



Genetic diversity of the *Rhododendron* subsection *Ledum* species in Sakhalin Island: data from chloroplast DNA markers variability

Diana R. Iunusova^{1*}, Maria A. Polezhaeva¹, Elena A. Marchuk²

Diana R. Iunusova^{1*}
e-mail: dianaiunusova@mail.ru

Maria A. Polezhaeva¹
e-mail: polezhaevam@mail.ru

Elena A. Marchuk²
e-mail: pimenova@botsad.ru

¹ Institute of Plant and Animal Ecology
UB RAS, Yekaterinburg, Russia

² Botanical Institute-Garden FEB RAS,
Vladivostok, Russia

* corresponding author

Manuscript received: 00.00.2023
Review completed: 00.00.2023
Accepted for publication: 00.00.2023
Published online: 00.00.2023

ABSTRACT

A high genetic variability was revealed in the populations of the *Rhododendron* subsection *Ledum* species complex in Sakhalin. In total, 94 individuals from natural populations of the four sympatric species of Labrador tea (*R. tomentosum*, *R. subarcticum*, *R. tolmachevii* and *R. hypoleucum*) were analyzed to evaluate the genetic variation in the territory of the Sakhalin Island and in two mainland populations. The haplotype diversity of five chloroplast intergenic spacers (*trnH-psbA*, *petB-petD*, *3'trnV(UAC)-ndbC*, *K2R-K707*, *atpB-rbcL*) was quite high. There were 16 haplotypes (h1–h16) identified; h1, h2 and h4 are the most common, the rest were rare and no species-specific ones were found. Analysis of Molecular Variance (AMOVA) indicate that about 37 % of the genetic diversity is due to among population differences ($F_{ST} = 0.37$, $p < 0.001$). Molecular phylogenetic analysis based on chloroplast DNA data did not reveal a clear species structure, but revealed a trend towards the isolation of two genetic groups: including a group of samples from the southern and the middle parts of Sakhalin Island.

Keywords: cpDNA haplotypes, Ericaceae, incomplete lineage sorting, Labrador teas, population structure, Russian Far East, species complex

РЕЗЮМЕ

Юнусова Д.Р., Полежаева М.А., Марчук Е.А. Генетическое разнообразие видов багульника на острове Сахалин: данные изменчивости маркеров хлоропластной ДНК. Изучено генетическое разнообразие 94 образцов из природных популяций четырех симпатричных видов багульников (подсекция *Ledum* рода *Rhododendron*: *R. tomentosum*, *R. subarcticum*, *R. tolmachevii* и *R. hypoleucum*) на территории о-ва Сахалин и в двух материковых популяциях. Изменчивость видов по пяти маркерным фрагментам хлоропластной ДНК (*trnH-psbA*, *petB-petD*, *3'trnV(UAC)-ndbC*, *K2R-K707*, *atpB-rbcL*) оказалась довольно высокой. Выявлено 16 гаплотипов (h1–h16): h1, h2 и h4 – самые распространенные, остальные редкие, видоспецифичных не обнаружено. Анализ молекулярной дисперсии (AMOVA) показал, что около 37 % генетического разнообразия приходится на межпопуляционные различия ($F_{ST} = 0,37$, $p < 0.001$). Анализ родства, проведенный по данным хлоропластной ДНК, не выявил четкой видовой структуры, однако выявила тенденцию к обособлению двух генетических групп: включающей группу выборок из южной и средней частей о-ва Сахалин.

Ключевые слова: гаплотипы хлДНК, Ericaceae, неполная сортировка линий, багульник, структура популяции, Дальний Восток России, видовой комплекс

Population studies of plants based on genetic data provide important information about the origin of species, changes in their ranges, hybrid contacts, etc. These data can also be applicable for solving practical tasks including biodiversity monitoring, conservation programs and rapid identification of plant species used as raw materials for herbal medicines. Effective identification is especially important for closely related plant species with high polymorphism within each of the species and/or high interspecific morphological similarity.

The Labrador teas are a group of highly polymorphic evergreen shrubs common in the Northern hemisphere. All parts of these plants contain high levels of essential oils and other metabolites, causing their wide use in traditional medicine (Dampc & Luczkiewicz 2013, 2015). Practical application determines the need to study the level of genetic variability of the species and to develop markers for identification. However, nomenclature and taxonomy of the Labrador teas are still not clarified enough, and there are

many subspecies, varieties and races distinguished (Krylov 1937, Tolmachev 1953, Mazurenko & Khokhryakov 1987). The problem of delimiting Labrador tea species is associated with the gradual transition of their morphological features and with their adaptive plasticity. These plants can both inhabit subarctic tundra and be dominant in forest undergrowth or swamps. In Russian-language literature the Labrador Teas belong to the genus *Ledum* (Komarov 1932, Bush 1952, Tolmachev 1974, Voroshilov 1982, Khokhryakov & Mazurenko 1991, Cherepanov 1995) closely related to the genus *Rhododendron* in the family Ericaceae. However back in 1990 Kron & Judd (1990) proposed to include this group within the genus *Rhododendron* as a lower taxonomic rank in subsection *Ledum* based on a detailed analysis of morphology. Subsequently, this fact was confirmed by molecular genetic data (Kurashige et al. 2001, Goetsch et al. 2005, Hart et al. 2017). The phylogenetic revision including samples from the North American and the Eurasian species confirmed the monophyletic origin of the *Ledum* subsection

