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GENETICS

Genetic Divergence of Closely Related Species *Oxytropis strobilacea*, *Oxytropis adamsiana*, and *Oxytropis vassilczenkoi* (Series *Strobilacei* Section *Orobia* Family Fabaceae) from Asian Russia

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Abstract—Genetic diversity and divergence of closely related species *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi* series *Strobilacei* section *Orobia* (Fabaceae) from Asian Russia were studied based on nucleotide polymorphism of cpDNA intergenic spacers *psbA-trnH, trnL-trnF,* and *trnS-trnG*, as well as ITS nrDNA. Most populations are characterized by a moderate or high level of chloroplast genetic diversity (*h* varies from 0.600 to 1.000). In total, 65 chlorotypes have been identified, no shared chlorotypes were found in the taxa, which confirms the status of *O. vassilczenkoi* as a separate species. Two phyletic lineages have been identified in *O. strobilacea*, which indicates ongoing intensive diversification processes. One of the seven ITS ribotypes is shared for the three species, which is probably due to their common origin and relatively recent divergence.

Keywords: Fabaceae, *Oxytropis*, *Orobia*, genetic diversity, divergence, chloroplast DNA, ITS

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INTRODUCTION

Section *Orobia* Bunge, the largest section of the genus *Oxytropis* DC., includes about 110 species in Europe, Asia, and America (Malyshev, 2008a). In the Asian Russia, this section is represented by 75 species and subspecies (Malyshev, 2012). In *Flora of the USSR* (Vasilchenko et al., 1948), the sect. *Orobia* is divided by the authors into five multispecies series: *Sordidae* Vass., *Uralensis* Vass., *Ambiguae* Vass., *Longirostrae* Vass., and *Songoricae* Vass. In 2005, based on a thorough typification and revision of species assigned to the series *Uralensis*, Knyazev (Knyazev, 2005), proposed changes in the systematics of the sect. *Orobia*. Taking into account the complex of morphological features, seven Siberian species related to *O. uralensis* (L.) DC. (*O. adamsiana* (Trautv.) Jurtzev, *O. vassilczenkoi* Jurtzev, *O. ambigua* (Pall.) DC., *O. arctica* R. Br., *O. wologdensis* Knjasev, *O. karga* Saposhn., and *O. subnutans* (Jurtz.) Jurtz.) and *O. strobilacea* Bunge were assigned into a separate series *Strobilacei* Knjasev with *O. strobilacea* as a type species.

Among representatives of the ser. *Strobilacei, O. strobilacea* is the most widespread mountain–steppe species; its range covers Northeast Kazakhstan, South Siberia, Northern Mongolia, and Chinese Altai (Malyshev, 2008a). The species is distributed in the insular steppes of Altai, southern Krasnoyarsk krai and the Republic of Tuva, and the steppes of the Angara River, Buryatia, and Transbaikalia; it occurs in the southern part of the Far East in the basins of the Zeya and Aldan rivers (Pavlova, 1989; Peshkova, 2001). The northern margin of its range is about 56° N. This is a very polymorphic species, the morphological characters of which, such as the degree of maturation, the length of peduncles, bracts, etc. vary greatly. For *O. strobilacea,* different variants of the chromosome number are known ($2n$ = 16, 32, 48, and 64), which may be a manifestation of racial differentiation in a species with a wide range, as well as when inhabiting several altitudinal mountain belts (Malyshev, 2008a, 2008b).

To the north, the ser. *Strobilacei* is represented by two closely related species: *O. adamsiana* found in the Stanovoi Highlands, Taimyr, and in the basins of the Lena (lower reaches), Yana, and Indigirka rivers and *O. vassilczenkoi* found east of the Kolyma River on the

Chukchi Peninsula and in the Koryak Highlands (Yurtsev, 1986; Pavlova, 1989; Peshkova, 2001; Malyshev, 2008a). *O. adamsiana* and *O. vassilczenkoi* were first described as independent species in 1959 (Yurtsev, 1959); previously, they were assigned to *O. strobilacea*. *O. adamsiana* represents the arcto-hypoarctic and alpine race of *O. strobilacea*; it is a tetraploid with $2n = 32$ (many locations) and $2n = 48$ for the population from the Udokan Ridge (Malyshev, 2008a). The Anadyr-Chukotian endemic *O. vassilczenkoi* ($2n = 32$) is closely related to *O. adamsiana,* but differs significantly in a number of characters, and represents the most isolated arctic race of *O. uralensis* (Yurtsev, 1959, 1986; Malyshev, 2008a). However, there is the opinion (Kozhevnikov, 1980) that *O. vassilczenkoi* is not an independent species, but a subspecies *O. adamsiana* ssp. *vassilczenkoi* (Jurtz.) Ju. Kozhevn. Phenetic analysis of species of the sect. *Orobia* showed the relative proximity of *O. adamsiana* and *O. vassilczenkoi* and the isolation of *O. strobilacea* (Malyshev, 2008b). The latter species belonged to a branch that is significantly distant from that with *O. adamsiana* and *O. vassilczenkoi* on the dendrogram of differences constructed based on the analysis of 47 qualitative morphological characters (Malyshev, 2008b).

The reconstruction of phylogenetic relationships of *Oxytropis* species, (including randomly selected single samples of *O. strobilacea, O. adamsiana,* and *O. vassilczenkoi*), based on sequencing of nuclear and chloroplast genome markers showed that relationships of species, even at the section level, have not been resolved (Kholina et al., 2016; Shavvon et al., 2017).

