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The migration history of the Kamchatka rhododendron (*Therorhodion camtschaticum s.l.*) indicates two distinct dispersal routes towards Eastern Beringia

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ABSTRACT

The Kamchatka rhododendron (*Therorhodion camtschaticum s.l.*) is a small, cold-resistant arcto-alpine shrub species with a controversial taxonomy and a wide Beringian distribution, from northern Eurasia to Arctic North America. Some authors regard *T. camtschaticum* and the closely related *Therorhodion glandulosum* as separate species while others suggest that they represent regionally differentiated subspecies. In order to resolve this relationship, we sampled 33 populations within the native species ranges, using three fragments of plastid DNA (ptDNA) and ten nuclear microsatellites (nSSR). We recovered two genetic lineages consistent with species rather than subspecies. The distribution of these genetic lineages are consistent with two different migration pathways of species from western Beringia to eastern Beringia: (i) for *T. glandulosum* a northern pathway from northern East Asia to the Seward Peninsula in northern Alaska; and (ii) for *T. camtschaticum* a southern pathway from the southern part of western Beringia to the Aleutian Islands and the seashore of south-east Alaska. The northern route extends from the Okhotsk seashore through the Kolyma region, Chukotka, and central and northern Kamchatka. The southern route begins at the Sikhote-Alin ridge, and goes through Sakhalin Island, the Kuril Islands, and southern Kamchatka. Demographic analysis using nSSR data inferred a divergence time between *T. camtschaticum* and *T. glandulosum* long before the Last Glacial Maximum.

Keywords: biogeography; nuclear microsatellites; north-east Asia; population structure; plastid DNA; *Therorhodion glandulosum*

INTRODUCTION

The history of arcto-alpine plant species is of particular current interest with regard to the phylogeography of the Northern Hemisphere, because arctic-alpine plants experience climate change at much higher rates than taxa at other localities globally (Rantanen *et al.* 2022). The current distribution and genetic diversity of cold-resistant plants reflects the history of climate fluctuations during the Late Pleistocene (Hewitt 2000, Alsos *et al.* 2022). Numerous studies have inferred refugia and dispersal routes of cold resistant flora in Europe (Brochmann *et al.* 2003, Birks and Willis 2008, Westergaard *et al.* 2011, 2019, Eidesen *et al.* 2013), North Asia (Abbott and Brochmann 2003, Polezhaeva *et al.* 2010, Semerikov *et al.* 2013, 2019, Ikeda *et al.* 2017, 2018, 2020, 2022), and North America (Tremblay and Schoen 1999, Eriksen and Topel 2006, Marr *et al.* 2008, Beatty and Provan 2010, Allen *et al.* 2012, 2015). Of particular relevance to current distributions are areas that were unglaciated during the Last Glacial Maximum (LGM) (Svendsen *et al.* 2004). The low sea levels during the peaks of the glacial periods exposed a vast land area extending from the McKenzie River in western Canada to the west as far as the Lena River in Siberia, referred to as Beringia (Hultén 1937). Beringia is considered a refugium for high latitude species based on both fossil pollen data (Brubaker *at al.* 2005, Lozhkin *et al.* 2007), and extant plant and animal genetic data (Abbott *et al.* 2000, Abbott and Brochmann 2003, Alsos *et al.* 2005, Loehr *et al.* 2006, Polezhaeva *et al.* 2010, Hope *et al.* 2013, Kohli *et al.* 2015, Fedorov *et al.* 2020, Winker *et al.* 2023). During its periodic emergences the Bering Land Bridge (BLB) served as a migration corridor between Asia and North America (NAm) for seed plants and other fauna (Sanmartín *et al.* 2001).

Such episodes have occurred since the Early Eocene, with asymmetrical dispersal documented from different time periods including the expansion of Arctic vegetation during the LGM (Wen *et al.* 2016, Graham 2018, Jiang *et al.* 2019). Recent biogeographic studies on Arctic plants reveal that populations of arcto-alpine plants from Beringia may have interacted repeatedly with populations in the peripheral Beringian territories of East Asia during the Pleistocene. For example, the genetic structure of the tundra plant species *Kalmia procumbens* (L.) Gift & Kron, *Phyllodoce aleutica* (Spreng.) A.Heller, and *Primula cuneifolia* Ledeb. point to a Beringian connection with the Japanese archipelago via the Aleutian Islands, Kamchatka, and the Kuril Islands (Ikeda *et al.* 2017, 2018, 2020). The pattern of range dynamics of arcto-alpine plants in the Beringian region may vary depending on the ecological characteristics of the species. When climatic conditions change, some species persist in the region, or shift their ranges in a southward or northward direction. A postglacial range expansion to Alaska from north-east Asia with a loss of genetic diversity was postulated for *Phyllodoce aleutica* (Ikeda *et al.* 2018) and *Primula cuneifolia* (Ikeda *et al.* 2022). Thus, amphiberingian taxa are good examples for the study of diversification and phylogeography in this region (Ickert-Bond *et al.* 2009).
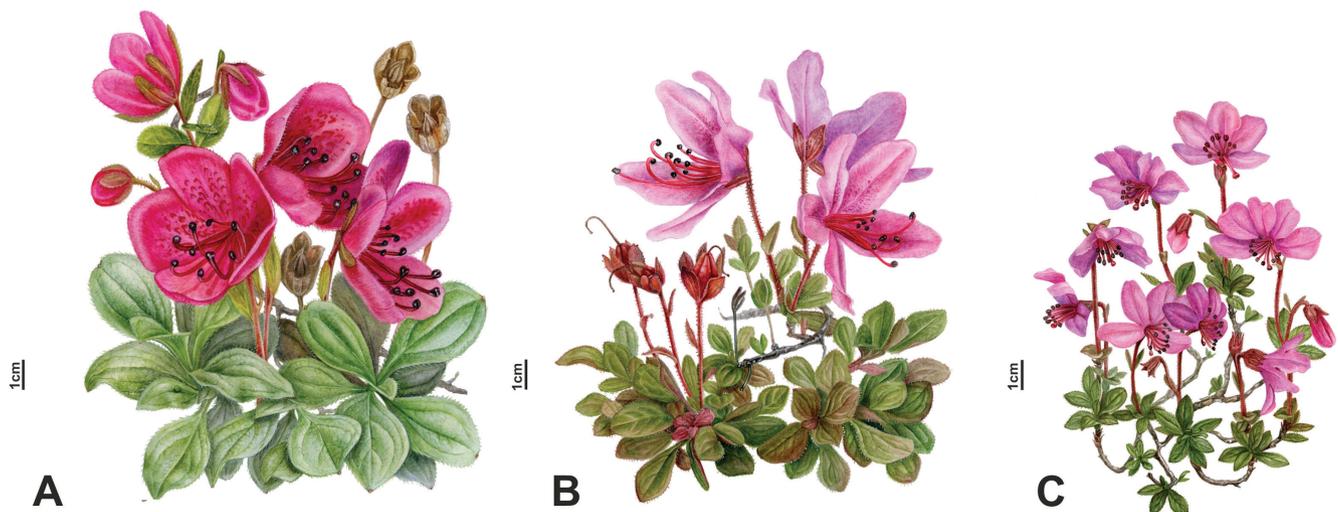
Here we elucidated the genetic structure and demographic history of two amphiberingian taxa—*Therorhodion camtschaticum* (Pall.) Small and *Therorhodion glandulosum* Standl. ex Small, which are both often referred to as the Kamchatka rhododendron (Fig. 1A, B). They are small, deciduous shrubs (up to 30 cm in height) with bright pink flowers that are widespread in high alpine and subalpine areas, often found on ridges and slopes, but they also are present at higher elevations of the Pacific forest belt (Viereck and Little 1972, 2007, Aleksandrova 1975). A third closely related taxon, *Therorhodion redowskianum* (Maxim.) Hutch., is a species with well-distinguishable morphological characters and only Asian distribution (Fig. 1C).

For some time *Therorhodion* was considered a subgenus of *Rhododendron* L. (Busch 1915, Hultén 1930, Viereck and Little 1972, Aleksandrova 1975, He and Chamberlain 2005, Usenko

2010, Yurtsev *et al.* 2010). However, numerous phylogenetic studies based on both molecular and morphological evidence (Kron 1997, Gao *et al.* 2002, Goetsch *et al.* 2005, Gillespie and Kron 2010), particularly seed and leaf morphology and pollen diversity (Wang *et al.* 2007a, b, Sarwar and Takahashi 2013) all support *Therorhodion* as a distinct taxon.