and revealed an incongruence between nuclear and plastid phylogenies indicating that interspecific hybridization events could happen in the past (Hart et al. 2017). All previous phylogenetic studies involved only few specimens for each species of the *Ledum* subsection. However because of the young evolutionary age and lack of reproductive isolation in this group, it is reasonable to investigate phylogenetic relationships at the population level, using more sample sets to determine the extent of intraspecific genetic diversity and to identify hybrid zones between species. There are 6 species of the Labrador tea distinguished in Russia (Khokhryakov & Mazurenko 1991, Cherepanov 1995). All these species are listed for the Sakhalin Island (Barkalov & Taran 2004), which, due to its meridional length, has a unique diversity of ecological conditions for the coexistence of boreal and temperate biota. In addition, Sakhalin is one of the main migration routes of biota in the Quaternary that is important for the formation of the flora of the entire region of the Northeast Asia (Krestov et al. 2004). This should therefore be a suitable region in which to compare levels of genetic variation between the species. For this study, we use samples of four species gathered in Sakhalin Island and in the mainland. *Rhododendron tomentosum* Harmaja (= *Ledum palustre* L.) is a circumboreal species, *R. subarcticum* Harmaja (= *L. decumbens* (Aiton) Lodd. ex Steud.) is a hypoarctic American-Asian species, *R. tolmachevii* Harmaja (= *L. macrophyllum* Tolm.) and *R. hypoleucum* (Kom.) Harmaja (= *L. hypoleucum* Kom.) are species with rather narrow ranges in the Far East (Harmaja 1990). It should be noted that the range boundaries of these species as well as their confinement to certain habitats are not very precise (Tolmachev 1953). In the current study we evaluated the effectiveness of chloroplast DNA (cpDNA) markers for the estimation of the genetic diversity level and the elucidation

of the relationships within subject. *Ledum* in Eurasia. The data obtained are the starting point for delimiting the species boundaries and revealing historical biogeography of the species complex in the Far East.

MATERIAL AND METHODS

We included sample sets from ten populations of *R. tomentosum*, two populations of *R. subarcticum*, four populations of *R. tolmachevii* and three populations of *R. hypoleucum* in the study (the collection sites and related geographic information are depicted in Fig. 1 and listed in Table 1). In total, 94 individuals were collected in the Sakhalin Island and in the mainland of the Primorye Territory and Magadan Region. Additionally voucher specimens were gathered from Sakhalin and Sikhote-Alin populations for species identification. All specimens are deposited in the herbarium collection of the Botanical Garden-Institute FEB RAS (VBGI). The assignment of the samples to each species were determined based on the morphology characters and geographic distribution, according to Tolmachev (1974). However, this assignment is very conventional, because when collecting samples in nature, we noticed that their morphological characters vary considerably, as well as their confinement to certain environmental conditions. Total DNA was extracted from silica-dried leaves according to a hexadecyltrimethyl ammonium bromide (CTAB) procedure (Devey et al. 1996). Amplification was carried out with universal primers for five non-coding chloroplast DNA regions: *petB–petD* (Löhne & Borsch 2005), *3'trnV* (UAC)–*ndbC* (Shaw et al. 2007), *trnH–psbA* (Hamilton 1999), K2R–K707 (Jiang et al. 2016), *atpB–rbcL* (Manen et al. 1994) according to the protocol and temperature profile of PCR recommended by the authors. *Therorbodion camtschaticum* (Pall.) Small (genus *Rhododendron* subgenus *Therorbodion*)

Table 1. The collection sites, related geographic information and indices of genetic diversity of cpDNA in the samples of the Labrador teas. N_h – the number of haplotypes; N – the frequency of haplotypes; H – haplotype diversity.

Samples	Coordinates	N_h	N	H
<i>Rhododendron tomentosum</i>				
1. Sakhalin, Okha district, Sabo	53°08'53.6"N 142°56'10.6"E	10	h1:3 h2:6 h3:1	0.6444
2. Nogliki, Sakhalin	51°47'47"N 143°7'12"E	5	h3:1 h4:1 h5:1 h15:1 h16:1	1.0000
3. Sakhalin, Onor	50°11'51"N 142°43'34"E	2	h2:1 h3:1	1.0000
4. Sakhalin, Poronaysk district, Gastello	49°6'28"N 142°57'15"E	3	h1:1 h2:1 h7:1	1.0000
5. Sakhalin, Makarovo	48°48'26"N 142°52'30"E	3	h1:1 h2:2	0.6667
6. Sakhalin, Krasnogorsk	48°25'20"N 142°6'12"E	3	h4:2 h9:1	0.6667
7. Sakhalin, Dolinsk district, Sokol	47°14'43"N 142°43'43.2"E	7	h1:4 h4:1 h6:1 h12:1	0.7143
8. Sakhalin, Starodubskoe, Naiba	47°24'21"N 142°47'21"E	2	h1:1 h2:1	1.0000
9. Sakhalin, Chekhov Peak	47°0'20"N 142°50'27"E	2	h4:2	0
Mean <i>R. tomentosum</i>		40	11 haplotypes	0.7435
<i>Rhododendron tolmachevii</i>				
10. Sakhalin, Uskovo	50°57'27"N 142°38'60"E	7	h2:5 h4:2	0.4762
11. Sakhalin, Nabil Range	50°50'48"N 142°58'51"E	3	h2:3	0
12. Sakhalin, Zhdanko Range	48°14'26"N 142°34'25"E	7	h3:7	0
13. Sakhalin, Mt. Tsapko	48°5'57"N 142°30'39"E	2	h1:2	0
Mean <i>R. tolmachevii</i>		19	4 haplotypes	0.1191
<i>Rhododendron hypoleucum</i>				
14. Sakhalin, Izvestkovy	49°58'37"N 143°22'37"E	6	h1:3 h8:3	0.6000
15. Sakhalin, Leonidovo	49°17'30"N 142°52'38"E	7	h2:6 h4:1	0.2857
16. Primorye Territory, Sikhote-Alin	45°48'34"N 136°36'17"E	8	h1:1 h4:2 h10:1 h11:1 h13:3	0.8571
Mean <i>R. hypoleucum</i>		21	7 haplotypes	0.5809
<i>Rhododendron subarcticum</i>				
17. Sakhalin, Lomonosovsky district, Mt. Krasnov	48°44'7"N 142°6'50"E	9	h1:5 h2:2 h4:2	0.6667
18. Magadan Region	59°33'25"N 150°48'29"E	8	h1:2 h2:3 h4:1 h14:2	0.8214
Mean <i>R. subarcticum</i>		17	4 haplotypes	0.74405