This work continues the study of the genetic diversity, population structure, and evaluation of the degree of divergence between closely related *Oxytropis* species (Kholina et al., 2018, 2019, 2020, 2021a, 2021b, 2021; Kozyrenko et al., 2020). This study aims to examine the genetic diversity and divergence of *O. strobilacea, O. adamsiana,* and *O. vassilczenkoi* ser. *Strobilacei* sect. *Orobia* from Asian Russia, to clarify the taxonomic status of *O. vassilczenkoi,* as well as to reconstruct the phylogenetic relationships based on the variability of nucleotide sequences of IGS *psbA-trnH* + *trnL-trnF* + *trnS-trnG* cpDNA and ITS nrDNA.

MATERIALS AND METHODS

In total, 108 plants were analyzed: *O. strobilacea* (55 specimens), *O. adamsiana* (16), and *O. vassilczenkoi* (37) from 16 natural localities (Table 1; Fig. 1). The names of species and sections are given according to (Malyshev, 2008a).

Methods for DNA extraction, amplification, and sequencing of cpDNA IGS *psbA-trnH, trnL-trnF,* and *trnS-trnG* are given in our previous works (Kholina et al., 2018, 2021b). The ITS region of nrDNA was amplified with primers ITS1 and ITS4 under the conditions given in (Mir et al., 2010). The nucleotide sequences of direct and reverse chains were determined on an ABI 3500 genetic analyzer (Applied Biosystems, United States) at the Joint-Use Center "Biotechnology and Genetic Engineering" of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia). The sequences of the four DNA regions were assembled and edited using the Staden Package v1.5 (Bonfeld et al., 1995), then aligned in SeaView v4.7 (Gouy et al., 2010).

The matrix of combined sequences of three IGS cpDNA was used to calculate the haplotype (h) and nucleotide (π) diversity (for populations with five or more samples), to assess the degree of divergence (Dxy) between populations/species based on nucleotide substitutions, and to analyze molecular variance (AMOVA) using the Arlequin software packages v3.5 (Excoffier and Lischer, 2010) and DnaSP v5 (Librado and Rozas, 2009). The statistical significance (p) of fixation indices (Φ_{ST}) was assessed based on 1023 permutations. The cpDNA and ITS haplotypes were identified in DnaSP v5.

The haplotype network was built in the program Network v5.0 (Bandelt et al., 1999), each deletion/insertion, regardless of its size, was encoded as one mutational event. For cpDNA haplotypes, the median joining (MJ) algorithm was used, for ITS haplotypes, the reduced median (RM) algorithm. The nucleotide sequences of *psbA-trnH, trnL-trnF,* and *trnS-trnG* cpDNA (GenBank accession numbers LT856572, LT856585, LT856598, respectively) and ITS nrDNA (LR898464), obtained earlier for *O. glabra* (Lam.) DC. sect. *Mesogaea* Bunge (Kholina et al., 2018, 2021b), were used as an outgroup.

RESULTS

Nucleotide sequences of IGS *psbA-trnH, trnL-trnF,* and *trnS-trnG* cpDNA of 108 samples of the studied species are characterized by low nucleotide variability and different lengths due to the presence of short indels (4–10 nucleotides) and mono- and dinucleotide repeats. The length of the combined sequences of the three regions after alignment was 2440 sites. In total, 17 nucleotide substitutions were identified; twelve of them are parsimony informative. There are no species-specific molecular markers.

All studied populations (with five or more samples) of three species are characterized by a high haplotype diversity (h varies from 0.600 to 1.000) and low and moderate nucleotide diversity (π varies from 0.0005 to 0.0047); there are no monomorphic populations found (Table 1). Nucleotide divergence (Dxy) is one of the indicators of the degree of genetic disunity of populations/species. In *O. strobilacea,* the highest Dxy values are found between the STR1 and STR2 populations, on the one hand, and all the others, on the other hand (Table 2). In *O. adamsiana,* the largest differ-

Table 1. Studied populations of *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi* and parameters of genetic diversity according to cpDNA data

Population location (number of samples)	Coordinates (N, E)	Code	Chlorotype	Diversity (standard error is given in brackets)	
				haplotype	nucleotide
O. strobilacea					
Republic of Buryatia, Zaigraevskii district, near the village Zaigraevo (5)	51.87514° N 108.24616° E	STR1	U1–U3	0.800 (0.164)	0.0009 (0.0007)
Republic of Buryatia, Eravninskii district, near the village Komsomolskoe (10)	52.478983° N 111.086773° E	STR2	U1–U7	0.867 (0.107)	0.001 6 (0.0010)
Republic of Buryatia, Kurumkanskii district, near the village Maysk (15)	54.61287° N 110.77431° E	STR3	U8–U18	0.952 (0.040)	0.003 2 (0.0018)
Republic of Buryatia, Kurumkanskii district, Dzherginsk Reserve, Ukshaki tract (5)	55.203529° N 111.448749° E	STR4	U19–U23	1.000 (0.127)	0.0042 (0.0027)
Republic of Buryatia, Tunkinskii district, near the village Torah (6)	51.76222° N 102.95333° E	STR5	U24–U26	0.600 (0.215)	0.0013 (0.0009)
Republic of Buryatia, Tunkinskii district, near the village Mondy (3)	51.69750° N 100.86746° E	STR6	U27–U29	–	–
Republic of Buryatia, Tunkinskii district, near the village Zun-Murino (11)	51.74499° N 102.86646° E	STR7	U26, U27, U30–U36	0.96 4 (0.051)	0.0025 (0.0015)
Total (55)				0.977 (0.009)	0.0049 (0.0025)
O. adamsiana					
Central Taimyr, Byrranga Mountains, near the northern shore of the Ledyanaya Bight, Taimyr Lake (1)	74.48824° N 99.6955° E	ADAM1	U37	–	–
Southwestern Taimyr, Putorana Plateau, southern tip of Ayan Lake (3)	68.99894° N 94.49041° E	ADAM2	U37	–	–

