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PLANT GENETICS

Modern State of Populations of Endemic *Oxytropis* Species from Baikal Siberia and Their Phylogenetic Relationships Based on Chloroplast DNA Markers

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Abstract—The genetic diversity and population structure of the endemic species of Baikal Siberia *Oxytropis triphylla*, *O. bargusinensis*, and *O. interposita* were studied for the first time on the basis of the nucleotide polymorphism of intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of chloroplast DNA. All populations of these species were characterized by a high haplotype (0.762–0.924) and relatively low nucleotide (0.0011–0.0022) diversity. Analysis of the distribution of variability in *O. triphylla* and *O. bargusinensis* showed that there was no significant genetic differentiation between populations of each species; the gene flow was 4.43 and 8.91, respectively. The high level of genetic diversity in the studied populations indicates a relatively stable state of these populations. A study of the phylogenetic relationships of closely related species confirms the concept of the origin of *O. bargusinensis* and *O. tompudae* as a result of intersectional hybridization of the species of the sections *Orobia* and *Verticillares*.

Keywords: Oxytropis, Fabaceae, endemic species, genetic diversity, population structure, phylogenetic relationships, intergenic spacers, chloroplast DNA

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INTRODUCTION

In recent years in Baikal Siberia, the anthropogenic press on wildlife has intensified and great changes in the structure of natural landscapes have taken place: large areas of floodplains of big rivers of steppe and forest-steppe territory have been transformed into arable land or flooded during the construction of hydroelectric stations, and the remaining part continues to be intensively exploited, which leads to fragmentation and the decrease in the number of plant populations, the elimination of individual species, the general and local depletion of the flora, and the irreplaceable loss of genetic resources of plant life. This situation became reflected in the increase in the number of plant species that are endangered and in need of protection. Thus, the *Red Book of the Republic of Buryatia* included 139 rare plant species in 1988 [1], 225 species in 2002 [2], and 251 species in 2013 [3]. *The Red Book of Irkutsk Oblast* included 167 rare plant species in 2001 [4] and 172 species in 2010 [5].

The most vulnerable elements of regional floras are usually endemic, relict, and also some useful (ornamental, medicinal, and food) plants. Territories surrounding Lake Baikal are characterized by an increased level of endemism. This is facilitated by the mountain relief, which creates, on one hand, a mosaic of microclimates and, on the other hand, long-term isolation of individual steppe habitats confined to the southern slopes or bottoms of the depressions. These circumstances favor the formation of new species or the preservation of relicts of different ages [6, 7].

Endemic plants of the Baikal region include representatives of the genus *Oxytropis* DC. [8], which was probably formed at the end of the Miocene–Early Pliocene in Southern Siberia on the basis of a macromutant that emerged in one of the ancient *Astragalus* species [9]. *Oxytropis triphylla* (Pall.) Pers. is an endemic of Baikal Siberia, a relict, and belongs to the section *Xerobia* Bunge. This species is included in the *Red Book of the Russian Federation* in status 3 (R), a rare species, narrow-range endemic [10], and in the *Red Books of Irkutsk Oblast* [5] and the Republic of Buryatia [3] in status 3 (NT), a rare species, relict probably of Pliocene age. According to G.A. Peshkova [11], *O. triphylla* is the most ancient species of the section *Xerobia* and belongs to the relicts of the ancient Mediterranean (Miocene–Pliocene) flora and has the Angarid confinement. *O. triphylla* is found in the Olkhonsky district of Irkutsk oblast: most of the popula-

tions of this species are noted in the Tazheran steppe; there are small populations at the northern extremity of the Olkhon Island [5, 11]; on the east coast of Lake Baikal, this species was found for the Barguzin depression [12] and along the valley of the Uda River [13]. The ontogenetic structure of *O. triphylla* coenopopulations was extensively studied and a complex assessment of their state was carried out [13]. The species *O. bargusinensis* Peschkova and *O. interposita* Sipl. belong to the section *Verticillares* DC. [8] and are young endemics of the Quaternary Period [6]. *O. bargusinensis* is an endemic of the Northern Transbaikal and occurs in the Bodaibinsky and Barguzinsky districts, at the Shilka River near the mouth of the Kaltia River, and at the Alla River [8]. This species is included in the *Red Book of Irkutsk Oblast* [5], category 4 (I), a species with an undetermined status. According to G.A. Peshkova [14], *O. bargusinensis* is a hybrid that has intermediate characteristics between the parental species *O. sylvatica* (Pall.) DC. of the section *Orobia* Bunge and *O. turczaninovii* Jurtz. of the section *Verticillares*, and is also close to *O. tompudae* M. Popov, a hybridogenic species of the same pair. However, phenotypic analysis of the species of the section *Verticillares* on the basis of 54 diagnostic traits showed that *O. bargusinensis* did not show proximity to the prospective parent *O. turczaninovii* [15]. Earlier, M.G. Popov [16] considered *O. tompudae* as the largest race (jordanon) of the linneon *O. oxyphylla* (Pall.) DC., and then G.A. Peshkova [17] noted the proximity of *O. tompudae* to *O. sylvatica*, but it is distinguished from the latter by whorled leaves and longer calyx teeth. *O. interposita* was noted only in Buryatia in the valley of the Alla River near the hot springs at the foot of the Barguzinsky Ridge [8]. Previously, this species was included in the *Red Book of the Republic of Buryatia* (2002) in status 3 (R), a rare species, narrow-range endemic of the Barguzinsky Ridge [2]. It is presumably an intermediate species between the sections *Verticillares* and *Polyadena* Bunge [18]. External similarity links *O. interposita* to *O. muricata* (Pall.) DC. of the section *Polyadena*. The section *Polyadena* combines glandular species of whorled-leaf oxytropes, but *O. interposita* differs from *O. muricata* by the lack of glandularity, greater shoot height, and the presence of pubescence on inflorescences and legumes [18]. In terms of the morphological traits, *O. interposita* partly resembles *O. varlakovii* Serg. of the section *Polyadena*, but the latter have warty legumes [8]. According to L.I. Malyshev [15], *O. interposita* is probably a mutant. There are no data on the state of *O. bargusinensis* and *O. interposita* populations.