Kamchatka rhododendrons have been recognized either as two subspecies/varieties or two distinct species: *Therorhodion camtschaticum* (Pall.) Small (syn. *Rhododendron camtschaticum* Pall. or var. *pallasianum* Kom.) and *Therorhodion glandulosum* Standl. ex Small [syn. *Rhododendron camtschaticum* subsp. *glandulosum* Standl. (Hult.) or var. *pumilum* E.Busch] (Busch 1915, Hultén 1930, Viereck and Little 1972, 2007, Aleksandrova 1975). Morphologically the two species are very similar, and they are found on both sides of the Bering Strait and the southern periphery of Beringia over a wide latitudinal range. Sometimes *T. glandulosum* is considered as a northern race of *T. camtschaticum* adapted to harsher conditions. The main characters of taxonomic utility are the presence of glandular foliar trichomes and a glabrous corolla in *T. glandulosum*, in contrast to *T. camtschaticum* with simple foliar trichomes and a pubescent corolla. However, the study of herbarium specimens, and observations in the field (Polezhaeva M.A., personal observations), show that simple trichomes are also found in *T. glandulosum.* The corolla type is not known in samples lacking flowers.

Different authors have interpreted the taxonomic relationships between *T. camtschaticum* and *T. glandulosum* in different ways. Russian floristic treatments and some English language reports consider the taxa as subspecies (Hultén 1968, Viereck and Little 1972, Aleksandrova 1975, Yurtsev *et al.* 2010, Takahashi 2015). In contrast, more recent descriptions separate *T. camtschaticum* and *T. glandulosum* at the species level (Viereck and Little 2007, Kron and Judd 2009, Elven *et al.* 2011). There are no clear distributional boundaries of the two species, but the literature suggests that *T. glandulosum* is distributed primarily in north-east Asia along the Okhotsk seashore, the Kolyma region, Chukotka, central and northern Kamchatka, and in North America in Alaska (mostly on the Seward Peninsula and the lower Yukon Valley) (Ohwi 1965,

Figure 1. Drawings of the three species of *Therorhodion*. A, *T. camtschaticum*. B, *T. glandulosum*. C, *T. redowskianum.* Drawings by Maria Polezhaeva.

Aleksandrova 1975, Hohryakov 1991, Mochalova and Yakubov 2004, Yakubov and Chernyagina 2004, Kron and Judd 2009). The range of *T. camtschaticum* encompasses southern Alaska, the Aleutian Islands, and northern Japan as well as southern localities of the Russian Far East (mostly the Kuril Islands and southern Kamchatka). Suggested areas of sympatry of the two taxa in Russia are Kamchatka, where *T. glandulosum* is mentioned for the central part of the peninsula and *T. camtschaticum* or both species for the southern areas, and the zone of transition of the Okhotsk coast to the coast of the Tatar Strait, where both species could hypothetically grow together.

Several recent studies have focused on the genetic diversity and biogeography of *Therorhodion* species. We screened the diversity of several plastid DNA (ptDNA) fragments in populations from Kamchatka, where *T. camtschaticum* and *T. glandulosum* co-occur, and revealed high differentiation between these two taxa (Polezhaeva *et al.* 2020). Hata *et al.* (2017) used seven nuclear loci to evaluate the hypothesis of a plant refugium in the Aleutian Islands. Their study revealed genetic similarities between southern Kamchatka and the Aleutian populations of *T. camtschaticum*. This outcome refutes this hypothesis and instead proposes a recent colonization of this area. A strong differentiation between Beringian and Japanese populations was also shown. Finally, a phylogenetic study by Oliver *et al.* (2017) assessed the evolutionary history of the genus. They included *Therorhodion redowskianum* (Maxim.) Hutch. for the first time (Fig. 1C) and evaluated the dispersal history in the context of the availability of the BLB over time. Whereas these studies addressed biogeography and evolutionary history of *Therorhodion*, other questions remain. In particular the persistence of these species in eastern Beringia during the LGM or migration from western Beringia or southern parts of the Pacific region remain unresolved. The lack of population-level sampling to fully characterize each of the different geographic areas has hindered fully elucidating the migration history and population structure of these species in different regions of Beringia.

In the present study, we used both ptDNA sequences and nuclear microsatellite (nSSR) data and a broad geographic sampling approach across the distributional range of *T. camtschaticum s.l.* in East Asia (excluding Japan) and Alaska. We addressed the following questions: (1) what is the geographic structure of genetic variation in *T. camtschaticum* and *T. glandulosum* and how does it vary, based on the plastid and nuclear markers? (2) Did the populations of these species persist in western North America (NAm) during the LGM and does NAm harbour unique lineages or do they reveal genetic similarity with nearby territories? (3) How did these lineages diversify over time and disperse into their present geographic range? For the last point we also re-used nSSR diversity data for *T. redowskianum*, which we obtained previously (Polezhaeva *et al.* 2022). Analysing the genetic structure of *Therorhodion* species provides new insights on the diversification processes and evolutionary history of the arctic-alpine flora in the Pacific region.

MATERIAL AND METHODS

Population sampling and DNA extraction

Samples of *T. camtschaticum* and *T. glandulosum* were collected from an area covering most of the two species' distributional ranges in North America (Alaska) and north-east Asia. The sample set contained 4–34 individuals per sample site (representing the population) depending on the accessibility and collecting conditions of the populations (Table 1; Figs 2A, 3A). We collected fresh material for DNA analysis without depositing herbarium vouchers. However, we provide information on herbarium vouchers of *T. camtschaticum* and *T. glandulosum* in herbaria of the Far East of Russia and Alaska, USA, from the locations where our sets of leaf samples were collected for the study, or the nearest locations (Supporting information, Data S1).

Individuals were collected at least 5–10 m apart from one another, thus minimizing the collection of asexually (clonally) reproduced plants. We also included a sample set from Kamchatka from a previous study (Polezhaeva *et al.* 2020). All individuals collected were analysed for variation in nuclear microsatellite loci (see below). For analysis of ptDNA variation we randomly chose 2–18 individuals per population which yielded a good PCR product. In total we examined 33 populations and 265 individuals (Table 1) for ptDNA variability and 30 populations and 510 individuals (Table 1; Figs 2A, 3A) for nSSR variability. Collected leaves were stored in silica gel prior to DNA extraction.

Total genomic DNA was extracted from silica gel-dried leaves or from air-dried leaves (population samples 30a and 30b) using the CTAB method according to Devey *et al.* (1996). The quality and yield of DNA isolated was checked using a spectrophotometer.

Analysis of plastid DNA

For detection of ptDNA polymorphisms the intergenic spacers *psb*A-*trn*H (AH), *trn*K–*mat*K (matK), and *trn*V-*ndh*C (VC) were chosen. To improve amplification as compared to universal primers often used, we designed new primers based on the *Rhododendron pulchrum* Sweet chloroplast genome sequence (NCBI number MN182619.1). The sequences of the primers used are as follows: *psb*A(rp)-GCTCCATCTACAACCGGATAAGA, *trn*H(rp)-TCTTTCTTTCAGAGTGGGCGA; K707(rp)-CAGGAAAGGCTCTTTAAACACCC, K2R(rp)-AAATCCTCTACCCTTGCCTCAA; *ndh*C(rp)-CGAGAAGGTCTACGGTTCGAG, *trn*V(rp)-TCGATCAAGTACGGGAAACTCA. The thermocycling conditions for all fragments were: 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 2 min, plus a final extension of 72 °C for 7 min. The PCR products were checked on 1.0% agarose gels, cleaned with an ExPure Kit (Nimagen, Netherlands) and then used as templates for sequencing using a Brilliant Dye v.3.1 Kit (Nimagen). We obtained sequences of these fragments for 2–18 individuals from each population using a NANOFOR 05 Genetic Analyzer (Syntol, Russia). The sequences of fragments for each combined haplotype were placed in GenBank with NCBI accession numbers OR701351 – OR701368; OR683694 – OR683702 (Supporting information, Data S2).

Analysis of nuclear SSR

Since there are no specific microsatellite loci available for *Therorhodion*, we tested primers developed for *Rhododendron aureum* Georgi (Kwak *et al.* 2015) and *Rhododendron ferrugineum* L. (Charrier *et al.* 2014). Primers were initially tested in simplex reactions on eight DNA samples from different parts of the Kamchatka rhododendron distribution to check for the

Table 1. Sampling localities and summary of genetic diversity statistics for studied populations of *T. camtschaticum* and *T. glandulosum* from the wild

Population ID, name, location	Lat./Long.	ptDNA		nSSR						
		$N1$[a]	$Hapl(N)$[b]	$N2$[a]	A_a[c]	$A_{r(10)}$[d]	A_e[e]	H_o[f]	H_e[g]	F[h]
T. camtschaticum										
1 BER Bering Island, Commander Islands	55.15 / 166.25	5	h1:5	6	2.3	-	1.79	0.233	0.314	0.261
2 UN Unalaska Island, Alaska	53.91 / -166.32	3	h1:3	3	2.1	-	1.82	0.367	0.328	-0.159
3 AD Adak Island, Alaska	51.81 / -176.50	10	h1:10	12	3.1	3.2	1.89	0.275	0.394	0.311
4 AG Agattu Island, Alaska	52.48 /173.71	5	h1:5	4	1.9	-	1.74	0.325	0.313	0.023
5 BUL Buldir Island, Alaska,	52.35 / 175.93	4	h1:4	10	2.5	2.5	1.60	0.300	0.306	-0.024
6 GAR Gareloi Island, Alaska	51.79 / -178.86	5	h1:4	4	1.7	-	1.38	0.100	0.216	0.479
7 KIS Kiska Island, Alaska	52.05 / 177.61	5	h1:5	5	2.0	-	1.71	0.260	0.256	0.025
8 KOD Kodiak Island, Alaska	57.46 / -153.43	10	h1:10	10	2.1	2.1	1.57	0.200	0.269	0.285
9 AV Mt. Avacha, Kamchatka	53.25 / 158.83	10	h1:7 h2:3	10	3.4	3.4	2.35	0.480	0.441	-0.057
10 MUT Mt. Mutnovsky, Kamchatka	52.43 /158.18	10	h1:10	17	6.0	5.2	3.88	0.512	0.623	0.255
11 YLIZ Yelizivo surr., Kamchatka	53.16 /158.13	10	h1:6 h2:4	30	5.1	4.1	2.99	0.477	0.475	-0.033
12 SAHZ Mt. Zhdanko, Sakhalin	48.25 /142.58	12	h5:12	34	5.3	4.0	3.20	0.538	0.548	0.047
13 SAHC Mt. Chekhov, Sakhalin	47.01 /142.84	14	h4:3 h5:11	30	4.3	3.8	2.92	0.447	0.506	0.205
14 OR Cape Orlova, Khabarovsk krai	51.425 /140.885	10	h6:10	23	3.7	3.2	2.65	0.387	0.392	-0.003
15 DEK De-Kastri, Khabarovsk krai	51.49/140.81	11	h6:11	22	4.9	3.9	2.96	0.418	0.425	0.048
16 KUNM Mt. Mendeleev, Kunashir Island, Kuril Islands	44.01 /145.76	7	h1:7	12	4.4	4.2	3.04	0.433	0.508	0.109
17 SKUR Uzhno-Kurilsk, Kunashir Island, Kuril Islands	44.025/145.865	7	h1:7	20	3.8	3.3	2.52	0.465	0.493	0.072
18 SHIK Shikotan Island, Kuril Islands	43.76 / 146.69	13	h6:13	27	7.9	5.8	4.59	0.474	0.550	0.140
18a Iturup Island, Kuril Islands	45.07 / 147.86	6	h1:3 h2:3	-	-	-	-	-	-	-
T. glandulosum										
19 CHG, Thermal springs, Chukotka	64.80/-173.40	4	h3:4	14	3.7	3.5	2.38	0.400	0.442	0.065
20 CHS, Sireniki, Chukotka	64.406/-173.924	4	h3:4	17	3.5	3.1	2.07	0.306	0.369	0.120
21 CHV, Volchja River, Chukotka	64.76/177.70	4	h3:4	8	2.4	-	1.59	0.200	0.230	0.123
22 CHU, Uelen, Chukotka	66.12/-169.88	4	h3:4	17	3.6	3.2	2.19	0.369	0.426	0.132
23 CHA, Arinay, Chukotka	62.70 /179.28	7	h3:7	24	4.9	3.8	2.49	0.438	0.459	0.041
24 SEW Seward Peninsula, Alaska	65.50 /-167.00	18	h3:18	12	2.9	2.7	1.92	0.292	0.333	0.080
25 ZAV, Zavjalova Island, Magadan dist.	59.066 /150.65	9	h3:9	24	8.8	6.3	4.35	0.554	0.597	0.114
26 KV Mt. Kamennyi Venez, Magadan dist.	59.52 / 150.67	7	h3:7	17	7.6	6.1	4.60	0.580	0.629	0.068
27 MAG, Magadan surr., Magadan dist.	59.58 /150.75	7	h3:7	24	5.4	4.4	3.50	0.525	0.556	0.079

Table 1. Continued

Population ID, name, location	Lat./Long.	ptDNA		nSSR						
		$N1^a$	$Hapl(N)^b$	$N2^a$	A_a^c	$A_{r(10)}^d$	A_e^e	H_o^f	H_e^g	F^h
T. camtschaticum										
28 NUK Nukla, Magadan dist.	59.582 / 151.128	10	h3:10	20	6.0	4.8	3.13	0.500	0.536	0.041
29 ARM, Arman, Magadan dist.	59.69 / 150.33	11	h3:11	26	8.5	6.3	4.57	0.550	0.599	0.150
30 ES Esso, Kamchatka Peninsula	55.93 / 158.69	13	h3:10 h7:3	28	5.2	4.0	2.85	0.454	0.531	0.157
30a Koryakia, Kamchatka Peninsula	59.577 / 163.08	2	h3:2	-	-	-	-	-	-	-
30b Ayan, Khabarovsk krai	56.468 / 138.022	8	h3:8	-	-	-	-	-	-	-

