045	1.192	7.303	0.2195	0.4425	0.7765	5.05E+04	6.65E+04	4.08E+05	8.67E+06		
<i>J. sabina</i> vs. <i>J. semiglobosa</i>	HiPt	1.73	0.386	0.002	0.022	1.306	1.238	8.85E+04	1.97E+04	1.02E+02	1.27E+07	0.02445	0.2999
	HPD95Lo	1.09	0.194	0	0	0.418	0.4605	5.58E+04	9.92E+03	0.00E+00	4.71E+06		
	HPD95Hi	2.686	0.742	3.234	3.154	0.562	2.998	1.37E+05	3.80E+04	1.65E+05	3.07E+07		

Note, All estimates include the per gene mutation rate, which is equal to the geometric mean of the mutation rate of all the loci;  $\theta_1$  = effective population size of the first species;  $\theta_2$  = effective population size of the second species,  $\theta_A$  = effective population size of the ancestral population; m1 = population migration rate from the first to the second species; m2 = population migration rate from the second to the first species; T = time since species divergence; the population migration rate:  $2Nm = \theta \times m/2$ ;  $G=50$ ;  $N=\theta/4\mu G$ ;  $T=t/\mu$ .

**Supplementary Table S8.** Maximum-likelihood estimates (MLE) and the 95% highest posterior density (HPD) intervals of demographic parameters from multiple population IMA2 multi-loci analyses.

Comparison		$\theta_1$	$\theta_2$	$\theta_3$	$\theta_A$	$\theta_B$	t1	t2	N1	N2	N3	NA	NB	T1(year)	T2(year)
MAE	HiPt	0.4795	0.9995	0.4015	0.9995	0.0565	1.23	1.698	2.68E+04	5.58E+04	2.24E+04	5.58E+04	3.15E+03	1.37E+07	1.90E+07
	HPD95Lo	0.2805	0.8075	0.2115	0.0235	0.0035	0.37	1.29	1.57E+04	4.51E+04	1.18E+04	1.31E+03	1.95E+02	4.13E+06	1.44E+07
	HPD95Hi	0.7725	0.9995	0.7255	0.9995	0.9445	2.37	3.998	4.31E+04	5.58E+04	4.05E+04	5.58E+04	5.27E+04	2.65E+07	4.46E+07
MCA	HiPt	0.422	0.874	1.774	0.018	3.998	1.093	1.692	3.12E+04	6.46E+04	1.31E+05	1.33E+03	2.95E+05	1.61E+07	2.50E+07
	HPD95Lo	0.214	0.582	1.218	0	0.614	0.5925	1.302	1.58E+04	4.30E+04	9.00E+04	0.00E+00	4.53E+04	8.75E+06	1.92E+07
	HPD95Hi	0.698	1.27	2.502	2.914	3.998	1.853	4.997	5.16E+04	9.38E+04	1.85E+05	2.15E+05	2.95E+05	2.74E+07	7.38E+07

Note, All estimates include the per gene mutation rate, which is equal to the geometric mean of the mutation rate of all the loci;  $\theta_1$  = effective population size of the first species;  $\theta_2$  = effective population size of the second species,  $\theta_A$ (MAE) = effective population size of ancestral population between *J.sabina* and *J.semiglobosa*;  $\theta_A$ (MCA) = effective population size of ancestral population between *J.microsperma* and *J.sabina-J.semiglobosa*;  $\theta_B$  = effective population size of ancestral population;  $m_1$  = population migration rate from the first to the second species;  $m_2$  = population migration rate from the second to the first species; T = time since species divergence; G=50; N= $\theta/4\mu$ G; T= $t/\mu$ ; MAE: *J.microsperma*, *J.sabina*, *J.semiglobosa*; MCA: *J.microsperma*, *J.tibetica* complex, *J.sabina-J.semiglobosa*.

**Supplementary Table S9.** Prior distribution for the demographic parameters that were applied in the DIYABC program. Uniform distribution (UN) with 2 parameters: min and max; Gamma distribution (GA) with 3 parameters: min, max, shape; Log-Uniform (LU) distribution with 2 parameters: min and max; Ancestral effective population size (N3); Effective population size after expansion (N4); Effective population size after bottleneck (N1 and N2); Ancestral population expansion time (t4) and three population bottleneck times (t3, t2, t1).