Assessment of the viability and adaptive potential of rare and endemic species under changing environmental conditions and development of a strategy for conservation of natural populations require knowledge of the genetic diversity and the population structure of species. Analysis of the chloroplast genome, which in the family Fabaceae Lindl. s. l. is inherited by the maternal line [19], not only enables reliable information about the current state of the species to be obtained but also contributes to the solution of disputable taxonomic and phylogenetic problems. So, using markers of chloroplast DNA (cpDNA), we studied in detail the populations of the endemic of the western coast of Lake Khanka *O. chankaensis* Jurtz. and formulated practical recommendations for the conservation of this species [20] and confirmed the independence of closely related species *O. chankaensis* Jurtz. and *O. oxyphylla* (Pall.) DC. of the section *Verticillares* [21], and *O. coerulea* (Pall.) DC. and *O. mandshurica* Bunge of the section *Janthina* Bunge, as well as the species status of *O. czukotica* of the section *Arctobia* Bunge [22].

The purpose of this investigation is to study the genetic diversity and population structure of the endemic species *O. triphylla*, *O. bargusinensis*, and *O. interposita* and to clarify their phylogenetic relationships to closely related species on the basis of analysis of nucleotide polymorphism of intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of cpDNA.

MATERIALS AND METHODS

The material of the study consisted of 62 plants of the species *O. triphylla*, *O. bargusinensis*, and *O. interposita* of the subgenus *Oxytropis* Bunge from five natural populations (Table 1). For the reconstruction of phylogenetic relations, representatives of *O. sylvatica*, *O. tompudae*, and *O. varlakovii* of the subgenus *Oxytropis* and *O. glabra* (Lam.) DC. of the section *Mesogaea* Bunge of the subgenus *Phacoxytropis* Bunge were included in the analysis as an outgroup (Table 1). A total of 75 plants were analyzed. In this paper, we take the names of species, sections, and subgenera as treated by L.I. Malyshev [8].

Individual preparations of total DNA were extracted from leaf tissue according to the procedure [23]. For the analysis of genetic variability, intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of cpDNA that we used earlier [22] were selected. Amplification of regions was carried out using universal primers, reaction conditions, and temperature regimes recommended for these regions [24, 25]. Nucleotide sequences of forward and backward chains, obtained after cyclic sequencing of amplification products, were determined using an ABI 3130 genetic analyzer (Applied Biosystems, United States), then edited and assembled using the software Staden Package v. 1.5 [26]. Thus, nucleotide sequences of the three cpDNA intergenic spacers were determined for 75 plants of the *Oxytropis* species. The sequences of haplotypes of the intergenic spacers were deposited in the database ENA (European Nucleotide Archive) with accession numbers LT856461–LT856598. For each sample, the sequences of the regions were manually aligned using the program SeaView v. 4 [27].

Table 1. Studied populations of *Oxytropis* species, distribution of haplotypes, and accession numbers of nucleotide sequences in the ENA database

Species, location (number of samples)	Latitude, longitude	Population code	Haplotype	Accession number in ENA *psbA–trnH/trnL–trnF/trnS–trnG*
		O. triphylla		
Republic of Buryatia, Kurumkansky district, near Sakhuli village (12)	54.45353° N, 110.45081° E	TRIKUR	H1	LT856461/LT856494/LT856527
			H2	LT856462/LT856495/LT856528
			H3	LT856463/LT856496/LT856529
			H4	LT856464/LT856497/LT856530
			H5	LT856465/LT856498/LT856531
			H6	LT856466/LT856499/LT856532
			H7	LT856467/LT856500/LT856533
			H8	LT856468/LT856501/LT856534
Republic of Buryatia, Khorinsky district, near Udinsk village (6)	52.08312° N, 108.98470° E	TRIHOR	H1	LT856469/LT856502/LT856535
			H6	LT856470/LT856503/LT856536
			H9	LT856471/LT856504/LT856537
			H10	LT856472/LT856505/LT856538
		O. bargusinensis		
Republic of Buryatia, Kurumkansky district, Alla River valley (15)	54.70861° N, 110.68113° E	BARKUR	H11	LT856473/LT856506/LT856539
			H12	LT856474/LT856507/LT856540
			H13	LT856475/LT856508/LT856541
			H14	LT856476/LT856509/LT856542
			H15	LT856477/LT856510/LT856543
			H16	LT856478/LT856511/LT856544
Republic of Buryatia, Barguzinsky district, near Urzhil village (15)	54.06054° N, 110.35923° E	BARBAR	H11	LT856479/LT856512/LT856545
			H13	LT856480/LT856513/LT856546
			H14	LT856481/LT856514/LT856547
			H16	LT856482/LT856515/LT856548
			H17	LT856483/LT856516/LT856549
			H18	LT856484/LT856517/LT856550
			H19	LT856485/LT856518/LT856551
			H20	LT856486/LT856519/LT856552
			H21	LT856487/LT856520/LT856553
			H22	LT856488/LT856521/LT856554
O. interposita, Republic of Buryatia, Kurumkansky district, Alla River valley (14)	54.70987° N, 110.68060° E	INTKUR	H23	LT856489/LT856522/LT856555
			H24	LT856490/LT856523/LT856556
			H25	LT856491/LT856524/LT856557
			H26	LT856492/LT856525/LT856558
			H27	LT856493/LT856526/LT856559
O. sylvatica, Republic of Buryatia, Khorinsky district, near Udinsk village (3)	52.11708° N, 109.13360° E	SYLVAT	S1	LT856560/LT856573/LT856586
			S2	LT856561/LT856574/LT856587
			S3	LT856562/LT856575/LT856588
O. varlakovii, Zabaikalsky krai, Aginsky district, near Lake Nozhy (3)	50.80440° N, 114.82984° E	VARLAK	V1	LT856563/LT856576/LT856589
			V2	LT856564/LT856577/LT856590
			V3	LT856565/LT856578/LT856591
O. tompudae, Republic of Buryatia, Kurumkansky district, near Maysk village (6)	54.59956° N, 110.77882° E	TOMPUD	T1	LT856566/LT856579/LT856592
			T2	LT856567/LT856580/LT856593
			T3	LT856568/LT856581/LT856594
			T4	LT856569/LT856582/LT856595
			T5	LT856570/LT856583/LT856596
			T6	LT856571/LT856584/LT856597

Table 1. (Contd.)

Species, location (number of samples)	Latitude, longitude	Population code	Haplotype	Accession number in ENA *psbA–trnH/trnL–trnF/trnS–trnG*
O. glabra, Republic of Buryatia, Ivolginsky district, near Orongoi village	51.54689° N, 107.03040° E	GLABRA	G1	LT856572/LT856585/LT856598

Bold indicates unique haplotypes.

For genetic analyses, two matrices were composed. The matrix for population analysis included combined sequences of three cpDNA regions for each sample of the species *O. triphylla*, *O. bargusinensis*, and *O. interposita*. The number of haplotypes (*nh*) and their population frequencies, haplotype (*h*) and nucleotide (π) diversity, pairwise genetic distances (F_{ST}), the level of differentiation and distribution of genetic variability within and between populations (AMOVA, analysis of molecular variation), and tests for population stability of Tajima (*D*) and Fu (*F*s) were calculated using the program Arlequin v. 3.5 [28]. The gene flow (*Nm*) and the analysis of the degree of divergence (*K*s) between populations on the basis of nucleotide substitutions and the distribution of pairwise nucleotide differences (mismatch distributions) were carried out in the program DnaSP v. 5.0 [29]. The genealogical relationships of haplotypes were analyzed using the median-joining (MJ) method in the program Network v. 5.0 [30], encoding each deletion or insertion, regardless of their size, as a single mutational event.

To reconstruct phylogenetic relations, a second matrix was composed, which contained haplotypes of endemic species *O. triphylla*, *O. bargusinensis*, and *O. interposita* and sequences of the same regions of *O. sylvatica*, *O. tompudae*, *O. varlakovii*, and *O. glabra* (Table 1), as well as haplotypes H53–H57 of *O. oxyphylla* and H67 of *O. muricata* that we identified earlier [22]. Phylogenetic analyses of the sequences were carried out using the maximum likelihood (ML) and maximum parsimony (MP) methods using the software package PAUP v. 4.0b10 [31]. The optimal model of the evolution of nucleotide sequences for ML analysis was selected in the program Modeltest v. 3.06 [32] using hierarchical tests. For ML and MP analyses, a heuristic search of optimal topology was used. The statistical significance of the branching order was estimated using a bootstrap analysis of 1000 alternative trees (bootstrap percentage, BP, %). Bayesian analysis (Bayesian inference, BI) of phylogenetic relations was carried out using the program MrBayes 3.1.2 [33]. The analysis included 10 million generations of Markov chains with the selection of every one-thousandth of generated trees. The first 25% of trees (before –lnL values start plateauing) were excluded from the analysis (burn-in), and the remaining ones were used to construct a consensus phylogenetic tree and obtain estimates of a posteriori probability (PP) of its branches. The BP values less than 50% and PP values less than 0.95 were not considered and are not indicated in Fig. 3.

This work was carried out using the equipment of the Shared Research Center Biotechnology and Genetic Engineering of the Federal Research Center of Biodiversity of the Far Eastern Branch of the Russian Academy of Sciences.

RESULTS

Sequence polymorphism analysis for 62 samples of the endemic species *O. triphylla*, *O. bargusinensis*, and *O. interposita* revealed that the length of one region is different due to the presence of insertions/deletions (indels) and mono- and dinucleotide repeats. Thus, the length of *psbA–trnH* was 454 bp in all *O. bargusinensis* representatives except one (455 bp), 455 bp in *O. interposita*, and 460 bp in *O. triphylla* except two (462 bp). Differences are due to an indel (six nucleotides) and two variable mononucleotide repeats: poly-A (A_{10}–A_{12}) and poly-T (T_8–T_9) motifs. Sequences of this region were revealed to contain three variable parsimony informative sites. The length of the *trnL–trnF* spacer varies from 756 to 767 bp owing to the presence of a mononucleotide poly-T motif (T_9–T_{10}) and a dinucleotide TA motif containing from 6 to 11 repeats. Variability of this region is low; only one nucleotide substitution is parsimony informative. The length of *trnS–trnG* varies from 1182 to 1189 bp; the differences are due to the presence of variable poly-A (9–14 repeats) and three poly-T (3–19 repeats) motifs and short indels (2–3 nucleotides). The spacer sequences contained nine polymorphic sites, seven of which were parsimony informative. The lengths of the aligned matrices of the regions *psbA–trnH*, *trnL—trnF*, and *trnS–trnG*, including indels, were 463, 767, and 1194 bp, respectively. The length of the combined sequences of the three regions of cpDNA was 2424 sites, including 2375 monomorphic and 13 variable sites. Eleven nucleotide substitutions were parsimony informative.

The parameters of genetic variability of the investigated populations of the three species are presented in Table 2. The highest level of haplotype diversity was found in the *O. triphylla* population from the Kurumkansky district ($h = 0.924$), the lowest in the *O. bargusinensis* population ($h = 0.762$) from the same district. In general, all the studied populations are char-

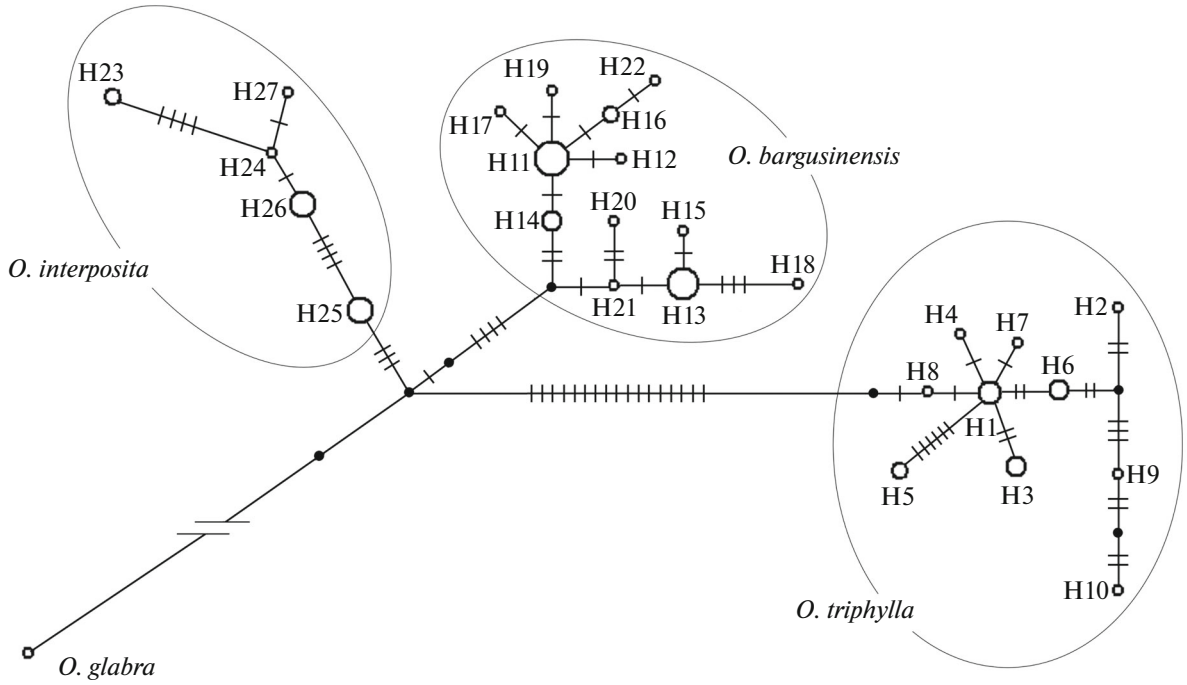

Fig. 1. The genealogical network of haplotypes of representatives of the species *O. triphylla*, *O. bargusinensis*, and *O. interposita* constructed using the MJ method. The size of circles reflects the frequency of occurrence of haplotypes, small black circles are median vectors, and cross lines are mutations. H1–H27 are haplotypes of *Oxytropis* species; see Table 1. An *O. glabra* sample was used as outgroup.

acterized by a high level of haplotype and a relatively low level of nucleotide diversity (Table 2). The divergence of nucleotide sequences is absent or very low between populations of one species and high between populations of different species (Table 3). A significant differentiation of the species under study and, consequently, a high degree of their genetic disunity is indicated by high pairwise genetic distances, whereas between populations of one species they are small and statistically insignificant (Table 3). Interspecies distances were 0.85833 for the pair *O. triphylla*–*O. bargusinensis*, 0.83517 for *O. triphylla*–*O. interposita*, and 0.75981 for *O. bargusinensis*–*O. interposita*. According to the AMOVA results (Table 4), the main part of all genetic variability (more than 83%) is due to the interspecific component and about 17% is due to the interpopulation component ($\Phi_{ST} = 0.83053$, $P < 0.0001$); the gene flow between the species was 0.15 migrants per generation. Analysis of the distribution of variability in *O. triphylla* and *O. bargusinensis* showed that there is no significant genetic differentiation between populations of each species ($\Phi_{ST} = 0.06278$, $P > 0.10$ and $\Phi_{ST} = 0.02729$, $P > 0.20$, respectively, Table 4); the gene flow was 4.43 and 8.91, respectively.

Nucleotide substitutions and indel variations in 62 sequences of the combined matrix revealed 27 haplotypes (H1–H27, Table 1), including ten haplotypes in *O. triphylla* (H1–H10), twelve in *O. bargusinensis* (H11–H22), and five in *O. interposita* (H23–H27). No common haplotypes for the three species were revealed. The greatest number of private haplotypes was identified in *O. bargusinensis*. To reveal genealogical relations among haplotypes, a network was constructed in which three divergent haplogroups are distinguished, each formed by haplotypes of only one species (Fig. 1). The central haplotype was not observed (hypothetical haplotype). The *O. triphylla* haplogroup is distanced from the central hypothetical haplotype by 20 mutational steps, *O. bargusinensis* haplogroup by seven, and *O. interposita* haplogroup by four mutational steps. *O. bargusinensis* haplotypes form two separate branches, but this distribution does not correspond to the population affiliation. Most haplotypes in the *O. triphylla* and *O. bargusinensis* haplogroups are 2–3 mutational steps from the neighboring one.

Population stability tests for all the populations except for the population *O. bargusinensis* BARBAR are statistically insignificant (data not shown), indicating that they are at the mutation–drift equilibrium. For the BARBAR population, the *F*s test had a negative statistically significant value (*F*s = –3.15432, $P < 0.05$) as a result of the presence of a large number of unique haplotypes (Table 1). The analysis of the distribution of pairwise nucleotide differences between haplotypes, used as a test for the population size changing, was carried out only for *O. bargusinensis*

Table 2. Parameters of genetic variability of populations of the species *O. triphylla*, *O. bargusinensis*, and *O. interposita* according to cpDNA data

Population code	Parameters				
	S	*I*	*nh*	*h* ± SD	π ± SD
O. triphylla					
TRIKUR	0	24	8	0.924 ± 0.058	0.0018 ± 0.0011
TRIHOR	0	25	4	0.800 ± 0.172	0.0022 ± 0.0014
In general, for the species	0	26	10	0.915 ± 0.0405	0.0020 ± 0.0011
O. bargusinensis					
BARKUR	4	20	6	0.762 ± 0.096	0.0011 ± 0.0007
BARBAR	4	21	11	0.895 ± 0.070	0.0016 ± 0.0009
In general, for the species	5	21	12	0.844 ± 0.046	0.0013 ± 0.0008
O. interposita					
INTKUR	0	29	5	0.769 ± 0.075	0.0015 ± 0.0009

S is the number of nucleotide substitutions; *I* is the number of indels; *nh* is the number of haplotypes; *h* is the haplotype diversity; π is the nucleotide diversity; SD is the standard deviation. For the population code, see Table 1.

Table 3. Nucleotide divergence (*K*s) and pairwise genetic distances (F_{ST}) between populations of the species *O. triphylla*, *O. bargusinensis*, and *O. interposita* according to cpDNA data

Population code	TRIKUR	TRIHOR	BARKUR	BARBAR	INTKUR
K_S					
TRIKUR	–	0.000 (0)	8.800 (7)	8.533 (7)	7.000 (7)
TRIHOR	0.00000	–	7.800 (6)	7.533 (6)	7.000 (7)
BARKUR	0.00368	0.00326	–	1.680 (0)	4.800 (3)
BARBAR	0.00357	0.00315	0.00070	–	4.533 (3)
INTKUR	0.00293	0.00293	0.00201	0.00189	–
F_{ST}					
TRIKUR	0.00000				
TRIHOR	0.06278 ns	0.00000			
BARKUR	0.87121*	0.88304*	0.00000		
BARBAR	0.84634*	0.85287*	0.02729 ns	0.00000	
INTKUR	0.84848*	0.83709*	0.77953*	0.73631*	0.00000

Above the diagonal is the average number of nucleotide differences (in parentheses indicate the number of fixed differences), below the diagonal is the average number of nucleotide substitutions per site; * $P < 0.0001$; ns is nonsignificant. The level of significance is determined on the basis of 1023 permutations. For the population code, see Table 1.

populations, since there are no nucleotide substitutions in the haplotypes of the other two species. The graphs (Fig. 2) have a bimodal distribution corresponding to the model of demographic equilibrium, that is, long-term population stability (constant population size).

The reconstruction of phylogenetic relations of the closely related species showed that the phylogenetic trees constructed by different methods (ML, MP, and BI) without taking into account indels did not differ in topology. The phylogram obtained as a result of the ML analysis is shown in Fig. 3. The *Oxytropis* species form two clades. Clade I with high support (93/87/1.00, bootstrap indices for ML and MP, and a posteriori probability for BI analyses, respectively) includes haplotypes of *O. triphylla* populations of the section *Xerobia*. Clade II with low resolution (65/71/–) includes all haplotypes of the other studied species in which three statistically supported clusters can be distinguished. One cluster (67/65/1.00) is formed by haplotypes of the species *O. interposita* and *O. varlakovii* belonging to the sections *Verticillares* and *Polyadena*, respectively, and the second cluster (63/70/0.99) is formed by *O. sylvatica* haplotypes of the section *Orobia* and five out of six *O. tompudae* haplotypes of the section *Verticillares*. The third cluster (62/60/0.97)

Table 4. Distribution of total genetic variability from cpDNA data

Variability	*d.f.*	SSD	CV	%	Fixation index
Species *O. triphylla*, *O. bargusinensis*, and *O. interposita*					
Between species	2	363.289	9.19605	83.05	Φ_{ST} = 0.83053*
Within species	59	110.711	1.87646	16.95	
Total	61	474.000	11.07251		
O. triphylla populations TRIKUR and TRIHOR					
Between populations	1	3.528	0.15386	6.28	Φ_{ST} = 0.06278 ns
Within populations	16	36.750	2.29688	93.72	
Total	17	40.278	2.45074		
O. bargusinensis populations BARKUR and BARBAR					
Between populations	1	2.267	0.04476	2.73	Φ_{ST} = 0.02729 ns
Within populations	28	44.667	1.59524	97.27	
Total	29	46.933	1.64000		

d.f. is degree of freedom; SSD is the sum of squares; CV is the absolute value of the variability component; % is the percentage of genetic variability; Φ_{ST} is the variability component associated with interspecific/interpopulation variability; * $P < 0.0001$; ns is nonsignificant. The level of significance is determined on the basis of 1023 permutations.

includes all the haplotypes of the *O. bargusinensis* and *O. oxyphylla* populations and one *O. tompudae* haplotype of the section *Verticillares*. *O. bargusinensis* haplotypes are distributed in two groups not related to the population affiliation.

DISCUSSION

The study of genetic polymorphism of endemic species represents a necessary stage in the development of a strategy for their conservation and recovery. It is considered that, in the small isolated populations of endemic species adapted to specific habitats, the level of genetic diversity is not high, and there is a danger of its further decline owing to gene drift and closely related crosses [34–36]. A low level of polymorphism does not provide for the adaptive potential of species and increases the risk of their disappearance [36]. In this context, biodiversity conservation requires adequate determination of the genetic reserve of a species.

Our study of populations of the endemic species *O. triphylla*, *O. bargusinensis*, and *O. interposita* carried using the data of nucleotide polymorphism analysis of intergenic spacers *psbA–trnH*, *trnL—trnF*, and *trnS–trnG* of cpDNA revealed high haplotype (*h* varied from 0.762 to 0.924) and relatively low nucleotide (π varied from 0.0011 to 0.0022) diversity (Table 2). The polymorphism level is comparable to or higher than that of other endemic species of the Fabaceae family, for example, in *O. chankaensis*, a narrow-range endemic of the western coast of Lake Khanka, according to the nucleotide polymorphism of intergenic spacers *trnL–trnF*, *petG–trnP*, and *trnS–trnG* of cpDNA, $h = 0.718$, $\pi = 0.0005$ [20]; in *Dalbergia nigra* (Vell.) Allemao ex Benth, an endangered tree, an endemic of the central Brazilian Atlantic forests, according to the variability of the spacer *trnV–trnM* and *trnL* intron, $h = 0.752$,

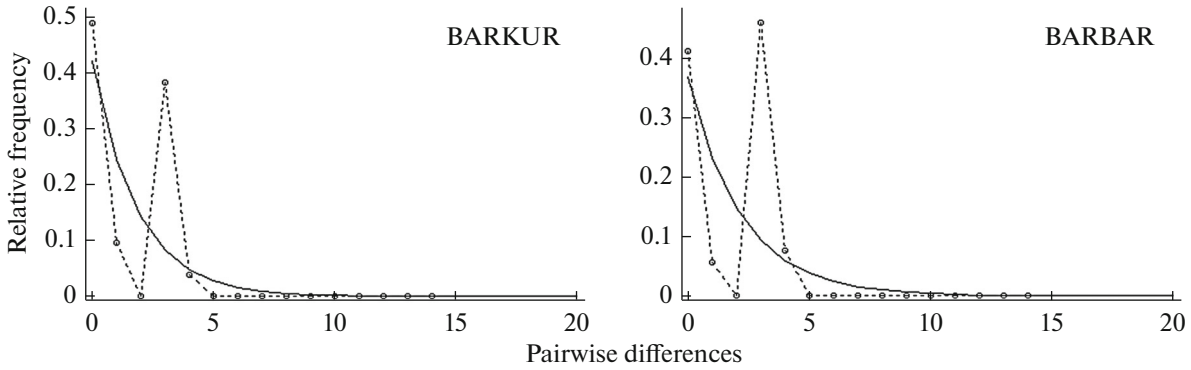

Fig. 2. Graphs of the distribution of pairwise nucleotide differences in *O. bargusinensis* populations. The solid line is the expected distribution; the dashed line is the observed distribution. For the population code, see Table 1.

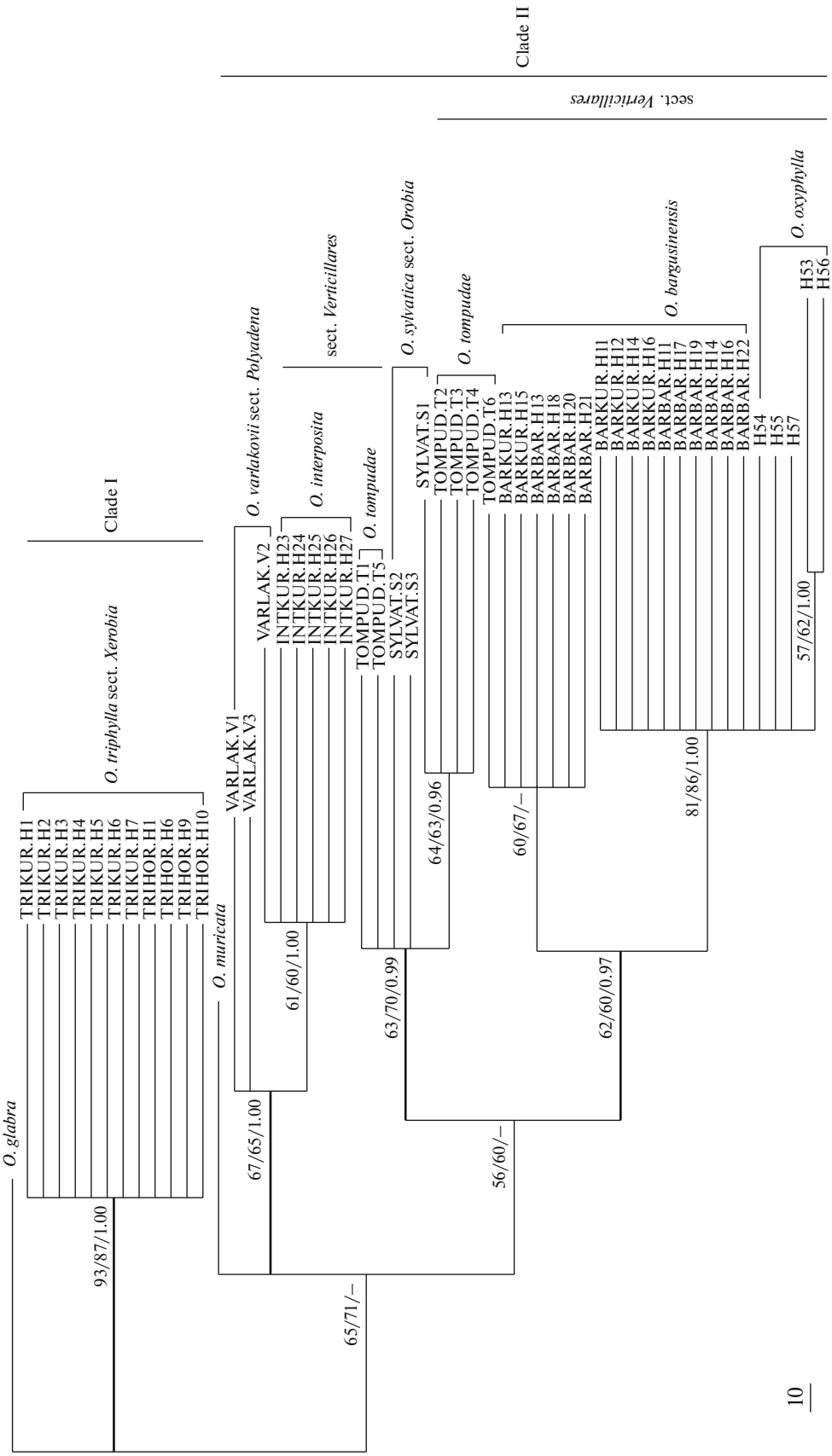

Fig. 3. The phylogenetic tree of representatives of the genus *Oxytropis* based on comparison of nucleotide sequences of cpDNA intergenic spacers by the ML method (GTR model, –Ln likelihood = 3599.82418). Numbers in the branch nodes denote BP values for ML and MP trees and PP values for Bayesian trees. Thick lines indicate the clusters considered in this work. For the population code, see Table 1. As outgroup, a sample of *O. glabra* was used.

$\pi = 0.0009$ [37]; as well as in representatives of other families, for example, in *Aconitum gimnandrum* Maximum. (Ranunculaceae), an alpine endemic of the Qinghai-Tibet Plateau, according to the polymorphism of the spacers *rpl*20–*rps*12 and *psbA*–*trnH* and intron *trnV*, *h* in the populations varied from 0 to 0.6000 and π varied from 0 to 0.0088 [38]; in *Centaurea borjae* Valdés B. & Rivas G. (Asteraceae), a narrow-range endemic of coastal cliffs in the northwest of the Iberian Peninsula, according to the analysis of the cpDNA spacer *trnT*–*trnF*, $h = 0.490$, $\pi = 0.0016$ [39]; in *Petunia exserta* Stehmann (Solanaceae), an endemic species that grows exclusively in rocky shelters in southern and southeastern Brazil, according to the nucleotide polymorphism of the spacers *psbA*–*trnH* and *trnS*–*trnG*, $h = 0.483$, $\pi = 0.001$ [40].

O. triphylla is characterized by maximum values of polymorphism (Table 2), while no significant genetic differences between the two geographically isolated populations of the species (Table 3, Fig. 3) were revealed. This may be a manifestation of ancestral polymorphism in the relict of the Miocene–Pliocene flora. According to G.A. Peshkova [11], in the past, under a warm and dry climate, the ancestral species of *O. triphylla* was widely distributed within the territory of Southern Siberia. Apparently, it was able to have a high level of genetic diversity and partly keep it to this day. The absence of population differentiation in *O. triphylla* may also be due to the persistent exchange of genes through a chain of intermediate local habitats. *O. triphylla* is strongly distinguished from the *Oxytropis* species that grow in Baikal Siberia by leaves of one or rarely two pairs of leaflets; in addition, inside the section *Xerobia*, it is isolated into a special oligotypic series *Triphyllae* [8, 11]. The phylogram (Fig. 3) indicates genetic isolation of *O. triphylla* from all the other studied *Oxytropis* species.

The range of *O. bargusinensis* is much smaller than that of *O. triphylla*; however, the levels of genetic diversity in the populations of these species are fairly close (Table 2). At the species level, the *O. bargusinensis* values are lower than those of *O. triphylla* but exceed known values for other endemic species (see above). A large number of haplotypes detected in closely located *O. bargusinensis* populations (Table 1, Fig. 1) indicate a rather high level of genetic diversity for the narrow-range species. The reason for this may be its hybrid origin from the pair *O. sylvatica* and *O. turczaninovii* [8, 14]. A species related to *O. bargusinensis* is another hybrid species *O. tompudae* of the section *Verticillares*. There are different opinions about the parental species of *O. tompudae*. Some authors [14] suggest that *O. tompudae*, like *O. bargusinensis*, is a hybrid of *O. sylvatica* and *O. turczaninovii*; others [8] suggest that *O. tompudae* is a hybrid of *O. sylvatica* and *O. oxyphylla*. In addition, L.I. Malyshev [8] considers *O. turczaninovii* to be the ecogeographical race of *O. oxyphylla*. Reconstruction of phylogenetic relations (Fig. 3) showed that *O. bargusinensis*, *O. oxyphylla*, and haplotype T6 of *O. tompudae* of the section *Verticillares* form a sister cluster to a cluster formed by five out of six haplotypes (T1–T5) of *O. tompudae* and *O. sylvatica* of the section *Orobia*. It can be assumed that the *O. sylvatica* species could have been parental by the maternal line for the five *O. tompudae* samples and by the paternal line for the *O. tompudae* haplotype T6. Earlier [41], according to the nucleotide polymorphism of the intergenic spacer *rpl*32–*trnL* of cpDNA in *Campanula baumgartenii* Becker (Campanulaceae) and ten hybrids *C. baumgartenii* × *rotundifolia*, it was found that *C. baumgartenii* was the parental species by the maternal line for eight hybrids and by the paternal line for two hybrids. On the basis of the results we obtained, it can be assumed that, for the *O. tompudae* haplotype T6, the parent by the maternal line was close to *O. bargusinensis*. Taking into account the opinions expressed earlier [8, 14], it could have been *O. turczaninovii* or *O. oxyphylla*. All haplotypes of *O. oxyphylla* form a single group with *O. bargusinensis* (Fig. 3). Even if *O. oxyphylla* is not directly a parental species for *O. bargusinensis*, it is obvious that *O. bargusinensis* originated from the representative of the *Verticillares* section by the maternal line. On the other hand, *O. bargusinensis* is separated from the prospective parental species *O. sylvatica*, which apparently indicates the paternal relationship. In general, the results of our study confirm the concept formed on the basis of the study of morphological traits about *O. bargusinensis* and *O. tompudae* originating as a result of intersectional hybridization.

The level of haplotype diversity of *O. interposita* is lower than that of *O. triphylla* and *O. bargusinensis* (Table 2), but higher than in other endemic species (see above), which is unusual for an extremely narrow species known only from one habitat in the valley of the Alla River. This is probably due to the polyploid origin of *O. interposita*: $2n = 24$ [42] and $2n = 32$ [43]. Previously, it was shown that an increase in the number of chromosomes by auto- or allopolyploidization leads to an increase in genetic polymorphism and an increase in the adaptive capacity of the species [44]. This could also have happened in the case of *O. interposita*. Clustering of haplotypes of *O. interposita* of the section *Verticillares* and *O. varlakovii* and *O. muricata* of the section *Polyadena* (Fig. 3) corresponds to their morphological similarity [8, 18]. Combining the haplotypes of the first two species suggests that *O. interposita* is a polyploid formed with the participation of the chloroplast genome of *O. varlakovii*. The genetic isolation of *O. interposita* from other species of the section *Verticillares* (*O. bargusinensis*, *O. oxyphylla*, and *O. tompudae*) is evident (Fig. 3). According to L.I. Malyshev [15], *O. interposita* is a mutant. On the basis of the data we obtained, it can be assumed that this species appeared as a result of polyploidization of a representative of the section *Polyadena*; then, under subsequent diploidization, a mutation could have arisen which manifested itself as suppression of

expression or loss of genes responsible for the presence of glands. Further studies are needed to address the question of the sectional affiliation of *O. interposita*.

Thus, the high level of genetic diversity in the studied populations of the endemic species of Baikal Siberia *O. triphylla*, *O. bargusinensis*, and *O. interposita* indicates their relatively stable state, but taking into account their small number, species vulnerability, and narrow ecological confinement, measures for habitat protection and genetic monitoring of population status are required. The hybrid origin of *O. bargusinensis* and *O. tompudae* as a result of cross-sectional hybridization of the species of the sections *Orobia* and *Verticillares* was confirmed.

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