

Supplementary Information

Title:

Reconstructing the survival history of relict herbs endemic to the heavy snowfall regions of Japan during the Pleistocene

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Table S1 Sampling location and genetic diversity estimates for *Glaucidium palmatum*, *Ranzania japonica* and *Paris japonica*

Species	Region	Locality	Latitude	Longitude	Altitude (m)	Snow depth (cm)	N	h	Haplotypes
<i>Glaucidium palmatum</i>									
	Hokkaido	Mt. Yubari, Yubari City, Hokkaido Pref., Hokkaido Isl.	43.11	142.22	1301	117	1	1	gH37(1)
		Mt. Kannon, Samani Town, Hokkaido Pref., Hokkaido Isl.	42.13	142.91	92	41	3	3	gH28(1), gH29(1), gH30(1)
		Heiwa-waterfall, Sapporo City, Hokkaido Pref., Hokkaido Isl.	43.06	141.22	255	146	4	2	gH9(2), gH10(2)
		Kannon-waterfall, Furubira Town, Hokkaido Pref., Hokkaido Isl.	43.23	140.59	89	152	3	2	gH18(2), gH19(1)
		Mt. Mekunnai, Rankoshi Town, Hokkaido Pref., Hokkaido Isl.	42.90	140.54	794	432	4	2	gH22(3), gH23(1)
		Utasai, Kuromatsunai Town, Hokkaido Pref., Hokkaido Isl.	42.65	140.32	46	128	5	3	gH10(3), gH33(1), gH34(1)
		Sasayama, Esashi Town, Hokkaido Pref., Hokkaido Isl.	41.86	140.20	302	48	4	2	gH3(3), gH4(1)
		Mt. Hakodate, Hakodate City, Hokkaido Pref., Hokkaido Isl.	41.75	140.71	200	28	4	1	gH12(4)
	northern Honshu	Mt. Kodake, Fujisato Town, Akita Pref., Honshu Isl.	40.42	140.21	720	185	4	2	gH15(3), gH16(1)
		Yunosawa, Senboku City, Akita Pref., Honshu Isl.	39.81	140.79	740	319	5	2	gH35(4), gH36(1)
		Ooshimizu-enchi, Yurihonjo City, Akita Pref., Honshu Isl.	39.10	140.11	840	394	4	1	gH27(4)
		Mt. Kanegasaki-komagatake, Kitakami City, Iwate Pref., Honshu Isl.	39.20	140.92	1020	160	4	2	gH13(3), gH14(1)
		Mt. Gassan, Nishikawa Town, Yamagata Pref., Honshu Isl.	38.54	140.01	1589	450	4	2	gH5(3), gH6(1)
		Mt. Torihara, Asahi Town, Yamagata Pref., Honshu Isl.	38.27	139.96	1363	324	2	2	gH31(1), gH32(1)
		Mt. Iide, Kitakata City, Fukushima Pref., Honshu Isl.	37.87	139.71	1700	268	2	2	gH20(1), gH21(1)