<b>Priors for the demographic parameters</b>	
N1	UN~[50000-300000]
N2	UN~[300000 - 500000]
N3	UN~[1000000 - 2000000]
Ne	UN~[500000 - 1000000]
t1	UN~[100000- 1000000]
t2	UN~[1000000 - 3000000]
t3	UN~[4000000 - 8000000]
t4	UN~[17000000 - 20000000]
Constraint on parameter	$N1 < N2; N2 < N3; Ne > N3; t1 < t2; t2 < t3; t3 < t4$
<i>Priors for the mutation model</i>	
MEAN - $\mu$	UN~[ 1.00E-9,1.00E-8]
GAM - $\mu$	GA~[ 1.00E-9,1.00E-5, 2]
MEAN - K_C/T	UN~[0.05, 20]
GAM - K_C/T	GA~[0.05 , 20, 2]

**Supplementary Table S10.** Marginal posterior probability densities for the demographic parameters of all five demographic scenarios. Demographic scenarios 1 to 5 are represented in Fig. 8. The probability that simulated S is smaller than observed S [Prob. (Ssimul. < Sobs.)] is given for each summary statistic and was calculated from 10,000 virtual datasets simulated from the posterior distributions of parameters obtained under a given scenario. Significance levels are indicated by asterisks (\* and \*\* for  $Q < 0.05$  and  $Q < 0.01$ , respectively).

Statistics (S)	Observed S	Scenarios					Probability (Ssimul. < Sobs.)				
		1	2	3	4	5					
NHA	3.8750	0.3425	0.2750	0.7945	0.2750	0.6915					
NSS	5.7500	0.4799	0.6900	0.8175	0.7020	0.9555 *					
MPD	1.2011	0.4460	0.6670	0.8200	0.6825	0.9120					
VPD	3.9370	0.5080	0.8145	0.8480	0.8310	0.9550 *					
DTA	0.0000	0.4868	0.6340	0.7635	0.6095	0.6745					
PSS	5.7500	0.4799	0.6900	0.8175	0.7020	0.9555 *					
MNS	14.8447	0.5661	0.6840	0.9040	0.6455	0.7475					
VNS	106.8668	0.4163	0.3800	0.8030	0.3920	0.5840					

Abbreviations for the summary statistics are as follows: number of haplotypes (NHA), number of segregating sites (NSS), mean of pairwise differences (MPD), variance of pairwise differences (VPD), Tajima's D (DTA), private segregating sites (PSS), mean of numbers of the rarest nucleotide at segregating sites (MNS) and variance of number of the rarest nucleotide at segregating sites (VNS). The probability (Ssimul. < Sobs.) given for each summary statistic was calculated from 10,000 virtual datasets simulated from the posterior distributions of parameters obtained under a given scenario. Significance levels are indicated by asterisks (\* and \*\* for  $Q < 0.05$  and  $Q < 0.01$ , respectively).

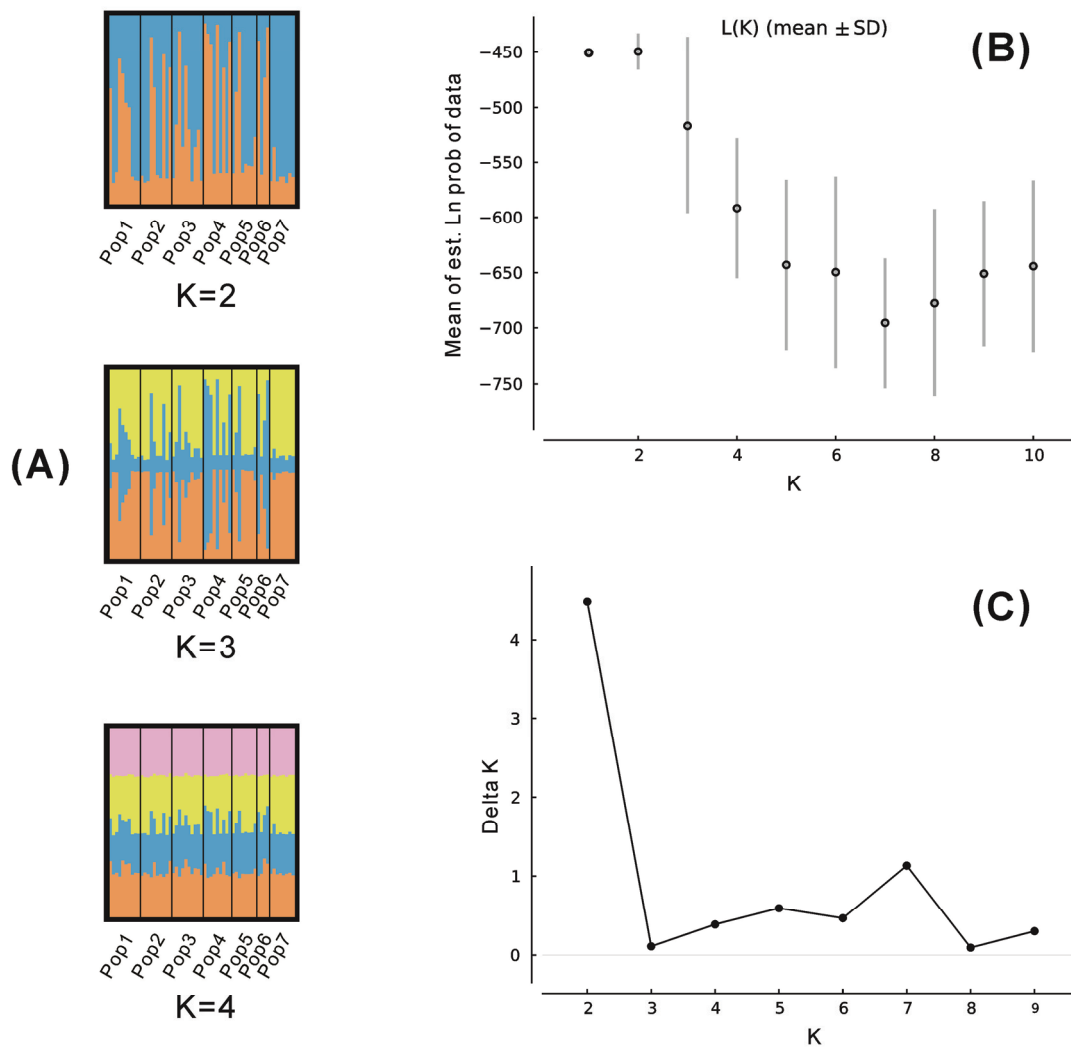
**Supplementary Table S11.** List of primers for the eight nuclear loci, their target sequence length, putative function, primer sequences and corresponding references<sup>1-6</sup>.