Table 1. (Contd.)

Population location (number of samples)	Coordinates (N, E)	Code	Chlorotype	Diversity (standard error is given in brackets)	
				haplotype	nucleotide
Southeastern Taimyr, the middle course of the Fomich River, northern shore of Besstochnoe Lake (1)	71.67283° N 108.30425° E	ADAM3	U38	–	–
Southeastern Taimyr, the middle course of the Kotuy River, the mouth of the Medvezhya River (1)	71.594564° N 102.663249° E	ADAM4	U39 (=H11*)	–	–
Republic of Buryatia, Bauntovskii district, near the village Uakit, left bank of the Uakit River(10)	55.56461° N 113.60958° E	ADAM5	U40–U47	0.956 (0.059)	0.0047 (0.0027)
Total (16)				0.933 (0.048)	0.0056 (0.0030)
O. vassilczenkoi					
Magadan oblast, Susuman district, settlement of Tal-Yuryakha, valley of the Arkagala River (14)	63.33147° N 146.66242° E	VAS1	U48–U52	0.769 (0.083)	0.0005 (0.0004)
Magadan oblast, Severo-Evenskii district, valley of the Pravaya Imlyaki River (1)	65.096498° N 160.104725° E	VAS2	U51 (=H46*)	–	–
Chukotka, Bilibinskii district, floodplain Yarkoveyem (9)	66.93205° N 167.00095° E	VAS3	U51, U53–U57	0.917 (0.073)	0.0013 (0.0009)
Kamchatka krai, Olyutorskii district, Vetveyskii Ridge, Seinav (13)	61.00551° N 166.04596° E	VAS4	U58–U65	0.897 (0.067)	0.0017 (0.0010)
Total (37)				0.935 (0.021)	0.0039 (0.0021)

The asterisk * indicates chlorotypes identified earlier (Kholina et al., 2016), accession numbers of nucleotide sequences of IGS *psbA-trnH, trnL-trnF,* and *trnS-trnG* cpDNA in GenBank/ENA/EMBL-EBI: H11–LN898575, LN898537, LN898649; H46–LN898519, LN898531, LN898643, respectively.