central Honshu	Mt. Handa, Koori Town, Fukushima Pref., Honshu Isl.	37.88	140.50	440	31	4	2	gH7(3), gH8(1)
	Mt. Aizu-komagatake, Hinoemata Village, Fukushima Pref., Honshu Isl.	37.02	139.34	1850	213	4	2	gH1(1), gH2(3)
	Mt. Naeba, Yuzawa Town, Niigata Pref., Honshu Isl.	36.85	138.70	1876	234	3	2	gH2(2), gH11(1)
	Mt. Yoneyama, Kashiwazaki City, Niigata Pref., Honshu Isl.	37.30	138.49	729	85	4	1	gH2(4)
	Mt. Kurosawa, Myoko City, Niigata Pref., Honshu Isl.	36.89	138.08	1780	238	4	1	gH1(4)
	Mt. Kurohime, Itoigawa City, Niigata Pref., Honshu Isl.	36.98	137.79	1034	127	1	1	gH17(1)
	Nagashiri-sawa, Hakuba Village, Nagano Pref., Honshu Isl.	36.74	137.78	1608	184	3	2	gH24(2), gH25(1)
	Oh'gi-sawa, Ohmachi City, Nagano Pref., Honshu Isl.	36.56	137.71	1575	112	3	1	gH26(3)
<i>Ranzania japonica</i>								
northern Honshu	Ajigasawa, Ajigasawa Town, Aomori Pref., Honshu Isl.	40.57	140.10	698	304	2	1	rH1(2)
	Yunosawa, Senboku City, Akita Pref., Honshu Isl.	39.81	140.79	740	319	2	1	rH1(2)
	Mt. Kanegasaki-komagatake, Kitakami City, Iwate Pref., Honshu Isl.	39.20	140.92	1020	160	5	1	rH1(5)
	Ooshimizu-enchi, Yurihonjo City, Akita Pref., Honshu Isl.	39.10	140.11	840	394	4	2	rH1(3), rH4(1)
	Oiri-sawa, Obanzawa City, Yamagata Pref., Honshu Isl.	38.52	140.57	601	281	3	1	rH1(3)
	Mt. Yudono, Tsuruoka City, Yamagata Pref., Honshu Isl.	38.52	139.96	880	397	4	4	rH5(1), rH6(1), rH7(1), rH8(1)
	Oishi-zawa, Oguni Town, Yamagata Pref., Honshu Isl.	37.96	139.80	436	199	2	1	rH1(2)
central Honshu	Danyoshi-shindo, Hinoemata Village, Fukushima Pref., Honshu Isl.	36.96	139.26	1426	268	6	2	rH1(5), rH2(1)

		Sotonosawa, Tookamachi City, Niigata Pref., Honshu Isl.	36.94	138.71	929	222	4	1	rH1(4)
		Mt. Togakushi, Nagano City, Nagano Pref., Honshu Isl.	36.79	138.08	1475	176	5	1	rH1(5)
		Nagashiri-sawa, Hakuba Village, Nagano Pref., Honshu Isl.	36.74	137.78	1650	184	7	2	rH1(6), rH3(1)
		Sarukura, Hakuba Village, Nagano Pref., Honshu Isl.	36.74	137.81	1124	172	6	1	rH1(6)
<i>Paris japonica</i>									
	northern Honshu	Mt. Jinba, Hirosaki City, Aomori Pref., Honshu Isl.	40.48	140.34	900	148	4	4	pH24(1), pH25(1), pH26(1), pH27(1)
		Naganuma, Kazuno City, Akita Pref., Honshu Isl.	39.98	140.82	1090	311	4	4	pH30(1), pH51(1), pH52(1), pH53(1)
		Mt. Mokko, Senboku City, Akita Pref., Honshu Isl.	39.94	140.85	1447	301	3	3	pH38(1), pH39(1), pH40(1)
		Mt. Yakeishi, Oushu City, Iwate Pref., Honshu Isl.	39.17	140.81	1200	435	3	3	pH30(1), pH61(1), pH62(1)
		Mt. Kamuro, Yuzawa City, Akita Pref., Honshu Isl.	38.91	140.50	1260	372	4	4	pH23(1), pH28(1), pH29(1), pH30(1)
		Mt. Gassan, Nishikawa Town, Yamagata Pref., Honshu Isl.	38.54	140.00	1438	441	4	4	pH10(1), pH11(1), pH12(1), pH13(1)
		Mt. Nishi-Azuma, Yonezawa City, Yamagata Pref., Honshu Isl.	37.75	140.15	1880	154	3	2	pH30(1), pH7(2)
		Mt. Iide, Kitakata City, Fukushima Pref., Honshu Isl.	37.84	139.67	1900	251	3	3	pH35(1), pH36(1), pH37(1)
	central Honshu	Mt. Aizu-komagatake, Hinoemata Village, Fukushima Pref., Honshu Isl.	37.01	139.33	1700	220	5	5	pH3(1), pH20(1), pH21(1), pH22(1), pH23(1)
		Mt. Hotaka, Kawaba Village, Gunma Pref., Honshu Isl.	36.80	139.14	2050	145	2	2	pH18(1), pH19(1)
		Mt. Naeba, Sakae Village, Nagano Pref., Honshu Isl.	36.85	138.68	1750	232	2	2	pH43(1), pH44(1)