Locus	Length (bp)	Putative function	PCR primers (5'-3')	Reference
nrDNA				
<i>CC1147</i>	612	Unknown	F:GCACCCATCTCACACTTGTC R:CCACCCTGTTTCATGTGATTCT	Kado et al. 2008
<i>CC2920</i>	685	Beta-1,3- glucanase like	F:CCGCCACATTCACGCCCTCT R:CGCAGTTCCAGTAGTTTCTC	Kado et al. 2008
<i>HemA</i>	909	Glutamyl-tRNA reductase	F:CTTCGGCAGCATCTCTTCAT R:AACCTTAGCCCTGATTTTCT	Tsumura et al. 1997
<i>Pgi</i>	372	Glucose-6- phosphate isomerase	F:GCCCAAATCCTCTGTGTCT R:GACTTCCTGAGCCAATAATG	Ujino-Ihara et al.2000
<i>CC1333</i>	386	GTP binding protein	F:CTCCTAGATCAGCGTATCACA R:CCCTTTCTTGACCAGCAG	Tani et al. 2003
<i>CC2241</i>	351	Glyceraldehyde-3- phosphatedehydrogenase	F:CAGATTTTCATTGGAGACAGC R:CAAATCTATGACTCGTGAAC	Tani et al. 2003
<i>Maldehy</i>	456	Putative malate dehydrogenase	F:CATATCCGTGATTGGGTGCTT R:CAGTGGCATCCAGTTTTTCCT	Dvornyk et al. 2002
<i>LHCA4</i>	733	LHC I type IV chlorophyll-binding protein	F:GGAGCTAGTGAACGGGAGGTG R:GAACGGGCCCTTTCCTGTTA	Dvornyk et al. 2002
cpDNA				
<i>trnT-trnL</i>	836	Unknown	F:CATTACAAATGCGATGCTCT R:GGGGATAGAGGGACTCGAAC	Taberlet et al. 1991
<i>trnL-trnF</i>	668	Unknown	F:CGGAATCGGTAGACGCTACG R:ATTTGAACTGGTGACACGAG	Taberlet et al. 1991