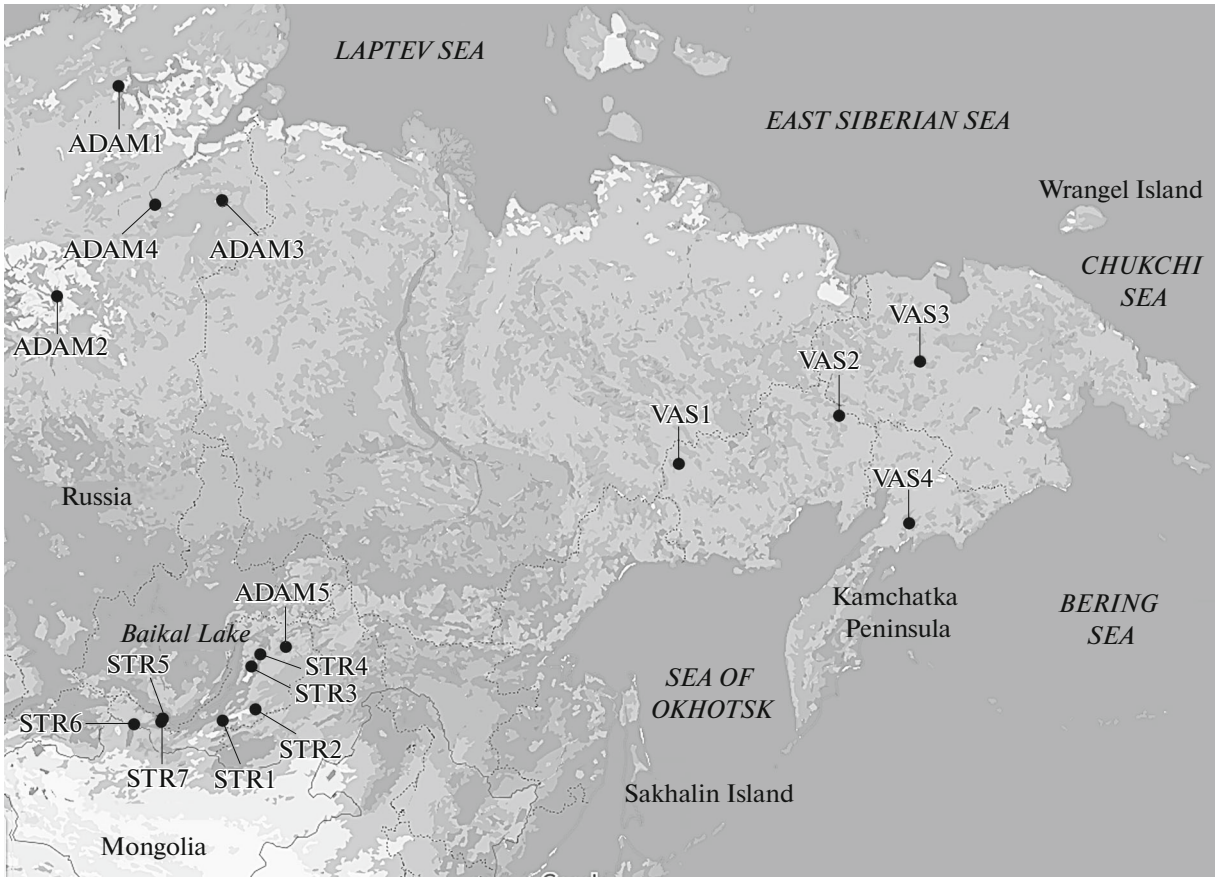

Fig. 1. Schematic map indicating the sampling sites of *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi* from 16 natural localities. The population code is similar to that in Table 1.

ences (0.00167) are registered between the ADAM1 and ADAM3 populations, as well as between ADAM2 and ADAM3 populations. In *O. vassilczenkoi,* only the VAS4 population is significantly distant (0.00205) from all others, between which there is no nucleotide divergence (Table 2). According to the AMOVA results (Table 3), in *O. strobilacea* and *O. adamsiana,* genetic variability is distributed almost equally between and within populations, while in *O. vassilczenkoi,* about 78% of all genetic variability is explained by interpopulation differences. The nucleotide divergence between *O. strobilacea* and *O. adamsiana,* as well as between *O. strobilacea* and *O. vassilczenkoi,* is 0.00170 for each pair, and between *O. adamsiana* and *O. vassilczenkoi* is 0.00091, but these interspecific *D*xy values correspond to the interpopulation level (Table 2). Hierarchical AMOVA (Table 3) evidences the low interspecies differentiation, when less than 28% of variability is explained by interspecies differences.

Analysis of 108 sequences reveals 65 haplotypes (chlorotypes), U1–U65 (Table 1), their sequences are deposited in DDBJ/ENA/GenBank-INSDC under the numbers OV260579–OV260641 (*psbA-trnH*), OV260679–OV260741 (*trnL-trnF*), and OV260768–OV260830 (*trnS-trnG*). *O. strobilacea* has 36 chlorotypes (U1–U36), *O. adamsiana*, 11 chlorotypes (U37–U47), and *O. vassilczenkoi,* 18 chlorotypes (U48–U65); no shared chlorotypes are found in taxa. In the median network of genealogical relationships, three haplogroups are distinguished (Fig. 2a), which diverge from a hypothetical chlorotype (not found in our study or an extinct ancestor) and are separated by 7–10 mutational steps. Two haplogroups are formed by chlorotypes of *O. strobilacea:* (1) haplogroup I with U1–U7 (populations STR1 and STR2), and (2) haplogroup II with U8–U36 (STR3–STR7). Haplogroup III is formed by all chlorotypes of *O. adamsiana* and *O. vassilczenkoi* (U37–U65); their distribution does not correspond to either the population or taxonomic level, which indicates the genetic homogeneity of this group (Fig. 2a). In haplogroups I and II, neighboring chlorotypes are connected mainly by 1–2 mutational steps, while in haplogroup III, some chlorotypes are separated from each other by 4–8 mutational steps (Fig. 2a). The presence of alternative connections (loop structures in the network) between chlorotypes in haplogroups II and III (Fig. 2a) does not allow one to establish unambiguously the relationship between the populations of each species. Only haplogroup I, including chlorotypes U1–U7 of populations STR1

Table 2. Nucleotide divergence between populations of *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi* according to cpDNA data

Population	STR1	STR2	STR3	STR4	STR5	STR6	STR7	ADAM1	ADAM2	ADAM3	ADAM4	ADAM 5	VAS1	VAS2	VAS3	VAS4
STR1	–	0.90 (0)	3.93 (3)	4.40 (3)	4.63 (3)	2.80 (2)	3.16 (2)	3.80 (3)	3.80 (3)	5.80 (5)	3.80 (3)	6.20 (5)	5.80 (5)	5.80 (5)	5.80 (5)	4.72 (3)
STR2	0.038	–	3.63 (3)	4.10 (3)	4.33 (3)	2.50 (2)	2.86 (2)	3.50 (3)	3.50 (3)	3.50 (3)	3.50 (3)	5.90 (5)	5.50 (5)	5.50 (5)	5.50 (5)	4.42 (3)
STR3	0.164	0.152	–	0.71 (0)	0.97 (0)	1.13 (1)	0.77 (0)	2.13 (2)	2.13 (2)	4.33 (4)	2.13 (2)	4.53 (4)	4.13 (4)	4.13 (4)	4.13 (4)	3.06 (2)
STR4	0.184	0.171	0.030	–		1.60 (1)	1.24 (0)	2.60 (2)	2.60 (2)	4.00 (4)	2.60 (2)	5.00 (4)	4.60 (4)	4.60 (4)	4.60 (4)	3.52(2)
STR5	0.193	0.181	0.040	0.060	–	1.83 (1)	1.43 (0)	2.83 (2)	2.83 (2)	4.83 (4)	2.13 (2)	5.23 (4)	4.83 (4)	4.83 (4)	4.83 (4)	3.76 (2)
STR6	0.117	0.104	0.047	0.067	0.077	–	0.36 (0)	1.00 (1)	1.00 (1)	3.00 (3)	1.00 (1)	3.00 (3)	3.00 (3)	3.00 (3)	3.00 (3)	1.92 (1)
STR7	0.132	0.120	0.032	0.052	0.061	0.015	–	1.36 (1)	1.64 (1)	3.36 (3)	1.36 (1)	3.76 (3)	3.36 (3)	3.36 (3)	3.36 (3)	2.29 (1)
ADAM1	0.159	0.147	0.089	0.109	0.119	0.042	0.057	–	3.60 (3)	4.00 (4)	0.00(0)	3.60 (3)	4.00 (4)	4.00 (4)	4.00 (4)	0.92 (0)
ADAM2	0.159	0.147	0.089	0.109	0.119	0.042	0.057	0.151	–	4.00 (4)	0.00 (0)	3.60 (3)	4.00 (4)	4.00 (4)	4.00 (4)	0.92 (0)
ADAM3	0.242	0.230	0.173	0.192	0.202	0.125	0.140	0.167	0.167	–	4.00 (4)	0.40 (0)	0.00 (0)	0.00 (0)	0.00 (0)	4.92 (4)
ADAM4	0.159	0.147	0.089	0.109	0.089	0.042	0.057	0.000	0.000	0.167	–	3.60 (3)	4.00 (4)	4.00 (4)	4.00 (4)	0.92 (0)
ADAM5	0.260	0.247	0.190	0.210	0.219	0.142	0.158	0.151	0.151	0.017	0.151	–	0.40 (0)	0.40 (0)	0.40 (0)	4.52 (3)
VAS1	0.242	0.230	0.173	0.192	0.202	0.125	0.140	0.167	0.167	0.000	0.167	0.017	–	0.00 (0)	0.00 (0)	4.92 (4)
VAS2	0.242	0.230	0.173	0.192	0.202	0.125	0.140	0.167	0.167	0.000	0.167	0.017	0.000	–	0.00 (0)	4.92 (4)
VAS3	0.242	0.230	0.173	0.192	0.202	0.125	0.140	0.167	0.167	0.000	0.167	0.017	0.000	0.000	–	4.92 (4)
VAS4	0.197	0.185	0.128	0.147	0.157	0.080	0.095	0.039	0.039	0.205	0.039	0.189	0.205	0.205	0.205	–

Values above the diagonal are the average number of nucleotide differences (the number of fixed differences); below the diagonal, the average number of nucleotide substitutions per site $\times 10^{-2}$. The population code is similar to that in Table 1.

Table 3. Distribution of genetic variability (AMOVA) in species *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi* according to cpDNA data

Source of dispersion	Genetic differences (%) between		
	groups	populations within groups	individuals in a population
Populations of *Oxytropis* species			
One group: (all populations of *O. strobilacea*)	–	54.35 *	45.65
One group: (all populations of *O. adamsiana*)	–	43.34**	56.66
One group: (all populations of *O. vassilczenkoi*)	–	77. 57 *	22.43
Three groups: (all populations of *O. strobilacea*), (all populations of *O. adamsiana*), (all populations of *O. vassilczenkoi*)	27.50*	45.16*	27.33*
Haplogroups identified in the network analysis			
Three groups: (I), (II), (III)	35.86*	37.64*	26.50*
Two groups: (I), (II)	47.34**	20.41*	32.25*
Two groups: (I), (III)	45.88**	36.87*	17.26*
Two groups: (I and II), (III)	27.09**	46.45*	26.46*
Two groups: (II), (III)	26.94**	43.43*	29.63*

The level of significance is marked as * $p < 0.0001$; ** $0.0009 < p < 0.05$. The level of significance is determined on the basis of 1023 permutations.

and STR2 of *O. strobilacea,* has specific markers, which are absent in all others: (1) two substitutions (A at positions 1468 and 2130 of the combined matrix, in all others, G and T, respectively), and (2) an insertion of four nucleotides (TTTA, positions 257–260). According to hierarchical AMOVA, about 36% of the variability is explained by differences between the three haplogroups (Table 3). However, it should be noted that the genetic differences between haplogroup I and haplogroups II and III are 1.7 times higher than those between haplogroups II and III (Table 3), which indicates the significant genetic isolation of haplogroup I.

Thus, the phylogenetic analysis of the genealogical relationships of chlorotypes and the presence of identified specific molecular markers indicates three evolutionary cpDNA branches/lineages in three closely related *Oxytropis* species of the ser. *Strobilacei* sect. *Orobia:* (1) *O. strobilacea*, populations STR1 and STR2; (2) *O. strobilacea,* populations STR3–STR7; and (3) *O. adamsiana–O. vassilczenkoi.*

Region ITS of nrDNA was amplified in 71 samples: *O. strobilacea* (40 specimens), *O. adamsiana* (14), and *O. vassilczenkoi* (17), representing all the cpDNA haplotypes identified in this work. Nineteen samples with intragenomic variability were excluded from further analysis. In 52 samples, ITS nucleotide sequences are characterized by the same length (603 bp) and low nucleotide variability: 597 sites are monomorphic, and six sites are variable and parsimony informative. Four substitutions (positions 119, 122, 166, and 227) are found in ITS1, and two (positions 466 and 564), in ITS2. Seven haplotypes (ribotypes) are identified and deposited in DDBJ/ENA/GenBank-INSDC under accession numbers OV257425–OV257433. *O. strobilacea* has three ribotypes (RU1–RU3); *O. adamsiana,* two ribotypes (RU3 and RU4); and *O. vassilczenkoi,* four ribotypes (RU3, RU5–RU7). The RU3 ribotype is shared to all three species; it is found most frequently in *O. strobilacea* (27 specimens), but only in nine specimens of *O. adamsiana* and in one specimen of *O. vassilczenkoi*. The median network of genealogical relationships of ribotypes has a star-like structure (Fig. 2b), the RU3 ribotype connected by single mutational steps with other ribotypes is located at its center (Fig. 2b). It should be noted that the RU1 ribotype has been determined only in the samples from the STR1 population of *O. strobilacea*, and the RU2 ribotype, only in STR2.

DISCUSSION

The analysis of nucleotide polymorphism of IGS *psbA-trnH* + *trnL-trnF* + *trnS-trnG* cpDNA in *O. strobilacea, O. adamsiana,* and *O. vassilczenkoi* testify a high level of genetic diversity (Table 1). High rates of genetic variability for *O. strobilacea* (Table 1) are general characteristic of polymorphic species with a wide range. In northern China, *O. diversifolia* E. Peter also has high genetic diversity ($h = 0.880$, $\pi = 0.0006$) according to the nucleotide polymorphism of five IGS cpDNA: *trnT-psbD, petN-psbM, trnS-trnG, psbE-petL,* and intron *rpl*16 (Wang et al., 2021). Widespread species *O. oxyphylla* (Pall.) DC. and *O. lanata* (Pall.) DC. are characterized by similar values of the genetic diversity parameters (Kholina et al., 2019). These species inhabit, just like the studied populations of

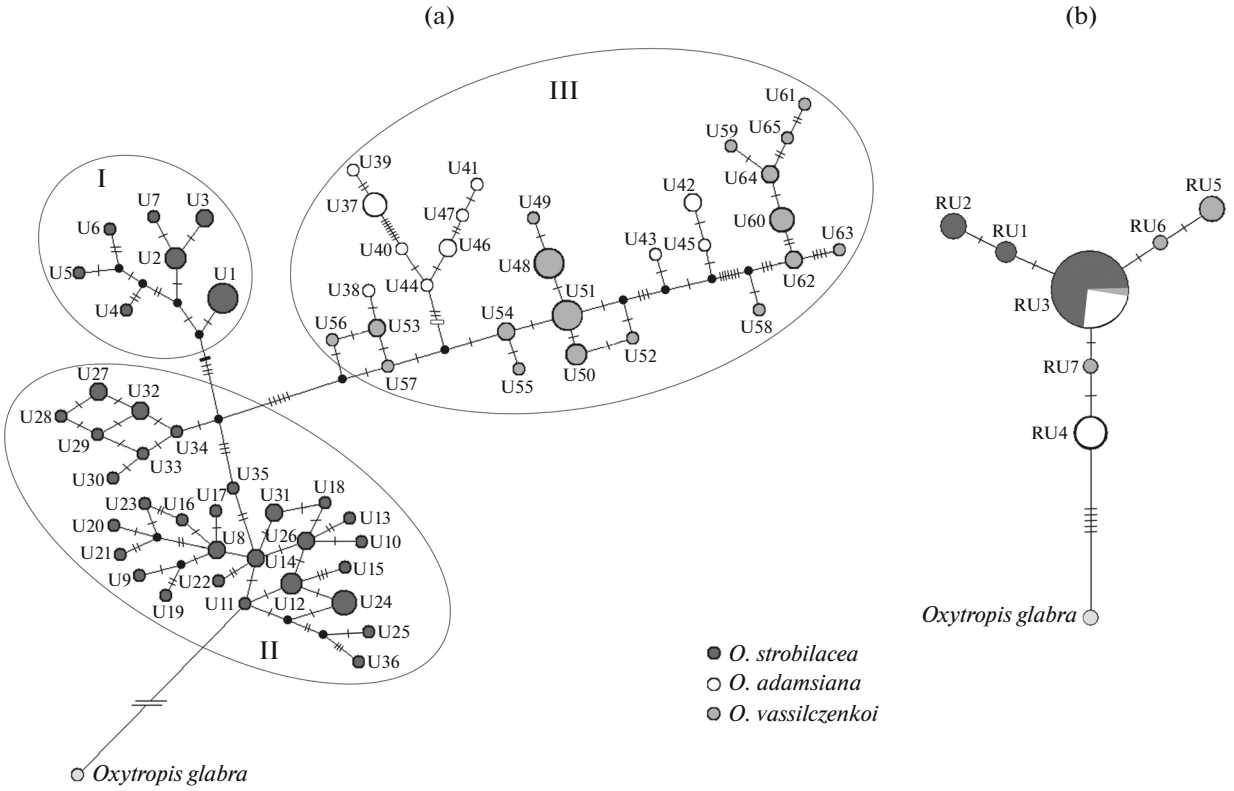

Fig. 2. Phylogenetic relationships of *Oxytropis strobilacea, O. adamsiana,* and *O. vassilczenkoi:* (a) genealogical network of chlorotypes cpDNA (U1–U65) constructed using the MJ method. The size of the circles reflects the frequency of occurrence of haplotypes. Small black circles are hypothetical chlorotypes. Transverse thin crossing lines on the branches are mutation events. Thick black and white crossing lines are indels, insertions, and deletions of nucleotides, respectively. The solid line indicates haplogroups I–III. Mutations for *O. glabra* (used as an outgroup) are not listed or considered; (b) genealogical network of ribotypes ITS nrDNA (RU1–RU7) constructed using the RM method. The size of the circles reflects the frequency of occurrence of ribotypes, and transverse thin intersecting lines on the branches are mutational events.

O. strobilacea and a highly polymorphic population ADAM5 of *O. adamsiana* studied here, the Baikal Siberia, one of the centers of speciation and diversity of the genus *Oxytropis* (Malyshev and Peshkova, 1984; Polozhiy, 2003). The Baikal center of speciation is characterized by uniqueness and richness of the flora species composition due to the orography and climate features of this region (Malyshev and Peshkova, 1984; Namzalov, 2009). Apparently, the level of polymorphism revealed in *O. strobilacea* and *O. adamsiana* populations may be associated with a variety of ecological and coenotic conditions of habitats, meeting the ecological needs of these species.

At the same time, a high level of haplotype diversity is unusual for the Anadyr–Chukotian endemic *O. vassilczenkoi,* inhabiting mainly the Arctic zone (Table 1). Endemic species are generally characterized by a low level of polymorphism. In populations of *O. neimonggolica* C.W. Chang & Y.Z. Zhao, a narrow-local endemic species native to northern China, *h* varies from 0.250 to 0.679 as measured by the variability of five IGS cpDNA (Wang et al., 2021). As for the arctic species, the previously studied *Oxytropis* species from sections *Arctobia* (Kholina et al., 2020) and *Gloeocephala* (Kholina et al., 2022) are characterized by the presence of monomorphic or weakly polymorphic populations with a high degree of divergence, which is largely explained by the history of the formation of species ranges. During the onset of glaciers, populations sharply reduced the area and effective population size (the "bottleneck" effect); during recolonization of post-glacial territories, populations were restored from refugia (the "founder" effect).

We assume that the unexpectedly high level of genetic diversity for the Arctic endemic *O. vassilczenkoi* is partly due to its origin from the highly polymorphic *O. strobilacea* and the preservation of ancestral polymorphism. A similar level of variability associated with the maintenance of ancestral polymorphism was reported earlier (Kholina et al., 2021) for the populations of the relict endemic species *O. triphylla* (Pall.) Pers. (h = 0.800–1.000) and for relict populations of *Astragalus onobrychis* L. (Plenk et al., 2020), in which the haplotype diversity varied as 0.833–1.000 according to polymorphism of the *atpI-H, ycf1,* and intron *rpL16* of cpDNA. In addition, the haplotype diversity

in *O. vassilczenkoi,* especially in the VAS4 population (eight haplotypes in the samples of 13 plants; Table 1), may indicate the existence of refugia in this area during climate fluctuations. Finally, the reproduction system of *Oxytropis* species (sexual reproduction and cross-pollination) and the gene exchange between existing populations may make a great contribution.

The absence of nucleotide divergence between *O. vassilczenkoi* populations from the Magadan region (VAS1, VAS2) and from Chukotka (VAS3) indicates that these localities are parts of the same regional metapopulation, which are intensively exchanging genes. The VAS4 population from the Olyutorskii District of Kamchatka krai differs significantly from the other three (*D*xy = 0.00205; Table 2), and the degree of divergence even exceed the interspecies difference (*D*xy = 0.00091, 0.00170; Table 2). A similarly high degree of nucleotide divergence has been found between the populations of *O. ochotensis* Bunge from Magadan oblast and Kamchatka (*D*xy = 0.00167), distanced from each other by about 800 km, as well as between the cpDNA phyletic lineages of *O. ruthenica* Vass. (*D*xy = 0.00188–0.00206) (Kozyrenko et al., 2020). The high degree of interpopulation differentiation in *O. vassilczenkoi* (77.57%; Table 3) is most likely associated precisely with the isolated position of the VAS4 population. This is also reflected in the median network of genealogical relationships of chlorotypes (Fig. 2a), in which the chlorotype group of the population VAS4 (U58–U65) occupies a terminal position on the branch forming haplogroup III. It should be noted that ribotypes RU6 and RU7 of ITS nrDNA are found only in the VAS4 population, but they are absent in the rest of the populations studied.

The indices of nucleotide divergence between *O. strobilacea* populations in some cases also exceed the interspecies values (Table 2), which indicates active microevolutionary processes in this species. This is also evidenced by the formation of two cpDNA phyletic lineages (Fig. 2a), one of which contains specific markers. The presence of two isolated phyletic lineages in *O. strobilacea*, the level of differentiation between which exceeds the level of differentiation between *O. strobilacea* and the other two species (Table 3), is rather unexpected given the compact geographic location of the *O. strobilacea* populations in the Baikal region (Fig. 1). To a large extent, such diversification may be due to the complex topography of the region. The influence of topography, as one of the leading factors of plant speciation, has been repeatedly noted in general earlier (Blanco-Pastor et al., 2019; Mahmoudi Shamsabad et al., 2019) and was found by us (Kholina et al., 2018) for *O. glandulosa* Turcz., a narrow-local endemic inhabiting the eastern coast of Lake Baikal. In *O. glandulosa,* two divergent phyletic lineages were found, each of which had specific markers, which allowed us to assume the presence of a cryptic species. In addition to the influence of relief and isolation of populations, the presence of chromosome races in *O. glandulosa* definitely contributed to the formation of phyletic lineages in this species; like that, the presence of chromosome races is characterized of *O. strobilacea* (2*n* = 16, 32, 48, and 64) (Malyshev, 2008a). Intraspecific divergence, resulting in the appearance of clearly distinct phyletic lineages, has been revealed for other *Oxytropis* species*: O. ruthenica* (Kozyrenko et al., 2020), *O. anadyrensis, O. borealis,* and *O. middendorffii* from section *Gloeocephala* (Kholina et al., 2022). Based on the nucleotide polymorphism of five IGS cpDNA, two phyletic lineages were identified in *Oxytropis leptophylla* (Pall.) DC. from northern China (Wang et al., 2021); these lineages are separated by 18 mutational steps in the genealogical network of haplotypes, and they form different isolated branches on the phylogenetic tree.

The low degree of differentiation established between three closely related species of the series *Strobilacei* (Table 3) is also characteristic of other closely related *Oxytropis* species, for example, for five species of the section *Orobia* (35.2%) (Kozyrenko et al., 2020). Based on polymorphism data of the IGS *ndhC-trnV* and *psbA-trnH*, genetic differences account for 16.18% of the total variability between relative species *Potentilla volgarica* Juz. and *P. eversmanniana* Fisch. ex Ledeb. (Schanzer et al., 2020), and according to *ndhJ-trnF* and *trnD-trnT*, for four closely related species *Indigofera* L., inhabiting East Asia, interspecific differences account for only 7.56% of the variability, and these species have shared haplotypes (Zhao et al., 2017).

Seven ribotypes of ITS nrDNA have been found in three species of the ser. *Strobilacei,* one of them is shared between all species, while none of 65 chlorotypes identified for these species turned out to be shared (Figs. 2a, 2b). A similar pattern is found in other plant species (Hou et al., 2017; Blanco-Pastor et al., 2019; Kozyrenko et al., 2020). Thus, for five species of the genus *Dendrobium* SW., 34 chlorotypes were identified according to nucleotide polymorphism of IGS *acc*D-*psa*I, *trn*C-*pet*N, and *rps*15-*ycf*1 cpDNA, and 25 ITS ribotypes; of the last two most frequently found ribotypes were shared between different species (Hou et al., 2017). The authors explained the presence of shared ribotypes by the manifestation of ancestral polymorphism of the progenitor species or by the existing gene flow, as well as by hybridization. In the case of species of the ser. *Strobilacei,* it is possible to assume the probability of hybridization between *O. strobilacea* and *O. adamsiana* in the zone of their sympatry. However, according to cpDNA data, these species are genetically isolated. Given the current distribution of *O. vassilczenkoi,* hybridization between the latter and the other two species is unlikely. Therefore, the presence of a shared ribotype in three species of the *Strobilacei* series may be associated to a greater extent with the polymorphism of the ancestral form, rapid adaptive radiation, and incomplete lineage sorting. Earlier, a shared ribotype was reported for six species of the genus *Oxytropis* belonging to three different

subgenera *Phacoxytropis, Tragacanthoxytropis*, and *Oxytropis* (Kholina et al., 2021b). This may be due to their common origin and the relatively recent rapid radiation noted for the genus *Oxytropis* (Shavvon et al., 2017). In some cases, rapid radiation of species may be accompanied by rapid isolation, as shown for the *Indigofera* species (Zhao et al., 2017). The incomplete lineage sorting of *O. adamsiana* and *O. vassilczenkoi* is also indicated by the chlorotype distribution which does not correspond to their taxonomic affiliation (haplogroup III, Fig. 2a). Previously, a similar pattern was revealed for four species of *Oxytropis* section *Polyadena* (Kholina et al., 2021a), as well as for three species of the *Acanthophyllum squarrosum* complex (Mahmoudi Shamsabad et al., 2019) and for 12 species of *Potentilla* (Schanzer et al., 2020).

The formation of phylogenetic relationships under the mutual influence of different evolutionary processes leads to the emergence of a complex pattern of the relationship between *Oxytropis* species of the ser. *Strobilacei* sect. *Orobia* as manifestations of the reticulate evolution characteristic of this genus. We have also noted a similar pattern in the relationships between *Oxytropis* species from other sections (Kholina et al., 2016, 2021a, 2021).

CONCLUSIONS

A high level of haplotype diversity has been found in the studied populations of *O. strobilacea, O. adamsiana,* and *O. vassilczenkoi* ser. *Strobilacei* sect. *Orobia* from Asian Russia; the level of nucleotide diversity varies from low to medium. The presence of two phyletic lineages of *O. strobilacea* indicates intensive diversification processes. The species *O. adamsiana* and *O. vassilczenkoi* are genetically distinct from *O. strobilacea*; they form a single genetic complex due to a common origin and incomplete lineage sorting, while the absence of shared chlorotypes confirms the status of *O. vassilczenkoi* as a separate species.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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