^aN, N_2 number of samples analysed for ptDNA and nSSR, respectively; $^bHapl(N)$ ptDNA haplotype number and quantity; ^cA_a mean number of allele per locus, ^dA_r mean allelic richness rarefied to a subsample of 10 individuals, ^eA_e mean number of effective alleles, ^fH_o observed heterozygosity, ^gH_e expected heterozygosity, ^hF Wright's fixation index.
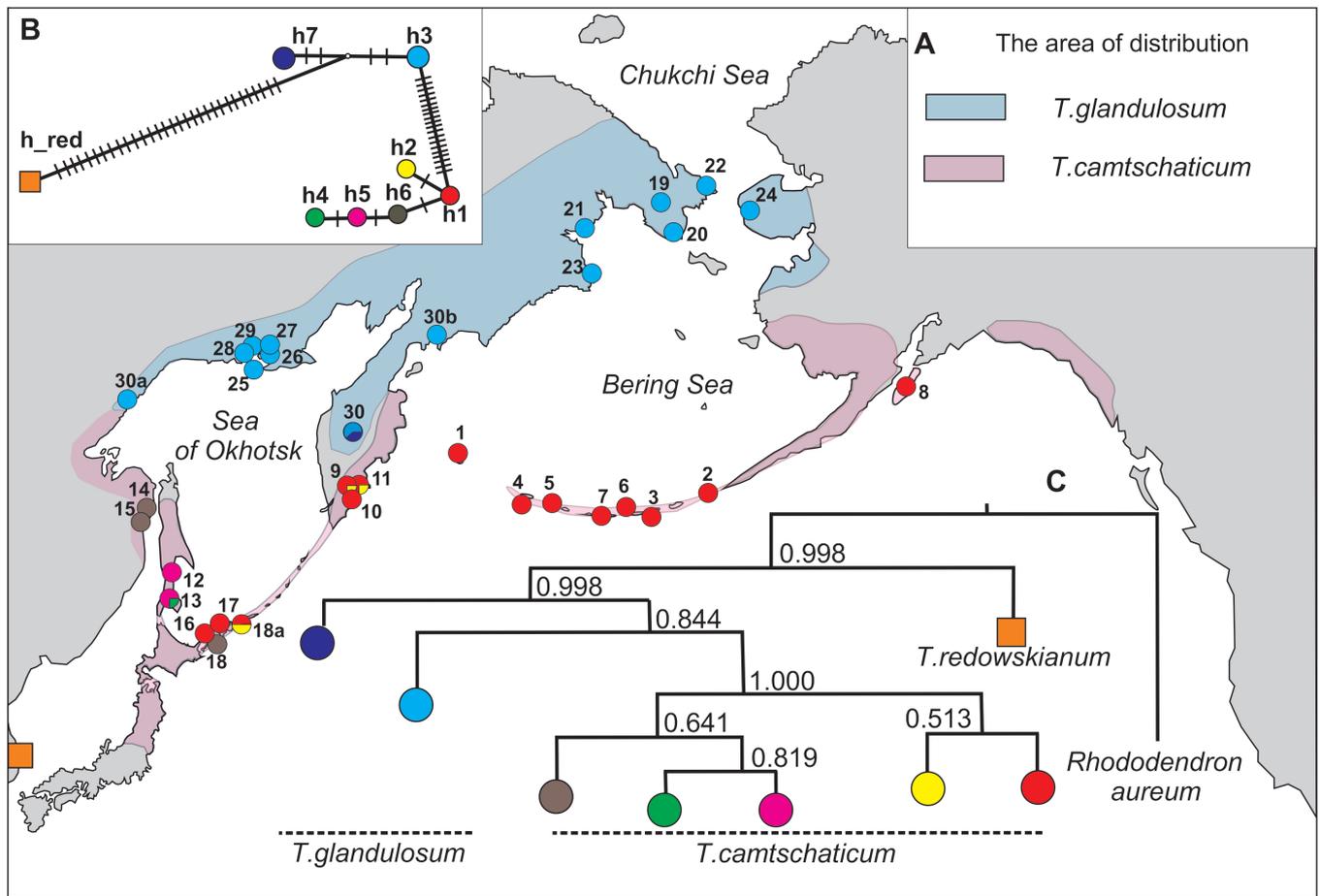
possibility of cross-amplification. After electrophoresis on a 6% denaturing polyacrylamide gel with subsequent silver staining, a set of 13 nSSR with polymorphisms and clear bands were selected. Primers for these loci were labelled by one of the fluorescent dyes FAM, HEX, or ROX (Syntol, Russia) and tested again in three multiplex panels on 16 samples from different geographical regions using capillary electrophoresis on a NANOFOR 05 Genetic Analyzer in the presence of a S-450 molecular weight marker (Gordiz, Russia). Multiplex PCR conditions were: 94 °C 3 min [98 °C 30 s – 57 °C (ramp 0.3 °C \s) 2 min – 72 °C 90 s] three cycles; [94 °C 30 s – 58 °C (ramp 0.3 °C \s) 2 min – 72 °C 90s] five cycles; [90 °C 30 s – 59 °C (ramp 0.3 °C \s) 2 min – 72 °C 75 s] 19 cycles; 90 °C30 s – 68 °C 30 min – 10 °C ∞.

In total 10 polymorphic loci showing high reproducibility with easy electrophoregrams interpretation were chosen: RA10, RA14, RA99, RA138, RA148 (Kwak *et al.* 2015) and RF6P2, RF74P1, RF14P3, RF46P2, RF113P2 (Charrier *et al.* 2014).

Genetic diversity and structure

Sequence alignment of ptDNA regions and accounting for variant sites were performed using BioEdit v.7.0.9 software (Hall 1999). Construction of the phylogenetic tree of haplotypes using sequences of the ptDNA regions for *T. camtschaticum* and *T. glandulosum* obtained earlier (NCBI numbers MN125545–125551, ON508844–508850) and the current study (OR683694–OR683702; OR701351–OR701368) was performed using MrBayes software v.3.1.2. (Ronquist and Huelsenbeck 2003). Each haplotype was represented by a combination of the sequences of the three ptDNA regions. Sequences of *T. redowskianum* and *Rhododendron aureum* (*Rhododendron* subgenus *Hymenanthes*) were included as outgroups. The relationships between ptDNA haplotypes were constructed using NETWORK v.4.6.1.2 (Bandelt *et al.* 1999).

For nSSR markers, allele sizes were determined using GENEMAPPER v.4.0. (Applied Biosystems, USA), first, automatically and then checked manually (Supporting information, Data S3). Departure from the Hardy–Weinberg equilibrium (HWE) at each locus and linkage disequilibrium (LD) were tested by randomization tests with 1000 permutations for each population at pairs of loci in GENEPOP (Raymond and Rousset 1995). Data were analysed after Bonferroni's correction for multiple testing. The frequency of null alleles was estimated with the second method of Brookfield (1996), as implemented in the MICRO-CHECKER 2.2.3 software (van Oosterhout *et al.* 2004). The diversity statistics for each population included the mean number of alleles per locus (*Aa*), the average number of effective alleles (*Ae*), and observed heterozygosity (*Ho*), and the expected heterozygosity (*He*) and Wright's fixation index were determined in GENALEX 6.5 (Peakall and Smouse 2012). To study the population structure, we first used principal coordinate analysis based on the pairwise Nei's genetic distances between populations (Nei *et al.* 1983) using GENALEX 6.5. Secondly, a Bayesian clustering approach was implemented using STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The number of potential clusters (*K*) was assessed with five replicates for each *K* value ranging from 1 to 10 with a burn-in length of 50 000 and 500 000 iterations of each chain using the admixture model along with the assumption of correlated allele frequencies

Figure 2. A, Approximate distribution pattern (adapted from Busch 1915, Hultén 1968, Vierek and Little 1972, Hohryakov 1991), geographical localities of the sampling sites, and distribution of ptDNA genotypes of *T. glandulosum* and *T. camtschaticum* in Beringia. Population numbers are explained in Table 1. B, NETWORK inferred phylogenetic relationships among haplotypes: *T. glandulosum* h3, h7; *T. camtschaticum* h1, h2, h4–h6, *T. redowskianum* h_red. C, Bayesian tree phylogeny based on the eight ptDNA haplotypes of *Therorhodion* of north-east Asia with *R. aureum* as the outgroup. Two lineages, *T. glandulosum* and *T. camtschaticum*, are marked.

between groups (Falush *et al.* 2003). The most likely number of clusters was estimated on the basis of the posterior probability of the data for a given K [LN $P\,(D)$] or the ad hoc statistic delta K described in Evanno *et al.* (2005) and was conducted with STRUCTURE Harvester (Earl and von Holdt 2012) and CLUMPAK (Kopelman *et al.* 2015). Additionally, we used BAPS 5.4 (Corander *et al.* 2004), which performs a population mixture analysis based on the clustering of individuals with non-identical allele frequencies, taking into account the geographical information of the samples. The program was run for $K = 2$–10 with 10 replicates for each value of K.
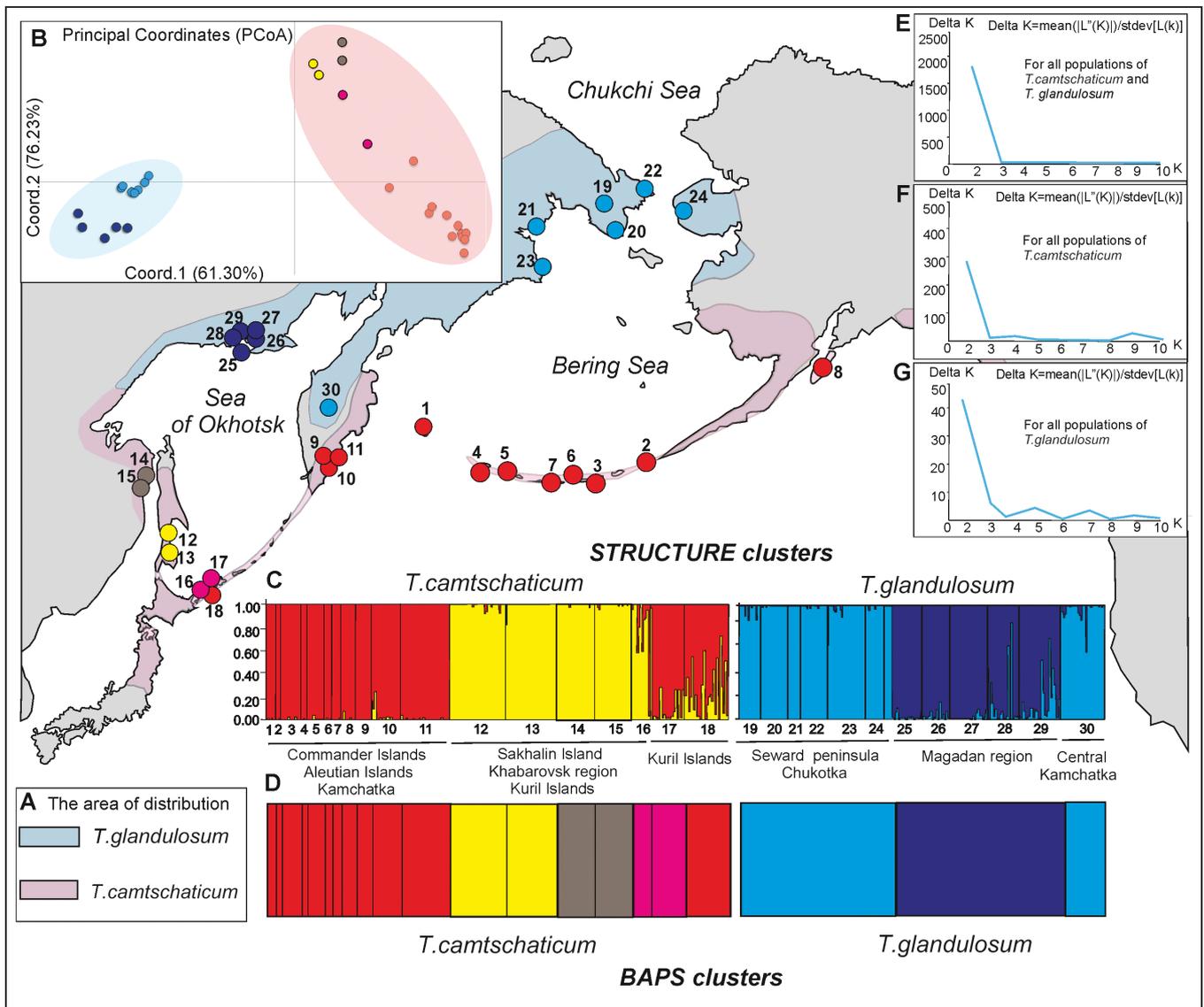
Hierarchical partitioning of diversity of both types of markers among species, populations, and individuals was based on analysis of molecular variance (AMOVA; Excoffier *et al.* 1992), using the program Arlequin v.3.5.1.2.

Approximate Bayesian computation

To explore the demographic history and the time of divergence of *Therorhodion* in the Pacific region several scenarios were compared using an Approximate Bayesian Computation (ABC) approach implemented in DIYABC 2.1.0 (Cornuet *et al.* 2014). We added the microsatellite diversity data of *T. redowskianum*,

obtained previously (Polezhaeva *et al.* 2022) with the same set of loci except RA99 and used the remaining nine loci, common for all three species, for the computation.

Effective population size (Ne) and the time of divergence (td) were estimated for the three taxa *T. camtschaticum*, *T. glandulosum*, and *T. redowskianum*. We tested four scenarios (Fig. 4): (1) *T. redowskianum* diverged first, then *T. camtschaticum*, then *T. glandulosum* diverged last; (2) *T. redowskianum* diverged from *T. camtschaticum*; (3) *T. redowskianum* diverged from *T. glandulosum*. Hypotheses 2 and 3 were put forward based on the assumption by Yurtsev (1968) that *T. redowskianum* originated as a derivative of the pre-oceanic *T. camtschaticum s.l.* Lastly, scenario (4) assumed a simultaneous divergence of the three lineages from a common ancestor. A total of 1×10^5 simulations were run for each scenario. The effective population sizes of *N1* 'T. redowskianum', *N2* 'T. camtschaticum', and *N3* 'T. glandulosum' ranged from 500 to 500 000. According to the ontogenetic study by Mazurenko (1980), *T. camtschaticum* first flowers in the wild at the age of 10–40 years, depending on conditions, and individuals can live up to 100 and even longer. Assuming a mean generation time as 10–40 years, the time of divergence t1 between *T. camtschaticum* and *T. glandulosum* was set as a minimum of 500

Figure 3. A, Approximate distribution pattern (adapted from Busch 1915, Hultén 1968, Vierek and Little 1972, Hohryakov 1991) and geographical localities of the sampling sites of *T. glandulosum* and *T. camtschaticum* in Beringia. Population numbers are explained in Table 1 and the colour of the sampling sites corresponds to the BAPS clusters. B, Principal coordinates analysis (PCoA) of the 30 studied populations. The colours correspond to the BAPS clusters. C, STRUCTURE assignment of individuals to ancestral groups: for 279 individuals of *T. camtschaticum* (K = 2), for 231 individuals of *T. glandulosum* (K = 2). D, BAPS assignment of genetically closest individuals to geographical groups for *T. camtschaticum* (K = 5), and *T. glandulosum* (K = 2). E–G, Plots for detecting the number of K groups in STRUCTURE harvester and CLUMPAK that best fit the data.
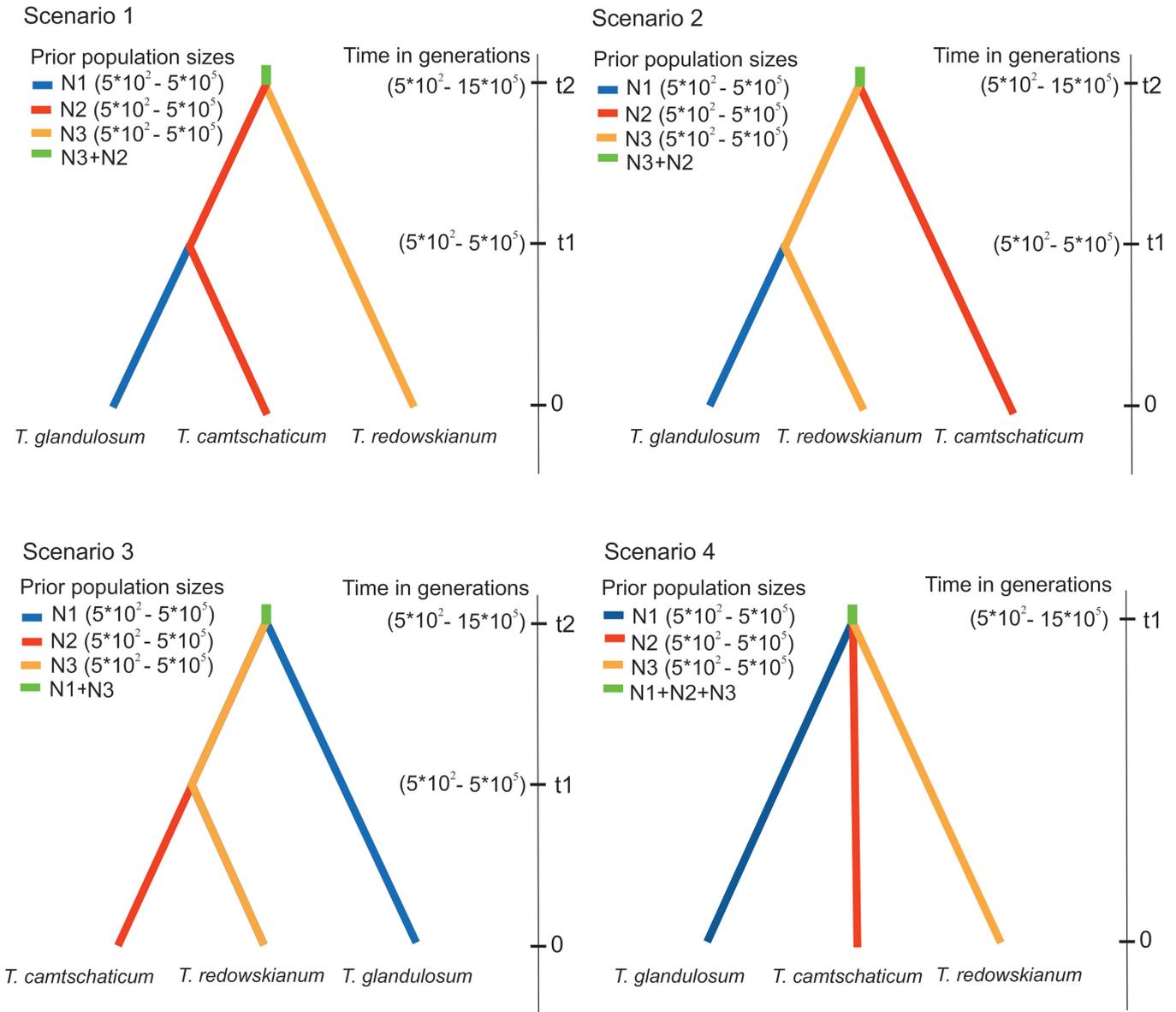
generations to a maximum of 500 000 generations. The time of divergence t2 between *T. redowskianum* and two other species was set as a minimum of 500 generations to a maximum of 1 500 000 generations. Since we used microsatellite variability data, we used a Generalized Stepwise Mutation (GSM) model and a mutation rate of 10^{-2} to 10^{-6} substitutions per locus per generation (Li *et al.* 2002). The DIYABC option 'pre-assessment of a priori combinations of scenarios' was used to initially test the scenarios, a priori values, and determine the optimal set of summary statistics. In total, five summary statistics were used. One-sample statistics included the mean number of alleles, mean size variant, and genetic diversity. Two-sample statistics included the classification index and shared allele distances. Next, the options