Mt. Kurosawa, Myoko City, Niigata Pref., Honshu Isl.	36.91	138.09	2110	217	7	6	pH9(1), pH23(2), pH31(1), pH32(1), pH33(1), pH34(1) pH41(1), pH42(1), pH45(1), pH46(1), pH47(1), pH48(1), pH49(1)
Nagashiri-sawa, Hakuba Village, Nagano Pref., Honshu Isl.	36.74	137.78	1633	184	7	7	pH54(1), pH55(1), pH56(1), pH57(1), pH58(1), pH59(1) pH4(1), pH5(1), pH6(1), pH7(1), pH8(1), pH9(1)
Oh'gi-sawa, Ohmachi City, Nagano Pref., Honshu Isl.	36.56	137.71	1575	112	6	6	pH60(1)
Mt. Chogatake, Azumino City, Nagano Pref., Honshu Isl.	36.29	137.73	2550	73	6	6	pH1(1), pH2(1)
Mt. Tateyama, Tateyama Town, Toyama Pref., Honshu Isl.	36.57	137.56	1994	181	1	1	pH14(1), pH15(1), pH16(1), pH17(1)
Amo, Hida City, Gifu Pref., Honshu Isl.	36.24	136.96	1495	147	2	2	
Mt. Hakusan, Hakusan City, Ishikawa Pref., Honshu Isl.	36.14	136.75	1913	248	4	4	

N, number of samples; *h*, number of haplotypes.

Snow depth, maximum snow depth of population location. Maximum snow depth values were extracted from Japan Meteorological Agency data with 30 arc-sec resolution in the latitudinal direction (<https://www.data.jma.go.jp/obd/stats/etrn/view/atlas.html>).

Table S2 Primer pairs used for each species

Region	Primer	<i>Glaucidium palmatum</i>	<i>Ranzania japonica</i>	<i>Paris japonica</i>
Z1	CPUP_1F_trnK-UUU	O	O	O
	CPUP_1R_trnR-UCU			
Z2	CPUP_2F_trnR-UCU	O	O	O
	CPUP_2R_rpoC2			
Z3	CPUP_3F_rpoC2	-	-	O
	CPUP_3R_trnD-GUC			
Z4	CPUP_4F_trnD-GUC	O	O	O
	CPUP_4R_psaA			
Z5	CPUP_5F_psaA	O	O	O
	CPUP_5R_trnF-GAA			
Z6	CPUP_6F_trnF-GAA	O	O	O
	CPUP_6R_rbcL			
Z7	CPUP_7F_rbcL	O	O	O
	CPUP_7R_trnW-CCA			
Z8	CPUP_8F_trnW-CCA	O	O	O
	CPUP_8R_petD			
Z9	CPUP_9F_petD	O	O	O
	CPUP_9R_trnI-CAU			
Z10	CPUP_10F_trnI-CAU	-	-	O
	CPUP_10R_rps7			
Z11	CPUP_11F_rps7	O	O	O
	CPUP_11R_rm4.5			
Z12	CPUP_12F_rm4.5	O	O	O
	CPUP_12R_trnL-UAG			
Z13	CPUP_13F_trnL-UAG	O	-	O
	CPUP_13R_rps15			
Z14	CPUP_14F_rps15	O	-	O
	CPUP_14R_trnR-ACG			
Z15	CPUP_15-1_1F_trnI-CAU			
	CPUP_15-1R_rps16	O	O	O
U4	rpoC2.520R	O	O	-
	petN.3R			
U5	trnC.GCA.47F	O	O	-
	psaB.2170F			
U11	rpl16.3R	O	O	-
	ycf2.5100R			
U12	ycf2.2300F	O	O	-
	rps12.190F			
U15	ndhI.194R	-	O	-
	trnN.GUU.7R			

Z1-Z15 (Zhang et al. 2016), U4-U15 (Uribe-Convers et al. 2014).