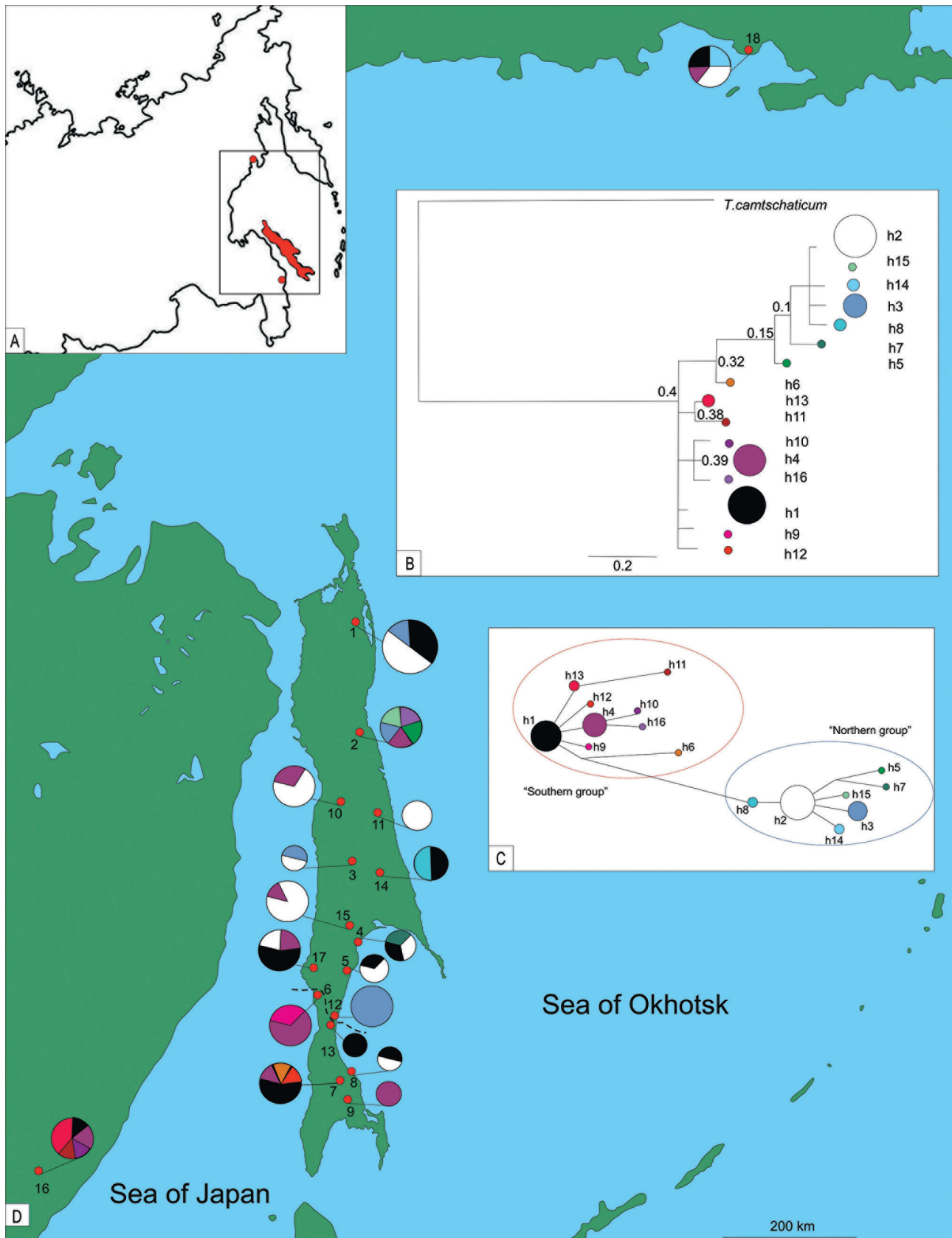


Figure 1 Genetic structure of the *Rhododendron* subject. *Ledum* populations in the Sakhalin Island and heighboring mainland: A – Sakhalin Island on the map; B – collection sites of the samples of the Labrador Teas in Sakhalin; the numbers correspond to the Table 1; the colors mark different haplotypes; the size of the circles corresponds to the frequencies of haplotypes; the dashed line corresponds to the “Poyasok” Isthmus. C – maximum parsimony network of cpDNA haplotypes (h1–h16) constructed in the NETWORK program; the size of the circle corresponds to the frequency of haplotype; the Southern and the Northern group are encircled. D – Phylogenetic tree of cpDNA haplotypes (h1–h17) constructed in the MrBayes program; h17 results from the sequences corresponding intergenic spacers of the outgroup specimen of *Therorodion camtschaticum* (Pall.) Small

was also collected as an outgroup taxon for phylogenetic analysis. These fragments from individuals of each sample were sequenced in NANOFOR 05 genetic analyzer (Sintol, Russia) at the Center for Collective Use “Modern Technologies for Ecological Research” of the IPAE, Ural Branch of RAS. The sequences were edited, assembled and aligned manually with the BioEdit software (Hall 1999). All sequences were deposited in Genbank under the following accession numbers: OQ689288 – OQ689371 (Table 2). Genetic diversity parameters, hierarchical partitioning of diversity among species, populations and individuals were evaluated by analysis of molecular variance (AMOVA) and calculated using Arlequin v. 3.5.1.2 (Excoffier & Lischer 2010). Phylogenetic relationships among the cpDNA haplotypes were evaluated by MrBayes v. 3.2.3. (Ronquist & Huelsenbeck 2003) on the basis of the model of nucleotide substitutions GTR + G + I. Insertions, inversions and deletions were considered as single events and were encoded as a binary data matrix consisting of zeros and ones. The relationships between cpDNA haplotypes were constructed using NETWORK v. 4.6.1.2 (Bandelt et al. 1999). Spatial analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) was used to identify population clusters. This method aims to identify clusters of populations that are maximally differentiated. A simulated annealing process was run to obtain the configuration of K groups that maximize FCT (the proportion of total genetic variance due to differences among groups of populations) and minimize single-population groups.

RESULTS

Alignment of five non-coding cpDNA regions resulted in a concatenated sequence 2115–2165 bp (base pairs) long. Only 21 variable sites were parsimoniously informative from 65 in total. There were indicated 60 SNPs and 5 indels (from 7 to 24 bp long) in analyzed sequences (Table 3). *Therorhodion camtschaticum* outgroup differed from haplotypes of subsect. *Ledum* by 46 mutations. There were 16 haplotypes derived from all polymorphic sites of all samples. The number (N_h) of haplotypes, the frequency of haplotypes (N) and the haplotype diversity index (H) for each population are listed in Table 1. A high range

of variability was detected for *R. tomentosum* populations, where H varied from 0 to 1 and N_h was 10. Populations of *R. hypoleucum* H = 0.28–0.85 and $N_h=8$ and *R. subarcticum* H = 0.53–0.82 and $N_h=5$ were moderate and high in variability. Populations of *R. tolmachevii* turned out to be the lowest in variability with H = 0–0.47 and $N_h=4$. Most of *R. tolmachevii* populations were monomorphic.

Haplotype distribution among acquired samples is shown in Fig. 1D. The most frequent haplotypes among all species were haplotypes h1, h2 and h4. Haplotype h3 was only shared among *R. tomentosum* and *R. tolmachevii*. The rest of haplotypes were rare and unique. Unique haplotypes were observed in the separate populations of *R. tomentosum*: h5, h15 and h16 in Nogliki (2, here and further the number of population in Table 1), h9 in Krasnogorsk (6), h6 and h12 in Sokol (7). *Rhododendron tolmachevii* had no species-specific haplotypes. *R. subarcticum* had a unique haplotype h14 in Magadan (18). *Rhododendron hypoleucum* had unique haplotypes h11 and h13 in Sikhote-Alin (16) and h8 in Izvestkovyi (14).

According to Maximum Parsimony analysis depicted by Median Network all cpDNA haplotypes splitted into two clusters. In this study we call them contingently the Southern group and the Northern group based on primarily distribution in the southern part of the island or in the middle and north parts of the island. The Southern and the Northern groups of haplotypes are five mutational steps away from each other. The Southern group includes nine sister haplotypes differing in 1–3 mutations, while the Northern group includes seven sister haplotypes differing in 1–2 mutations. Although haplotypes belonging to both clusters occur with varying frequencies in different populations across Sakhalin, southern haplotypes occurred in both populations of the mainland of Magadan (18) and Sikhote-Alin (16), none of northern haplotypes were observed in the mainland population from Sikhote-Alin (16). The topology obtained by Bayesian analysis supported two main clusters, however the clades had low statistical support from posterior probabilities (PPvalue 0.1–0.4).

An analysis of molecular variance (AMOVA) detected that as much as 37 % of the nucleotide variation was apportioned among populations and 62 % was apportioned within populations. Less than 1 % of genetic differentiation

Table 2. Accessions numbers of five cpDNA fragments for each haplotype in GenBank data base.

Haplotype	Accession number in GeneBank				
	<i>petB–petD</i>	<i>trnV–ndbC</i>	<i>atpB–rbcL</i>	<i>trnH–psbA</i>	K2R–K707
h1	OQ689288	OQ689304	OQ689321	OQ689338	OQ689355
h2	OQ689289	OQ689305	OQ689322	OQ689339	OQ689356
h3	OQ689290	OQ689306	OQ689323	OQ689340	OQ689357
h4	OQ689291	OQ689307	OQ689324	OQ689341	OQ689358
h5	OQ689292	OQ689308	OQ689325	OQ689342	OQ689359
h6	OQ689293	OQ689309	OQ689326	OQ689343	OQ689360
h7	OQ689294	OQ689310	OQ689327	OQ689344	OQ689361
h8	OQ689295	OQ689311	OQ689328	OQ689345	OQ689362
h9	OQ689296	OQ689312	OQ689329	OQ689346	OQ689363
h10	OQ689297	OQ689313	OQ689330	OQ689347	OQ689364
h11	OQ689298	OQ689314	OQ689331	OQ689348	OQ689365
h12	OQ689299	OQ689315	OQ689332	OQ689349	OQ689366
h13	OQ689300	OQ689316	OQ689333	OQ689350	OQ689367
h14	OQ689301	OQ689317	OQ689334	OQ689351	OQ689368
h15	OQ689302	OQ689318	OQ689335	OQ689352	OQ689369
h16	OQ689303	OQ689319	OQ689336	OQ689353	OQ689370

was detected among the four species of subsect. *Ledum*, 42 % of the genetic variation apportioned between the Southern and the Northern groups of populations (FCT = 0.42, $p < 0.05$).

Spatial analysis of molecular variance (SAMOVA) was run for $K = 2$ and divided all the samples in the Southern and the Northern groups depending on the prevalence of h1 and h2 respectfully. When the given number of K increases from 3 or more, the analysis resulted with single-population groups, reaching a maximum FCT=0.587 at $K = 18$ equal to the number of sample sets.

DISCUSSION

A high genetic variability of cpDNA was revealed in the populations of the subsection *Ledum* species complex in Sakhalin, but unexpectedly there was no differentiation between species. Previously cpDNA markers detected a high haplotypic diversity (14 haplotypes, four regions of cpDNA) and clear genetic structure among a complex of four closely related rhododendron species of Siberia and the Far East, *R. dauricum* L. s.l. (Polezhaeva et al. 2018). Also it was revealed that cpDNA markers could be species-specific for two sympatric and hybridizing *Rhododendron* species – *R. adamsii* Rehder and *R. parvifolium* Adams (Polezhaeva et al. 2022). But for the complex of Labrador teas in Sakhalin we obtained shared haplotypes and non-significant levels of genetic divergence between species. The same pattern of no genetic divergence among the four species of the *R. pseudochrysanthum* Hayata s.l. complex was observed in Taiwan (Chung et al. 2007). Of eight haplotypes detected using two regions of chloroplast DNA two haplotypes were shared among all the species and the rest haplotypes were observed in the single or several populations with no regard to the species. Thus, for different species of the same genus *Rhododendron*, chloroplast markers showed different degrees of resolution in determining the phylogenetic relationships among species.

Since the genetic variability of species is determined by both recent and historical evolutionary processes, we

can explain our pattern based on these factors. On the one hand, the geographical distribution of two shared ancestral haplotypes h1 and h2 with no clear resolution between populations can support the point of view on the *Ledum* species complex as a single highly polymorphic species with varieties (Sugawara 1940). Obtained here data of cpDNA diversity and phylogenetic topologies suggest that subsection *Ledum* species in Sakhalin are capable of genetic exchange, thus the current taxonomic treatment does not clearly reflect the distinct taxonomic units. Alternatively, our results can evidence the retention of an ancestral polymorphism, prior to divergence. An unresolved star pattern in the haplotype network suggests that rapid diversification occurred on the Sakhalin Island. Considering the absence of polymorphism in some populations and the presence of rare and unique haplotypes, which result in 37 % of differentiation between populations, these species may be undergoing the process of speciation due to genetic drift. We should also mention that the northernmost mainland population from the Magadan Region was not marked by haplotypic depletion; on the contrary, there was a unique haplotype. That is also true for the mainland population of Sikhote-Alin with three unique haplotypes, excepting the fact that all the haplotype from the population belonged to the Southern group of haplotypes.

The high genetic diversity may reflect both the high adaptive plasticity of species and the absence of past dramatic population decline events. According to our data, the genetic structure of the *Ledum* complex demonstrates a tendency for the isolation of two genetic groups on the Island. The distribution of high-frequency haplotypes h1 and h2 approximately corresponds to the phytogeographic boundary that runs along the Isthmus Belt “Poyasok”: to the south of it, the North Japan type flora becomes predominant and the presence of northern boreal species decreases (Pietsch et al. 2012). The formation of vegetation complexes on the Island is mainly caused by the geological history. Sakhalin was practically not glaciated during the all Quaternary. The Pleistocene was characterized by climate

Table 3. Segregating sites for sixteen haplotypes derived from 5 non-coding cpDNA markers. B – TTTATAGAAG; D – TTTATTCT; E – CATT TTTCT; F – TTTCGGAGATGGAAATTTT TAGTCG.

Haplotype	Nucleotide position																			
	<i>petB</i> – <i>petD</i>	<i>trnV</i> – <i>ndbC</i>					<i>atpB</i> – <i>rbcl</i>				<i>trnH</i> – <i>psbA</i>				K2R–K707					
	215–224	468	514–521	525	561	691	766	911	1002–1010	1222–1246	1228	1254	1299	1457	1594	1645	1659	1613	1627	2042
h1	B	A	-	A	C	C	A	T	E	-	A	G	C	A	T	G	C	C	G	C
h2	-	A	-	G	T	C	T	T	-	-	A	G	C	A	T	G	C	C	G	T
h3	-	A	D	G	T	C	T	T	-	-	A	G	C	A	T	G	C	C	G	T
h4	B	T	-	A	C	C	A	T	E	-	A	G	C	A	T	G	C	C	G	C
h5	-	A	-	G	T	C	T	T	-	-	A	G	C	A	A	G	C	T	G	C
h6	B	A	-	A	C	C	A	T	E	-	A	G	C	A	A	G	C	T	G	T
h7	-	A	-	G	T	T	T	T	-	-	A	G	C	A	A	G	C	C	G	T
h8	-	A	-	G	T	C	A	T	-	-	A	G	C	A	T	G	C	C	G	T
h9	B	A	-	A	C	C	A	T	E	F	A	G	C	A	T	G	C	C	G	C
h10	B	T	-	A	C	C	A	T	E	-	T	G	C	A	T	G	C	C	G	C
h11	B	A	-	A	C	C	A	T	E	-	A	A	C	T	T	A	C	C	G	C
h12	B	A	-	A	C	C	A	T	E	-	A	G	T	A	T	G	C	C	G	C
h13	B	A	-	A	C	C	A	T	E	-	A	G	C	T	T	G	C	C	G	C
h14	-	A	-	G	T	C	T	T	-	-	A	G	C	A	T	G	A	C	G	T
h15	-	A	-	G	T	C	T	T	-	-	A	G	C	A	T	G	C	C	T	T
h16	B	T	-	A	C	C	A	G	E	-	A	G	C	A	T	G	C	C	G	C

fluctuations and sea level alterations, which repeatedly led to merging of Sakhalin with Hokkaido and with the mainland (Pletnev 2004). Such unification events influenced the floristic composition of modern Sakhalin. It could be supposed that just in the Eopleistocene when the sea level was low and the climate was temperate the thermophilic flora invades Sakhalin from the South. When approximately three million years ago in the Northern Hemisphere a belt of arctic biome was formed the appearance of new niches because of the climate cooling contributed to rapid recolonization for plants inhabiting mountains (Matthews 1979). The subsection *Ledum* appears to be an evolutionary young group of the genus *Rhododendron* that was widely distributed in the arctic biome belt. Hart et al. (2017) suggested the scenario where an ancestor of the modern-day subsection *Ledum* species acquired a chloroplast genome from a mountain-inhabiting lepidote *Rhododendron*, possibly Himalayas (hotspot of the genus *Rhododendron*). The Labrador teas as a part of the Arctic flora, experiencing range expansion, entered Sakhalin from the north during periods of late Pleistocene cooling. This could explain why the Northern group haplotypes are absent in Sikhote-Alin population and in the south of the Island.

High genetic variability observed in the *Ledum* species complex in Sakhalin also reflects their adaptive plasticity. Except from mostly diploid *R. subarcticum* with $n=26$ (Lantai & Kihlman 1995) all the other Eurasian species are tetraploids with $n = 52$ (Gurzenkov 1973, Měsíček & Javůrková-Jarolímová 1992, Probatova et al. 2007, Theqvist 2011). Polyploidization is often associated with adaptive speciation (Alix et al 2017), therefore diversification events in Sakhalin might happen by recurrent range expansions possibly linked to climatic niche differentiation. Although polyploids rely more on vegetative spread leading to decrease of genetic variability, *Ledum* species in our study are highly polymorphic in both morphology and genetics. Possible explanation is an ability to form interspecific hybrids, as it is known that subsection *Ledum* species could form hybrids not only with rhododendrons of sister groups (lepidots) as *R. ferrugineum*, but they also hybridize with rhododendrons from section *Lapponica* (Kihlman 2004). For example, there is a natural hybrid between *R. subarcticum* and *R. lapponicum* in Greenland (Dalgaard & Fredskild 1993). Artificial hybrids between subsection *Ledum* and several other *Rhododendron* species combined under the name *Ledodendron* (Kihlman 2004, Theqvist 2013). Additionally, a number of studies have shown that hybridization of alpine rhododendrons leads to an increase in genetic diversity, and thus enhances adaptation to changing environmental conditions (Zha et al. 2008, Bruni et al. 2014).

CONCLUSIONS

Thus, the complex of sympatric species of the *Ledum* subsection in Sakhalin Island, unlike other representatives of the *Rhododendron* genus common in the Northeast Asia, turned out to be extremely diverse genetically. The species do not form a strong genetic structure and therefore it is difficult to select molecular markers for their identification. An absence of species-specific cpDNA markers might be

explained by recent diversification of subsection *Ledum* from the genus *Rhododendron* in the Northern Hemisphere. The expansion of the Arctic belt contributed to the rapid evolution of the taxon in a way that the diversification of the subsection coincided with a period of global cooling. Successful colonization of this group in the Northern Hemisphere resulted from ecological adaptability and the ability to hybridization. As a result, this complex of species have ancestral polymorphisms that have not yet been erased by genetic drift reflecting insufficient divergence time between taxa with recurrent gene flow or incomplete lineage sorting of ancestral variation. To further elucidate the relationships between *Ledum*, it is necessary to study more sample sets from Eurasia and approbated the new types of markers.

ACKNOWLEDGEMENTS

We are grateful to O.S. Korchagina, I.V. Frolov and V.V. Kotseruba for providing some of the sample sets from Sakhalin Island. To M.N. Koldaeva for botanical consultation and definition of the species and E.V. Hantemirova for supporting in the field work.

The molecular analysis was performed at the Center for Collective Use “Modern Technologies for Ecological Research” of the IPAE, Ural Branch of RAS.

Funding

This research was financially supported by a grant from the Russian Science Foundation, No 23-24-00173, <https://rscf.ru/en/project/23-24-00173/>.

LITERATURE CITED

- Alix, K., P.R. Gérard, T. Schwarzacher & J.S. Heslop-Harrison 2017. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Annals of Botany* 120(2):183–194.
- Bandelt, H.J., P. Forster & A. Rohlf 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16:37–48.
- Barkalov, V.Y. & A.A. Taran 2004. A checklist of vascular plants of the Sakhalin Island. In: *Flora and Fauna of Sakhalin Island (Materials of International Sakhalin Island Project)* (S.Yu. Storozhenko, ed.), pp. 39–66, Dal'nauka, Vladivostok (in Russian with English abstract). [Баркалов В.Ю., Таран А.А. 2004. Список видов сосудистых растений острова Сахалин // Растительный и животный мир острова Сахалин (Материалы Международного сахалинского проекта) / под ред. С.Ю. Стороженко. Владивосток: Дальнаука. С. 39–66].
- Bruni, I., F. De Mattia, S. Fluch, C. Ferrari, M. Corazza, E. Dinelli & M. Labra 2016. Genetic introgression of hybrid *Rhododendron* × *intermedium* Tausch is habitat mediated: Evidences from south-eastern Alps (Italy). *Plant Biosystems – An International Journal Dealing with All Aspects of Plant Biology* 150(3):449–458.
- Bush, E.A. & A.I. Poyarkova 1952. The family Ericaceae DC. In: *Flora SSSR, vol. 18* (B.K. Shishkin & E.G. Bobrov, eds), pp. 22–93. Izd-vo AN SSSR, Moscow (in Russian). [Буш Е.А., Пояркова А.И. 1952. Семейство Вересковые – Ericaceae DC. Флора СССР. Т. 18 / под ред. Б.К. Шишкина и Е.Г. Боброва. Москва: Изд-во АН СССР. С. 22–93].
- Cherepanov, S.K. 1995. *Vascular plants of Russia and neighboring countries*. Mir i sem'ya, Saint-Petersburg, 990 pp. (in

- Russian). [Черепанов С.К. 1991. Сосудистые растения России и сопредельных государств. Санкт-Петербург: Мир и семья. 990 с.].
- Chiang, T.Y., B.A. Schaal & C.I. Peng 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. *Botanical Bulletin of Academia Sinica* 39:245–250.
- Chung, J.D., T.P. Lin, Y.L. Chen, Y.P. Cheng & S.Y. Hwang 2007. Phylogeographic study reveals the origin and evolutionary history of a *Rhododendron* species complex in Taiwan. *Molecular Phylogenetics and Evolution* 42(1):14–24.
- Dalgaard, V. & B. Fredskild 1993. *Ledodendron vanhoeffeni* (syn.: *Rhododendron vanhoeffeni*) refound in Greenland. *Nordic Journal of Botany* 13(3):253–255.
- Dampc, A. & M. Luczkiewicz 2013. *Rhododendron tomentosum* (*Ledum palustre*). A review of traditional use based on current research. *Fitoterapia* 85:130–143.
- Dampc, A. & M. Luczkiewicz 2015. Labrador tea – the aromatic beverage and spice: a review of origin, processing and safety. *Journal of the Science of Food and Agriculture* 95:1577–1583.
- Devey, M.E., J.C. Bell, D.N. Smith, D.B. Neale & G.F. Moran 1996. A genetic linkage map for *Pinus radiata* based on RFLP, RAPD, and microsatellite markers. *Theoretical and Applied Genetics* 92:673–679.
- Dumolin-Lapegue, S., M.-H. Pemonge & R.J. Petit 1997. An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology* 6:393–397.
- Dupanloup, I., S. Schneider & L. Excoffier 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* 11:2571–2581.
- Excoffier, L. & H. Lischer 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10:564–567.
- Goetsch, L., A.J. Eckert & B.D. Hall 2005. The molecular systematics of *Rhododendron* (Ericaceae): a phylogeny based upon RPB2 gene sequences. *Systematic Botany* 30(3):616–626.
- Gurzenkov, N.N. 1973. Studies of chromosome numbers of plants from the south of the Soviet Far East. *Komarov Memorial Lectures* 20:47–61 (in Russian). [Гурзенков Н.Н. 1973. Исследование хромосомных чисел растений юга Дальнего Востока // Комаровские чтения. Вып. 20. С. 47–61].
- Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
- Hamilton, M.B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8:521–523.
- Harmaja, H. 1990. 1990. New names and nomenclatural combinations in *Rhododendron* (Ericaceae). *Annales Botanici Fennici* 27(2):203–204.
- Hart, A., K. Kron & E. Gillespie 2017. Molecular phylogenetic analysis of the north-temperate Labrador Teas (Ericaceae: *Rhododendron* subsect. *Ledum*) suggests a complex genetic history. *Journal of the Botanical Research Institute of Texas* 11(1):53–65.
- Khohryakov, A.P. & M.T. Mazurenko 1991. The family Ericaceae Juss. In: *Vascular Plants of the Soviet Far East. Vol. 5* (S.S. Kharkevich, ed.), pp. 119–166, Nauka, Saint-Petersburg (in Russian). [Хохряков А.П., Мазуренко М.Т. 1991. Семейство 68. Вересковые – Ericaceae Juss. // Сосудистые растения советского Дальнего Востока / отв. ред. С.С. Харкевич. Санкт-Петербург: Наука. Т. 5. С. 119–166].
- Kihlman, B. 2004. Hybrids between *Ledums* and lepidote *Rhododendrons*. *Journal of the American Rhododendron Society* 58:74–81.
- Komarov, V.L. 1932. *Plant Identifier of the Far East Region*. Leningrad, 622 pp. (in Russian). [Комаров В.Л. 1932. Определитель растений Дальневосточного края. Ленинград: Издательство Академии наук СССР. 622 с.].
- Krestov, P.V., V.Yu. Barkalov & A.A. Taran 2004. Phyto-geographical regionalization of the Sakhalin Island. In: *Flora and Fauna of Sakhalin Island (Materials of International Sakhalin Island Project)* (S.Yu. Storozhenko, ed.), pp. 67–92, Dal'nauka, Vladivostok (in Russian with English abstract). [Крестов П.В., Баркалов В.Ю., Таран А.А. 2004. Ботанико-географическое районирование острова Сахалин // Растительный и животный мир острова Сахалин (Материалы Международного сахалинского проекта) / под ред. С.Ю. Сторозенко. Владивосток: Дальнаука. С. 67–92].
- Kron, K.A. & W.S. Judd 1990. Phylogenetic relationships within the *Rhodoreae* (Ericaceae) with specific comments on the placement of *Ledum*. *Systematic Botany* 15(1):57–68.
- Krylov, P.N. 1937. *Flora of Western Siberia*. Izdanie Botanicheskoi Sektsii Tomskogo Obshchestva Ispytatelei Prirody, Tomsk, 331 pp. (in Russian). [Крылов П.Н. 1937. Флора Западной Сибири. Томск: Издание Ботанической Секции Томского Общества Испытателей Природы. 331 с.].
- Kurashige, Y., J.I. Etoh, T. Handa, K. Takayanagi & T. Yukawa 2001. Sectional relationships in the genus *Rhododendron* (Ericaceae): Evidence from mat K and trn K intron sequences. *Plant Systematics and Evolution* 228:1–14.
- Jiang, N., L. Man, W. Zhang, H.X. Dong, H.Y. Wang, M.R. Li, ... & M.Z. Sun 2016. Chloroplast view of the population genetics and phylogeography of a widely distributed shrub species, *Rhododendron dauricum* (Ericaceae). *Systematic Botany* 41(3):626–633.
- Johnson, L.A. & D.E. Soltis 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using matK sequences. *Annals of the Missouri Botanical Garden* 82:149–175.
- Lantai, K. & B. Kihlman. 1995. The chromosome number of *Ledum palustre* ssp. *decumbens* and of some related taxa. *Hereditas* 122(2):181–184.
- Löhne, C. & T. Borsch 2005. Molecular evolution and phylogenetic utility of the petD group II intron: a case study in basal angiosperms. *Molecular Biology and Evolution* 22(2):317–332.
- Manen, J.F., A. Natali & F. Ehrendorfer 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Plant Systematics and Evolution* 190:195–211.
- Matthews, J.V. 1979. Tertiary and Quaternary environments: historical background for an analysis of the Canadian insect fauna. *The Memoirs of the Entomological Society of Canada* 111(108):31–86.
- Mazurenko, M.T. & L.P. Khokhryakov 1987. Evolution of biormorphs and speciation in Far Eastern ledums. *Zhurnal Obshchei Biologii* 48(2):213–221 (in Russian). [Мазуренко М.Т., Хохряков Л.П. 1987. Эволюция биоморф и таксонообразование у дальневосточных багульников // Журнал общей биологии. Т. 48, № 2. С. 213–221].
- Měsíček, J. & V. Javůrková-Jarolímová 1992. *List of Chromosome Numbers of the Czech Vascular Plants*. Academia, Praha, 144 pp.

- Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106:283–292.
- Rambaut, A. & A. Drummond 2009. *FigTree v1.3.1: Tree figure drawing tool*. Institute of Evolutionary Biology, Edinburgh, UK.
- Pietsch, T.W., V.V. Bogatov, S.Yu. Storozhenko, A.S. Lelej, V.Yu. Barkalov, H. Takahashi, S.L. Joneson, S.K. Kholin, K.A. Glew, J.A. Harpel, ... & D.E. Stevenson 2012. Biodiversity and biogeography of Sakhalin Island. In: *Flora and fauna of North-West Pacific islands (Materials of International Kuril Island and International Sakhalin Island Projects)*, (S.Yu. Storozhenko, ed.), pp. 11–78, Dal'nauka, Vladivostok.
- Pletnev, S.P. 2004. Geological development of Sakhalin Island. In: *Flora and Fauna of Sakhalin Island (Materials of International Sakhalin Island Project)*, (S.Yu. Storozhenko, ed.), pp. 11–22, Dal'nauka, Vladivostok (in Russian with English abstract). [Плетнев С.П. 2004. Историко-геологическое развитие острова Сахалин // Растительный и животный мир острова Сахалин (Материалы Международного сахалинского проекта) / под ред. С.Ю. Сторозенко. Владивосток: Дальнаука. С. 11–22].
- Polezhaeva, M.A., E.A. Pimenova, N.A. Tikhonova & O.S. Korchagina 2018. Plastid DNA diversity and genetic divergence within *Rhododendron dauricum* s.l. (*R. dauricum* s.s., *R. ledebourii*, *R. sichotense* and *R. mucronulatum*; Ericaceae). *Plant Systematics and Evolution* 304:763–774.
- Polezhaeva, M.A., D.R. Iunusova, N.A. Tikhonova, A.N. Polezhaev & M.N. Koldaeva 2022. Genetic identification of closely related endangered *Rhododendron* species from East Asia. *Russian Journal of Genetics* 58(1):116–121.
- Probatova, N.S., V.Yu. Barkalov & E.G. Rudyka 2007. *Karyology of the flora of Sakhalin and Kuril Islands*. Dal'nauka, Vladivostok, 392 pp. (in Russian). [Пробатова Н.С., Баркалов В.Ю., Рудыка Э.Г. 2007. Кариология флоры Сахалина и Курильских островов. Владивосток: Дальнаука. 392 с.].
- Ronquist, F. & J.P. Huelsenbeck 2003. MrBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12):1572–1574.
- Shaw, J., E.B. Lickey, E.E. Schilling & R.L. Small 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94(3):275–288.
- Sugawara, Sh. 1940. *Illustrated flora of Saghalien with descriptions and figures of phanerogams and higher cryptogams indigenous to Saghalien. Vol. 4 vol. 4: Diapensiaceae–Asteraceae*, Tokyo, pp. 1439–1957.
- Taberlet, P.T., L. Geilly, G. Patou & J. Bouvet 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. *Plant Molecular Biology* 17:1105–1109.
- Theqvist, K. 2011. *Ploidy level of Ledum species*. <http://rosebayblog.blogspot.com/search/label/ledum> Last accessed 05.04.2023.
- Theqvist, K. 2013. *Hybridity of lepidotes in Rhododendron subsection Ledum based on some hybridization results*. <https://www.rhodogarden.com/cross/hybridity.html> Last accessed 06.04.2023.
- Tolmachev, A.I. 1953. Toward the cognition of Eurasian species of the genus *Ledum* L. *Botanicheskie Materialy Gербариya BIN AN SSSR* 15:197–207 (in Russian). [Толмачев А.И. 1953. К познанию евразийских видов рода *Ledum* L. // Ботанические материалы гербария БИН АН СССР. Т. 15. С. 197–207].
- Tolmachev, A.I. 1974. *Guide to the higher plants of Sakhalin and the Kuril Islands*. Nauka, Leningrad, 372 pp. (in Russian). [Толмачев А.И. 1974. Определитель высших растений Сахалина и Курильских островов. Ленинград: Наука. 372 с.].
- Voroshilov, V.N. 1982. *Guide to the plants of the Soviet Far East*. Nauka, Moscow, 674 pp. (in Russian). [Ворошилов В.Н. 1982. Определитель растений советского Дальнего Востока. Москва: Наука. 674 с.].
- Zha, H.G., R.I. Milne & H. Sun 2008. Morphological and molecular evidence of natural hybridization between two distantly related *Rhododendron* species from the Sino-Himalaya. *Botanical Journal of the Linnean Society* 156(1):119–129.