with the analyses of 'model checking', 'comparison of scenarios', and 'confidence in scenario choice' were used.

RESULTS

Plastid DNA

Three ptDNA fragments were combined into one contig with a total length of 1964 bp (HA 1–370; *mat*K 371–1140; VC 1141–1964). In total seven haplotypes were identified for Kamchatka rhododendrons: h1, h2, h4, h5, h6 for *T. camtschaticum*, h3 and h7 for *T. glandulosum*, and two haplotypes for the two outgroup taxa *T. redowskianum* (h_red) and *Rhododendron aureum* (Table 1; Fig. 2). The haplotype

Figure 4. Graphical representation of the four scenarios used for the DIYABC simulations (see text for details).

network (Fig. 2B) shows the relatedness between the haplotypes of *T. camtschaticum* that differ from each other by only one mutation. The haplotypes represented for *T. glandulosum* are also close (h3 and h7 differ by four mutations). These two lineages of haplotypes differ from each other by at least 20 mutations, which is comparable with the difference recovered from the haplotype of *T. redowskianum* (a minimum of 31 mutations). Using a Bayesian phylogenetic approach *T. redowskianum* is inferred as the earliest diverging lineage within *Therorhodion* (Fig. 2C). Further diverging haplotypes of *T. glandulosum*, h3 and h7, are distributed in populations of the Seward Peninsula in Alaska (24), in Chukotka (19–23), in the Magadan Oblast (25–29), and in central (30) and northern Kamchatka (30b). A separate and diverse clade includes all populations of *T. camtschaticum* with the most frequent haplotype h1 present in southern Alaska (8), the Aleutian Islands (2–6), the Commander Islands (1), most of southern Kamchatka (9–11), and the Kuril Islands (16, 17, 18a). A less

frequent haplotype h2 is found in southern Kamchatka (9, 11) and the Kuril Islands (18a). Haplotype h6 is present in the Khabarovsk Region (14, 15) and the Kurils (18). A separate subclade including haplotypes h4 and h5 is exclusively found in populations from Sakhalin (12, 13). AMOVA analyses (Table 3) revealed that the level of differentiation among all the populations surveyed was high $F_{ST} = 0.909$, $P < 0.001$ and about 51% of the total ptDNA variation occurred between the two species ($F_{CT} = 0.517$). It is noteworthy that the genetic variability of *T. camtschaticum* was much higher than that of *T. glandulosum* at all levels. In both lineages differentiation among populations was slightly higher than within populations: for *T. camtschaticum* we recovered a $F_{ST} = 0.876$ and $F_{SC} = 0.837$, and $F_{ST} = 0.536$ and $F_{SC} = 0.507$ for the *T. glandulosum* lineage. We also calculated the level of ptDNA differentiation within each species (Table 3). Populations of *T. camtschaticum* were divided into two closely related geographic groups: (1) populations from the Commander Islands the Aleutian Islands,

and Kamchatka and (2) populations from Sakhalin, the Khabarovsk Region, and the Kuril Islands. The level of differentiation between these groups was quite high with $F_{CT} = 0.240$ ($P < 0.001$). Populations of *T. glandulosum* were divided into two groups as well: group (1) included populations from the Magadan Region and group (2) included populations from central Kamchatka, Chukotka, and the Seward Peninsula. The level of differentiation between them was low and not significant $F_{CT} = 0.059$ ($P < 0.4$).

Nuclear SSR loci

An analysis of the variability of 10 microsatellite loci in the two studied species (30 populations, 510 samples in total, Table 1) revealed 207 allelic variants. The number of detected alleles per locus across all populations ranged from two (locus RF46P2) to 43 (locus RF74P1), the mean number of effective alleles ranged from 1.07 to 4.07 with an average 2.67. Within all populations, the observed heterozygosity (H_o) for each of the SSR loci ranged from 0.040 to 0.590, and the expected heterozygosity (H_e) ranged from 0.111 to 0.653 (Table 2).

In the 300 HWE tests across all loci in all populations (10 loci × 30 populations), seven loci (RA10, RA14, RA99, RA148, RF6P2, RF74P1, RF113P2) in 18 tests showed significant deviation from the HWE ($P < 0.01$). MICROCHECKER analysis in 18 tests from 300 tests suggested the presence of null alleles at all loci, but these loci differed between populations. In the 1350 LD tests performed, only 17 tests with several pairs of loci (RA99 and RA148, RA10, RA14 and RF74P1; RF74P1 and RA148, RA138, RA10, RA14 and RF113P2; RA148 and RA10, RA14 and RA138; RA14 and RA10, RA138; RA138 and RA10) exhibited significant departure from LD in different populations. These results indicate that no locus exhibits systematic deviations in the tests, and these instances likely reflect the individual characteristics of certain populations (e.g. missing data, occasional departures from random mating); therefore, all loci were retained in subsequent analyses.

In contrast to the ptDNA diversity, the values of the nSSR genetic parameters were slightly lower in *T. camtschaticum* as compared to *T. glandulosum* (Table 1). In *T. camtschaticum* the mean number of alleles per locus (A_a) ranged from 1.7 to 7.9

Table 2. Summary of genetic diversity statistics for the 10 SSR loci across 30 populations of *T. camtschaticum* and *T. glandulosum*

Locus	A^a	A_e^b	I^c	H_o^d	H_e^e	F_{IS}^f	F_{IT}^g	F_{ST}^h
RA10	38	3.90	1.31	0.590	0.619	0.047	0.366	0.335
RA14	27	3.34	1.23	0.550	0.594	0.073	0.354	0.303
RA99	20	3.47	1.26	0.559	0.611	0.084	0.373	0.315
RA138	23	2.41	0.88	0.463	0.461	-0.005	0.397	0.400
RA148	31	4.03	1.41	0.451	0.653	0.308	0.503	0.281
RF6P2	7	1.30	0.28	0.175	0.173	-0.012	0.687	0.691
RF14P3	5	1.17	0.19	0.085	0.111	0.232	0.839	0.790
RF46P2	2	1.07	1.30	0.040	0.053	0.246	0.915	0.887
RF113P2	11	2.43	0.90	0.451	0.491	0.083	0.301	0.238
RF74P1	43	3.63	1.29	0.584	0.589	0.009	0.344	0.338
Mean	16.99	2.67	0.88	0.395	0.435	0.106	0.508	0.458

[a]*A* total number of alleles, [b]*A_e* average number of effective alleles, [c]*I* Shannon's index, [d]*H_o* observed heterozygosity, [e]*H_e* expected heterozygosity, [f]*F_{IS}* inbreeding coefficient within individuals relative to the subpopulation, [g]*F_{IT}* inbreeding coefficient within individuals relative to the total, [h]*F_{ST}* inbreeding coefficient within subpopulations relative to the total.

Table 3. Hierarchical analysis of molecular variation (AMOVA) for populations of *T. camtschaticum* and *T. glandulosum*. All values are significant ($P < 0.001$). Zeros before the period are not shown

Source of variation	ptDNA			nSSR		
	d.f.	% of variation	Fixation indices[a]	d.f.	% of variation	Fixation indices[a]
Among two lineages	1	51.75	F_{CT} 0.517	1	27.70	F_{CT} 0.277
Among all populations	28	39.13	F_{ST} 0.909	28	16.75	F_{ST} 0.445
Within all populations	232	9.12	F_{SC} 0.811	990	55.55	F_{SC} 0.232
Among two clusters within the *T. camtschaticum* lineage	1	23.95	F_{CT} 0.240	1	17.42	F_{CT} 0.174
Among populations	16	63.63	F_{ST} 0.876	15	17.54	F_{ST} 0.350
Within populations	132	12.42	F_{SC} 0.837	540	65.04	F_{SC} 0.212
Among two clusters within the *T. glandulosum* lineage	1	5.87	F_{CT} 0.059[c]	1	2.56	F_{CT} 0.026[b]
Among populations	10	47.44	F_{ST} 0.536	10	13.40	F_{ST} 0.160
Within populations	100	46.39	F_{SC} 0.507	450	85.03	F_{SC} 0.138

[a]$P < 0.001$, [b]$P < 0.01$, [c]non-significant.

with an average of 3.7, while they ranged from 2.4 to 8.8 with an average of 5.1. in *T. glandulosum*. Values of the mean number of effective alleles per locus (A_e) were close for both species—on average 2.48 for *T. camtschaticum* and 2.97 for *T. glandulosum*. The observed heterozygosity (H_o) ranged from 0.1 to 0.538 with an average of 0.372 in *T. camtschaticum* and ranged from 0.2 to 0.580 with an average of 0.434 in *T. glandulosum*. The average expected heterozygosity (H_e) was lower in *T. camtschaticum* than in *T. glandulosum* (0.409 vs. 0.475). The pairwise F_{ST} (Supporting information, Data S4) throughout the populations of *T. camtschaticum* ranged from 0.047 to 0.363 and throughout the populations of *T. glandulosum* from 0.02 to 0.192. The highest pairwise F_{ST} values were observed between populations of *T. glandulosum* from Chukotka and the Seward Peninsula and populations of *T. camtschaticum* from the Commander and the Aleutian Islands (from 0.381 to 0.638).

Bayesian cluster analysis for all studied populations showed that the highest ΔK occurred at $K = 2$, according to two species (Fig. 3E). Additional cluster analysis for *T. camtschaticum* and *T. glandulosum* separately also revealed that the highest ΔK occurred at $K = 2$ (Fig. 3C, F, G). Principal coordinate analysis based on genetic Nei's distances between samples confirms a clear differentiation between the two taxa (Fig. 3B). The first and second coordinate accounted for 61.30% and 76.23% of the total variance, respectively. The population structure analysis considering geographical information of the samples in BAPS inferred the presence of seven clusters (Fig. 3D), which were geographically differentiated. The clusters of *T. glandulosum* corresponded to those in the STRUCTURE analysis and for *T. camtschaticum* separate clusters were detected in the Khabarovsk region, Sakhalin, and the Kuril Islands. AMOVA analysis revealed that the genetic differentiation between the two taxa was significant ($F_{CT} = 0.277$, $P < 0.001$), the same as between two clusters of *T. camtschaticum*, defined by STRUCTURE ($F_{CT} = 0.174$, $P < 0.001$), and between two clusters of *T. glandulosum* ($F_{CT} = 0.026$, $P < 0.008$) (Table 3).

Using the DIYABC program and the 'pre-evaluate scenario and priors' option, the principal component analysis (PCA) plot clearly showed that the observed data set was placed within the 1000 (1%) simulated data set (Supporting information, Data S5). This suggests that the chosen statistics are suitable for the priors and fit the model. All the summary statistics together with the PCA plots indicated a good fit of scenario 1 (Sc1) to the observed data (Supporting information, Data S5). Scenario 1 (Sc1) hypothesized the divergence of the different genetic groups of *Therorhodion* (Fig. 4), in particular that *T. redowskianum* was the earliest diverging clade with the highest posterior probability [$PP_{(direct\ approach)} = 0,77$, $PP_{(logistic\ approach)} = 0,98$, with the bias $0.232_{(direct\ approach)}$; $0.222_{(logistic\ approach)}$]. Demographical parameters from most appropriate scenario 1 are shown in Table 4. The analysis revealed a lower effective population size for N3 *T. glandulosum* 124.000 (95% CI [confidence interval]: 34.700–400.000) and N2 *T. camtschaticum*—231.000 (95% CI: 85.800–442.000) than for N1 *T. redowskianum*—318.000 (95% CI: 143.000–468.000) individuals. The recent divergence of *T. redowskianum* was inferred to 9.70×10^5 generations ago (95% CI: 3.9–14.5), and the divergence between *T. camtschaticum* and *T. glandulosum* was inferred at 1.52×10^5 generations ago (95% CI: 0.34–4.14). Assuming a broad generation time range of 10–40 years for *Therorhodion*, the divergence time for the divergence of the clade of *T. camtschaticum* and *T. glandulosum* from *T. redowskianum* was inferred at 9.7–38.8 Mya and the divergence time between *T. glandulosum* and *T. camtschaticum* was inferred at 1.52–6.0 Mya.

DISCUSSION

Taxonomy, genetic structure, and diversity of the Kamchatka rhododendron

Very few studies, based on a relatively small number of specimens, have been devoted to species delimitation of the Kamchatka rhododendron (Hata *et al.* 2017, Oliver 2017, Polezhaeva *et al.* 2020). In the present study, based on extensive material from throughout Beringia and neighbouring territories including both nuclear and plastid DNA data, we conclusively show that *T. camtschaticum* and *T. glandulosum* are well-distinguished genetic lineages corresponding to different species, with comparable levels of variability, and a high coefficient of differentiation. This high level of differentiation from maternally inherited ptDNA markers is comparable with other species complexes of rhododendrons from East Asia, such as *Rhododendron dauricum* L. *s.l.* and *Rhododendron pseudochrysanthum* Hayata *s.l.* (Huang *et al.* 2011, Polezhaeva *et al.* 2018). A lower level of differentiation from the biparently inherited nSSR markers reflects higher gene flow mainly via pollen then via seeds (Ennos 1994).

Based on the common set of nSSRs, *T. camtschaticum* ($H_e = 0.409$) and *T. glandulosum* ($H_e = 0.475$) have comparable genetic diversity when compared to *T. redowskianum* ($H_e = 0.523$) (Polezhaeva *et al.* 2022). The lowest level of genetic variability for *T. camtschaticum* was observed in the samples from the Aleutian and Commander Islands, and for *T. glandulosum* in some populations from Chukotka and the Seward Peninsula (Table 1). These differences can be explained by genetic drift in marginal populations (Pennington and Sexton 2024). When

Table 4. Demographical parameters from most appropriate Scenario 1 of divergence for *Therorhodion* by Approximate Bayesian Computation

Parameters	Median	95% confidence limits
Population size: N1 *T.redowskianum*	318.000	143.000–468.000
Population size: N2 *T.camtschaticum*	231.000	85.800–442.000
Population size: N3 *T.glandulosum*	124.000	34.700–400.000
Scaled divergence time:		
T1 *T.redowskianum from T.camtschaticum and T.glandulosum*	9.70 generations	3.9–14.5
T2 *between T.camtschaticum and T.glandulosum*	1.52 generations	0.34–4.14

considering life span, reproductive mode, and evolutionary history of each *Therorhodion* species, they show relatively moderate genetic diversity, akin to other outcrossing perennial woody plants (Nybom 2004).

Despite references in the literature for *T. glandulosum* and *T. camtschaticum* to occur sympatrically, we find no evidence of coexistence, hybridization, or intermediate traits based on nSSR or ptDNA markers in the Khabarovsk region (populations 14, 15), as well as in the Magadan region and Kamchatka, which are expected to be zones of sympatry for the two species. Both types of markers support that only *T. camtschaticum* is distributed throughout the entire southern periphery of Beringia, and it has a high genetic diversity in this area (Figs 2, 3). We can hypothesize a distribution boundary between the two species in the Khabarovsk Krai without hybridization, because in the STRUCTURE analysis there was no admixture of the *T. glandulosum* clusters in the clusters of *T. camtschaticum*. We do not consider this to be a limitation of the method, as it has been shown previously for a number of rhododendron species that it is possible to detect a hybrid origin of a species and to identify hybrid populations using nSSR markers (Yan et al. 2017, Zhang et al. 2020). Denser collection is probably required to better assess the possibility of hybridization between *Therorhodion* species, especially for *T. camtschaticum* in putative sympatry zones.

Divergence of *Therorhodion* taxa and migration to Alaska

Demographic analysis using nSSR data for Asian endemic *T. redowskianum* confirmed the sister relationship of this species to the rest, inferring a divergence time at 9.70×10^5 generations ago (95% CI: 3.9–14.5), while the divergence between *T. camtschaticum* and *T. glandulosum* is inferred at 1.52×10^5 generations ago (95% CI: 0.34–4.14). If we assume the average generation time as 10 years our divergence time estimates would be in the Miocene and Pleistocene at 9.70 Mya and 1.52 Mya, respectively. These estimates are close to previous divergence estimates by Oliver (2017) on the basis of diversity of ptDNA and nuclear fragments and a secondary constraint for *T. redowskianum* in the Miocene at 7.49 Mya (CI: 4.92–10.94 Mya), and between *T. camtschaticum* and *T. glandulosum* in the Pliocene at 5.37 Mya (CI: 3.50–7.83 Mya). In a recent reconstruction of the global phylogeny of rhododendrons using 3437 orthologous nuclear genes (Xia et al. 2022), the divergence time between *T. redowskianum* and *T. camtschaticum* was inferred in the Oligocene at 27.76 Mya (see fig. S4 in Xia et al. 2022). The differences in the inferred dates can be explained by different methodological approaches, including the fact that the exact mutation rate of different molecular markers for specific groups of organisms is unknown. Furthermore, we used an approximate time of generational change for *Therorhodion* species as 10 years without taking into account the realities of competitive existence of species in nature. It is known that flowering of these perennial shrubs can start already in the third to sixth year of life, but according to an ontogenetic analysis of plants in the wild, the transition to the generative stage usually begins on average by the age of 10 years, and in some individuals it can be delayed by up to 40 years in unfavourable conditions (Mazurenko 1980). Thus, if we consider a generation time of 40 years, our estimates shift to the earlier estimates of 38.8 Mya for the divergence of the *T. camtschaticum/T.*

glandulosum clade from *T. redowskianum* and 6 Mya for the divergence between these two species, respectively, and will be closer to those dates inferred by Xia et al. (2022). Taking the above assumptions about generation time and mutation rate, we can only speculate about the exact time of the divergence, but we can be certain that the divergence between *T. camtschaticum/T. glandulosum* occurred long before the LGM based on our data.

Although genetic differentiation of amphiberingian taxa into western and eastern clades has been shown previously for some animals and plants (Pruett and Winker 2005, Eidesen et al. 2007, Carlsen et al. 2010, Hope et al. 2013, Ikeda et al. 2020), it was less pronounced in other studies (Brunhoff et al. 2003, Fedorov et al. 2003, Kohli et al. 2015, Ikeda et al. 2018, Kurata et al. 2022), and our results did not support separate western and eastern Beringian clades for the Kamchatka rhododendron. The efficiency of nSSR markers to capture Late Pleistocene differentiation has been shown for the European alpine species *Rhododendron ferrugineum* L. with two distinct genetic lineages in the Alps and Pyrenees and a substructure in the Pyrenees lineage with a presumed origin during the LGM (Charrier et al. 2014). Likewise for the Asian *Rhododendron aureum* Georgi, whose main lineages are Siberian, north-eastern, and North Pacific, a divergence time was inferred before the LGM, and the age of the older (North Pacific) group was estimated to be about 165.2 Kya (51.6–352 Kya) (Polezhaeva et al. 2021). Considering that no specific divergent lineages for the Kamchatka rhododendron have been identified within western and eastern Beringia, a recent colonization likely occurred from Asia to North America. Divergence between *T. camtschaticum* and *T. glandulosum* may coincide with the emergence of the Arctic tundra biome dating approximately to the same time when the BLB periodically existed for the last time (stage BLB III, from 1.5 Mya to 10 Kya) (Sanmartín et al. 2001, Clark et al. 2006) and acted as a corridor for dispersal between the two continents. The small distributional area of species in eastern Beringia and the reduced genetic variability in their populations are consistent with a recent colonization from Asia to North America.

Analysis of the palaeofloras of eastern Beringia (western NAm) suggests that the source of subarctic taxa in this territory is the area of western Beringia (north-east Asia) (Strong 2021). Assuming that colonization occurred during the last land-bridge period approximately 35 700 Kya (Farmer et al. 2023), migration must have occurred at a high rate from nearby refugia as was shown for several Arctic plants (Alsos et al. 2007, Eidesen et al. 2007). The seeds of *Therorhodion* are small and easily dispersed by wind similar to those of other species of *Rhododendron* that can travel over 100 m (up to 1000 m) in high latitudes where the winds are strong (Stephenson et al. 2007, Wang et al. 2014). The nearest refugial areas for woody plants and Arctic flora are reconstructed for the coast of the Okhotsk Sea and the Upper Kolyma region (Shilo et al. 2007, Anderson et al. 2010), and the Kamchatka Peninsula (Hultén 1937, Ikeda et al. 2018, 2020).

Along with the BLB, the Aleutian Islands, a chain of volcanic islands across the southern Bering Sea, is another migration route at the southern margin of Beringia. The Aleutian arc did not serve as a land bridge later than 42–15 Mya (DeLong et al. 1978, McKenna 1983) and was ice-covered during the Late Wisconsin period from 26 000 to 11 000 BC (Hamilton 1994). However, the existence of insular refugia in the Aleutian Islands during the Late Wisconsin was suggested based on evidence

from animal phylogeographic studies (Congdon *et al.* 2000, Harlin-Cognato *et al.* 2006, Winker *et al.* 2023) and palaeoecological evidence on Arctic tundra (Frenzel *et al.* 1992). These refugia likely played a crucial role in the survival and distribution of species. However, both Hata *et al.* (2017) and ourselves were unable to identify a unique lineage in the Aleutian Islands for *T. camtschaticum*. Given the closeness of floras between southern Kamchatka and the Aleutian Islands (Hultén 1960, Krestov 2004, Garroutte *et al.* 2018), we conclude that *T. camtschaticum* may have used this route for long-distance colonization eastward from Asia to North America during or after the LGM with other snowbed species like *Phyllodoce aleutica* (Ikeda *et al.* 2018), *Primula cuneifolia* (Ikeda *et al.* 2020), and *Geranium erianthum* DC. (Kurata *et al.* 2022).

In summary, we clearly delimit the distributional boundaries of each *Therorhodion* species and their migration routes within the Beringian region. The range of *T. camtschaticum* encompasses the Sikhote-Alin ridge, Sakhalin Island, the Kuril Islands, and southern Kamchatka, and it used a southern route of migration across the Commander and Aleutian islands to the coast of south-east Alaska. *Therorhodion glandulosum* is distributed in the north of East Asia along the Okhotsk seashore, the Kolyma region, central and northern Kamchatka, and Chukotka, and it used a northern route for migration to the Seward Peninsula (Alaska) via the BLB. These findings align with previous research from rock ptarmigans (*Lagopus mutus* Montin) in the Beringian region which suggests that Aleutian rock ptarmigans might have descended from an Okhotsk refugium (Holder *et al.* 2000) via glacial vicariance (Hewitt 2000). Similarly, our data support an out-of-Asia origin for *Therorhodion*, which has found backing in several other Beringian taxa such as *Smelowskia* (Brassicaceae, Carlsen *et al.* 2010) and the Arctic alpine *Saxifraga oppositifolia* L. (Abbott *et al.* 2000, DeChaine 2009, Ickert-Bond *et al.* 2009).

SUPPORTING INFORMATION

Supplementary data is available at *Botanical Journal of the Linnean Society* online.

CREDIT STATEMENT

Maria A. Polezhaeva (Conceptualization, Funding acquisition, Data curation, Formal Analysis, Supervision, Validation, Visualization Writing—original draft, Writing—review & editing), Makar V. Modorov (Formal Analysis, Software, Validation), Olga A.Mochalova (Resources, Validation) and Stefanie M. Ickert-Bond (Resources, Validation, Writing—review & editing).

CONFLICT OF INTEREST

The authors have declared there are no conflicts of interest.

DATA AVAILABILITY

The ptDNA fragments sequences for each haplotype are available in the NCBI database; numbers of herbarium vauchers, microsatellite genotypes, files with DIYABC analyses data all are available in supplementary data.

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