Reference:

Uribe-Convers S, Duke JR, Moore MJ, Tank DC (2014) A long PCR-based approach for DNA enrichment prior to next-generation sequencing for systematic studies. *Appl Plant Sci* 2: 1300063

Zhang T, Zeng CX, Yang JB, Li HT, Li DZ (2016) Fifteen novel universal primer pairs for sequencing whole chloroplast genomes and a primer pair for nuclear ribosomal DNAs. *J Syst Evol* 54: 219-227

Table S3 Sequences of outgroup species used

Species	outgroup species	Accession number
<i>Glaucidium palmatum</i> (Ranunculaceae + Berberidaceae)		
	<i>Aconitum kusnezoffii</i>	MK569468
	<i>Adonis sutchuenensis</i>	MK569470
	<i>Anemone raddeana</i>	MK569472
	<i>Coptis chinensis</i>	MK569483
	<i>Eranthis stellate</i>	MK569487
	<i>Hydrastis canadensis</i>	MK569495
	<i>Ranzania japonica</i>	MH423072
<i>Ranzania japonica</i> (Berberidaceae)		
	<i>Achlys triphylla</i>	MH423064
	<i>Berberis amurensis</i>	KM057374
	<i>Berberis bealei</i>	KF176554
	<i>Caulophyllum robustum</i>	MH423066
	<i>Nandina domestica</i>	DQ923117
	<i>Podophyllum peltatum</i>	MH423071
	<i>Vancouveria hexandra</i>	MH423073
<i>Paris japonica</i> (Melanthiaceae)		
	<i>Chionographis japonica</i>	KF951065
	<i>Paris verticillata</i>	MH796669
	<i>Paris quadrifolia</i>	KX784051
	<i>Paris marmorata</i>	KX784047
	<i>Paris dunniana</i>	KX784042
	<i>Veratrum patulum</i>	KF437397
	<i>Xerophyllum tenax</i>	KM078035
	<i>Ypsilandra thibetica</i>	MH796671

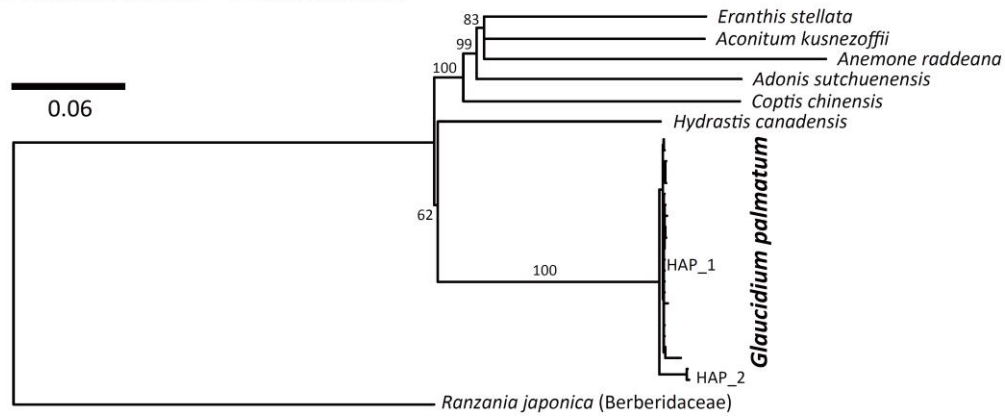
Table S4 Number of sites (bp) determined with no missing, and polymorphisms detected

	With outgroups			Without outgroups				
	number of sites	number of SNPs	Number of haplotypes*	number of sites	number of SNPs	Number of haplotypes	Number of shared haplotypes among populations	Number of singleton haplotypes
<i>Glaucidium palmatum</i>	65,091	2344	30 (7, 23)	115,061	244	37	3	21
<i>Ranzania japonica</i>	66,363	286	15 (7, 8)	113,592	15	8	1	7
<i>Paris japonica</i>	56,918	1347	61 (8, 53)	84,388	115	62	3	58

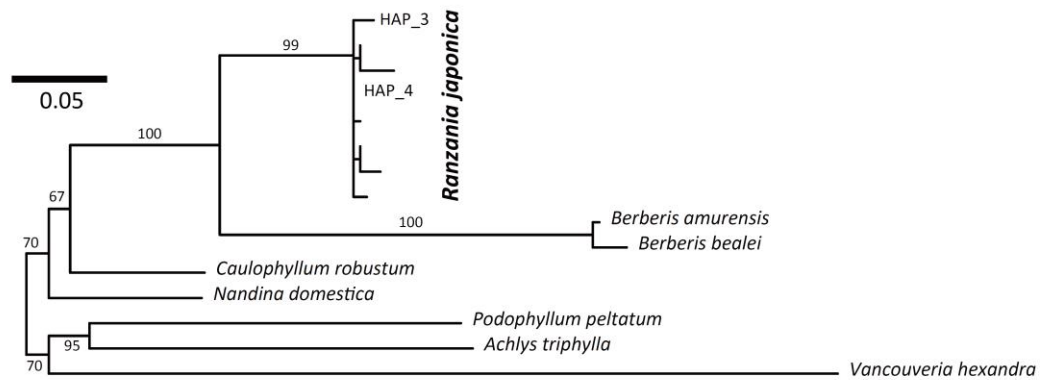
* Numbers of haplotypes for outgroups and the study species, respectively, are shown separately in parentheses.

SNP, single nucleotide polymorphism.

a Ranunculaceae + Berberidaceae



b Berberidaceae



c Melanthiaceae

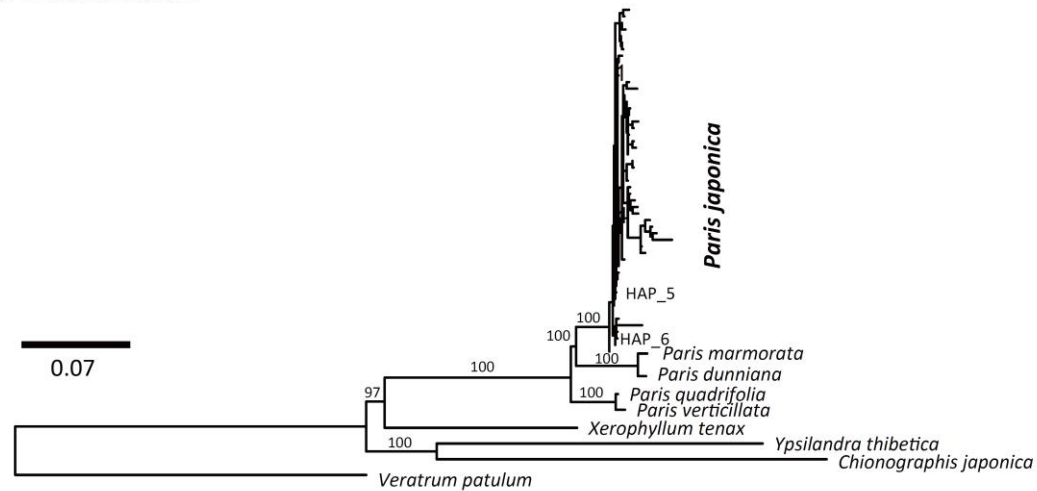


Fig. S1 Relationships between haplotypes from **a** Ranunculaceae including *Glaucidium palmatum* and Berberidaceae, **b** Berberidaceae including *Ranzania japonica*, and **c** Melanthiaceae including *Paris japonica*, estimated by a maximum likelihood method implemented in RAxML. Bootstrap probabilities are shown above nodes.

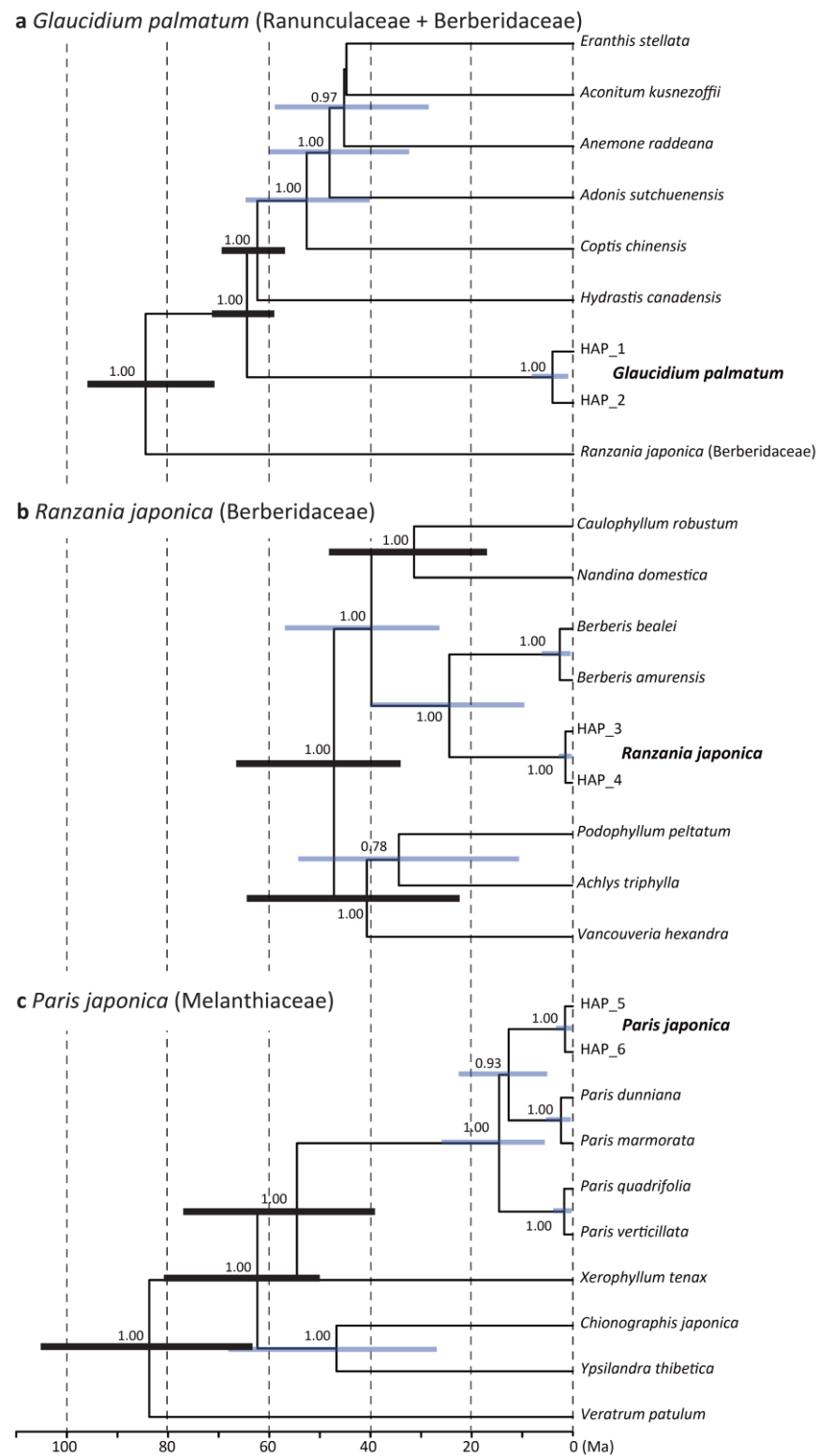


Fig. S2 Phylogenetic relationships and divergence time estimates for **a** Ranunculaceae and Berberidaceae, **b** Berberidaceae, and **c** Melanthiaceae. All posterior probabilities are shown above the nodes. Bars shown the 95% highest posterior density (95%HPD) of divergence time. Calibrated nodes are shown by black bars.

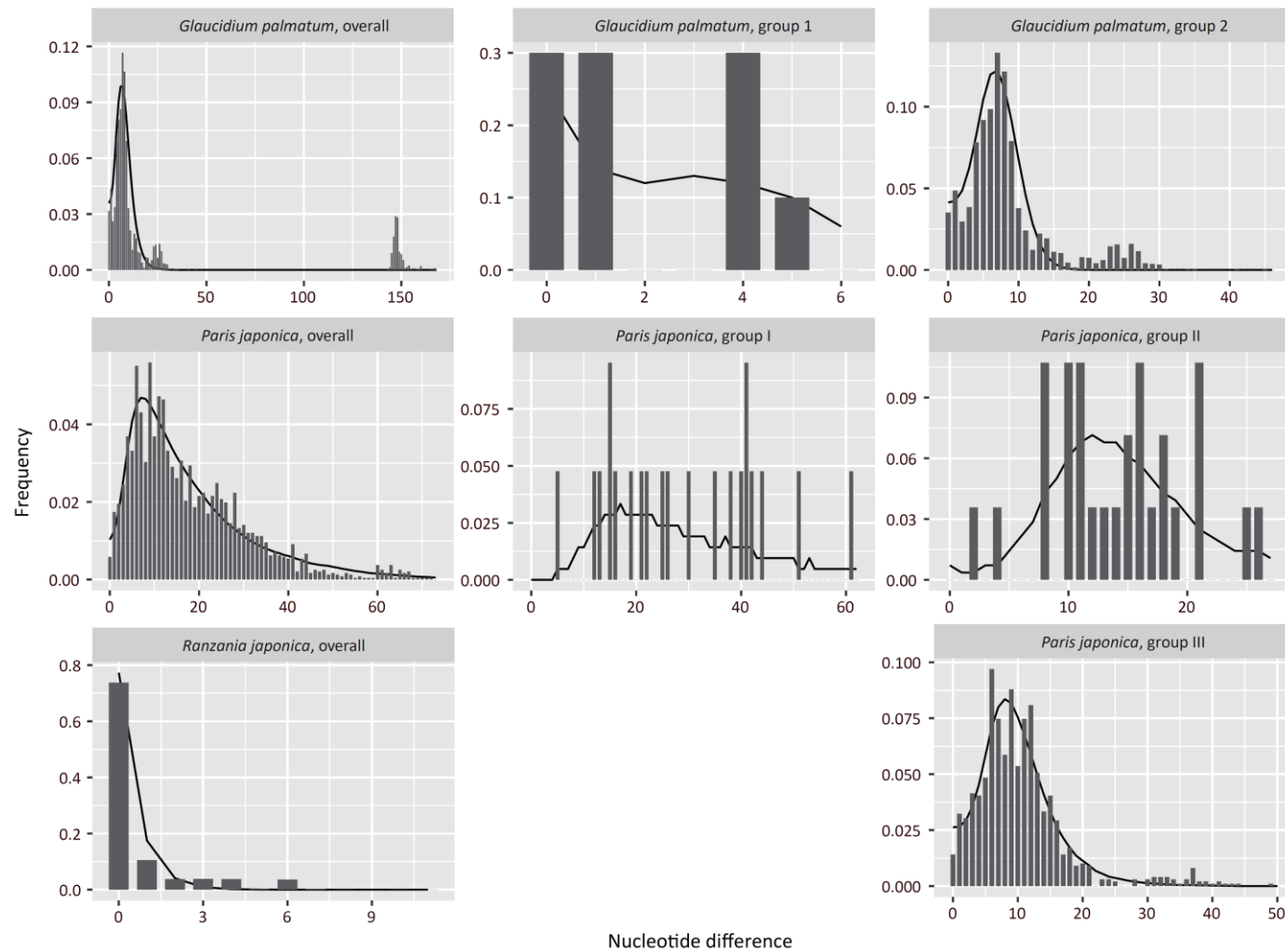


Fig. S3 The mismatch distribution for each study species and group. Bars show observed nucleotide differences between samples, and lines show the expected distribution under a sudden population expansion model.