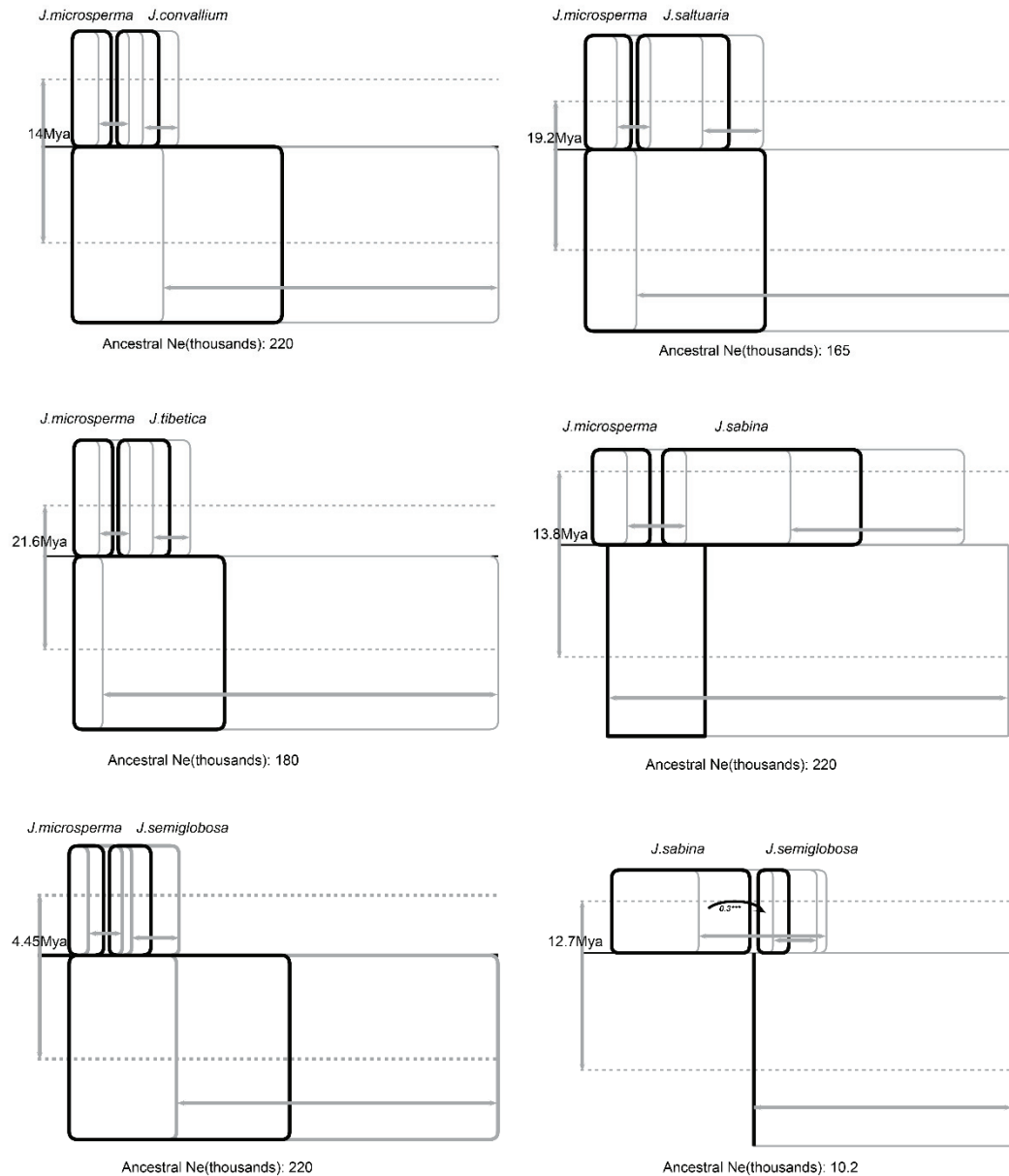
References:

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**Supplementary Figure S1.** (A) Bayesian clustering for individuals of *Juniperus microsperma* based on eight nuclear loci, assuming two, three and four clusters (K=2, 3, 4); (B) Mean LnPD values of nine runs that assumed two to ten clusters (K=2–10); (C) Delta K values of eight runs that assumed two to nine clusters (K=2–9), and for each K value, results of the run with the highest value of LnPD were used. Thin vertical lines in (A) represent boundaries among populations; open circles and gray bars in (B) represent the mean value and the standard deviation of LnPD for each run.



**Supplementary Figure S2.** Histories for six species pairs, *Juniperus microsperma* and each of its five congeners, are represented as boxes (for both sampled and ancestral populations). Boxes spanning from the top border of each subfigure represent living populations, whereas other boxes represent ancestral populations. For each box, its horizontal and vertical widths represent effective population size and time, respectively. Curved arrows linking boxes represent migration between populations. Time is represented on the vertical axis in each figure, with the sampled species names provided at the top of each figure at the most recent time point. For all figures, the 95% highest posterior density intervals are shown with gray arrows for population sizes (i.e. box widths) and splitting times (dotted lines). Only those population migration rates that were found to be statistically significant using a

likelihood-ratio test are shown, in which case the estimated value of  $2Nm$  is provided in addition to the significance level: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .