



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

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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, hybridization, Labrador tea, 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 выборки из южной и средней частей о-ва Сахалин.

Ключевые слова: гаплотипы хлДНК, Ericaceae, гибридизация, 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, in some cases the assignment was rough, 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. *Therorhodon camtschaticum* (Pall.) Small (genus *Rhododendron* subgenus *Therorhodon*)

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 samples; 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>		37	10 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

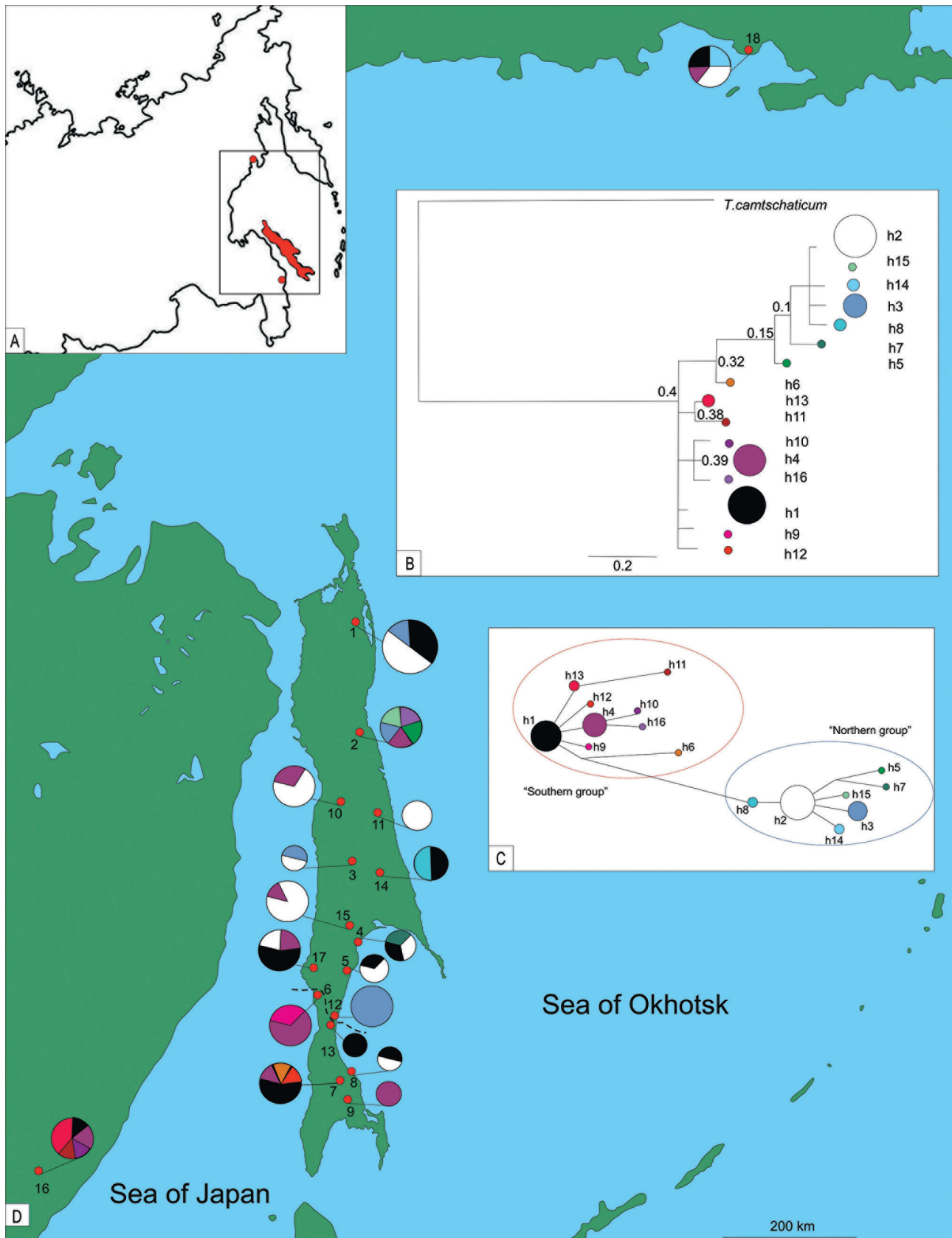


Figure 1 Genetic structure of the *Rhododendron* subject. *Ledum* populations in the Sakhalin Island and heighboring mainland: A – Sakhalin Island on the map; B – collection sites of the samples of the Labrador Teas in Sakhalin; the numbers correspond to the Table 1; the colors mark different haplotypes; 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 (Syntol, 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 MrBayers 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). *Therorbodion 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_i) of samples in each population, 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 the total number of haplotypes was 10. Populations of *R. hypoleucum* $H = 0.28–0.85$ with 7 haplotypes and *R. subarcticum* $H = 0.53–0.82$ with 4 haplotypes were moderate and high in variability. Populations of *R. tolmachevii* turned out to be the lowest in variability with $H = 0–0.47$ had 4 haplotypes. Most of *R. tolmachevii* populations were monomorphic.

Haplotype distribution among 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 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

Table 2. Accession 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

within populations. Less than 1 % of genetic differentiation was detected among the four species of subsect. *Ledum*, 42 % of the genetic variation apportioned between the Southern and the Northern groups of populations ($F_{CT} = 0.42$, $p < 0.05$).

Spatial analysis of molecular variance (SAMOVA) divided all the samples in the Southern and the Northern groups depending on the prevalence of h1 and h2 respectively, when $K=2$ ($F_{CT} = 0.52$). When K increases from 3 or more, the analysis resulted with single-population groups.

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. Firstly, the geographical distribution of two shared ancestral haplotypes h1 and h2 with no clear resolution between populations supports the perspective of 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 in 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 – CATTCTTCT; F – TTTCGGAGATGGAAATTTTGTAGTCG.

Haplotype	Nucleotide position																			
	<i>petB-petD</i>	<i>trnV-ndbC</i>					<i>atpB-rbcL</i>				<i>trnH-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 test new types of markers.

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