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GENETICS

Variability of Chloroplast DNA in *Oxytropis* Section *Polyadena* (Fabaceae) from Asian Russia: Population Analysis and Phylogenetic Relationships

A. B. Kholina[a, *], M. M. Kozyrenko[a], E. V. Artyukova[a], and T. E. Pozdnyakova[b]

[a] *Federal Scientific Center of East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, 690022 Russia*

[b] *St. Petersburg State Agrarian University, St. Petersburg–Pushkin, 196601 Russia*

**e-mail: kholina@biosoil.ru*

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Abstract—Nucleotide polymorphism of the intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of chloroplast DNA was analyzed in populations of six *Oxytropis* species of the section *Polyadena* from Asian Russia. A low level of nucleotide diversity (0.0001–0.0014) was found, except for two *O. glandulosa* populations (0.0036 and 0.0059). It was noted that the haplotype diversity varies from 0.133 to 0.911. It was established that high interspecific genetic distances and detected species-specific nucleotide substitutions and indels indicate a significant differentiation of the chloroplast genomes of *O. muricata*, *O. microphylla*, *O. trichophysa*, and *O. glandulosa* (lineages 2, 3). The species *O. pseudoglandulosa*, *O. muricata*, *O. varlakovii*, and *O. glandulosa* (lineage 1) form a weakly differentiated complex, which is probably caused by their relatively recent divergence.

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INTRODUCTION

The species of the section *Polyadena* Bunge of the genus *Oxytropis* DC., (Fabaceae) are acaulescent plants with a verticillate leaflets and tubercular glands on the legumes, leaves, stipules, and calyxes, with a purple–violet, pink, or whitish yellow corolla (Malyshev, 2008). The section includes ~10 species, of which six species grow in Asian Russia: *O. glandulosa* Turcz., *O. physocarpa* Ledeb., *O. microphylla* (Pall.) DC., *O. muricata* (Pall.) DC., *O. trichophysa* Bunge, and *O. varlakovii* Serg.; the last four are also found in northern Mongolia, while *O. microphylla* is also found in Northern Manchuria (Malyshev, 2008). *O. pseudoglandulosa* Gontsch. ex Grub., a member of the section *Polyadena*, also grows in Mongolia (endemic to Mongolia) (Ulziikhutag, 2003). The species *O. glandulosa* (narrow local endemic to Buryatia), *O. trichophysa*, and *O. physocarpa* (endemic to Altai and Tyva) are included in the Red Book of the Russian Federation (2008) as rare species. *O. glandulosa* is also included in the Red Book of the Republic of Buryatia (2013). The relics of Miocene–Pliocene flora *O. microphylla* and *O. varlakovii* (Peshkova, 1972) are included in the Red Book of Irkutsk Oblast (2010), the first as a vulnerable species and the second (subendemic of the southern part of Eastern Siberia) as an endangered species. The Altai–Sayan subendemic *O. muricata* is included in the Red Book of Krasnoyarsk Krai (2012) as a vulnerable species, the number of which is decreasing on the northern border of the range. It is assumed (Malyshev, 2008) that *O. microphylla* originated from hybridization between *O. muricata* and *O. lanata* (Pall.) DC. section *Verticillares* DC. In addition, *O. muricata* appears similar to *O. interposita* Sipl. section *Verticillares*, but the latter is characterized by the absence of glandularity, a higher height of the shoots, and the presence of pubescence on inflorescences and legumes (Siplivinskii, 1966). In morphology, *O. varlakovii* is partially similar to *O. interposita*, but the first has warty legumes; *O. varlakovii* is also close to *O. muricata* and to *O. glandulosa*, but differs from the first by shorter sepals and more hairy legumes and from the second by a light corolla (Malyshev, 2008).

It is known that many members of the genus *Oxytropis* (including the species of the section *Polyadena*) are used in the traditional medicine of Siberia, Mongolia, Central Asia, and Kazakhstan as efficient medicines having wound healing, diuretic, and vasodilating properties. Chemical studies of plants demonstrated the presence of biologically active substances, such as flavonoids (kaempferol, ramnazin, robinin, isorobinin, baicalein, etc.), alkaloids (muricatin, muricatinin, muricatid, etc.), phenolcarboxylic acids, triterpenoids, sesquiterpene lactones, coumarins, and saponins (Povydysh et al., 2010). Flavonoids have

vasodilating, antipyretic, hypotensive, and choleretic properties, while coumarins (found in *O. glandulosa*) exhibit antitumor activity (Povydysh et al., 2010). Three alkaloids were isolated from the aerial part of *O. pseudoglandulosa* (Purevsuren et al., 2002). It was demonstrated that an extract of *O. pseudoglandulosa* can be a potent remedy for the treatment of occlusive vascular diseases (Lee et al., 2018).

At present, a sharp decrease in the number of individuals (up to complete extintion of populations in some habitats) occurs due to intensive human economic activity. The results of coenopopulation studies of *O. microphylla*, *O. varlakovii*, *O. trichophysa*, and *O. glandulosa* have demonstrated that a moderate pasture digression does not harm them, and they are vulnerable because of a small population size within a limited area in combination with a narrow ecological amplitude (Selyutina and Sandanov, 2016). Therefore, the species of the section *Polyadena* need measures to preserve and restore natural populations. First of all, it is necessary to determine the level of genetic variability and to describe the gene pool of the species, since reconstruction of the historically established population genetic structure is one of the decisive factors in the species recovery. Intergenic spacers *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* of chloroplast DNA (cpDNA) are efficient and reliable molecular markers for the population analysis and reconstruction of phylogenetic relationships of closely related *Oxytropis* species (Kholina et al., 2018a, 2018b, 2019). According to sequencing of these regions, estimation of the state of natural populations of some rare and endemic *Oxytropis* species is given (Kholina et al., 2018a, 2018b), and the phylogenetic relationships of the species of the sections *Verticillares* (Kholina et al., 2019) and *Orobia* (Kozyrenko et al., 2020) were clarified.

The aim of this work was to study the genetic diversity of *Oxytropis* section *Polyadena* from Asian Russia and to reconstruct the phylogenetic relationships of closely related species based on analysis of the polymorphism of nucleotide sequences of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA.

MATERIALS AND METHODS

One hundred twenty-nine plants from 13 natural populations of the species *O. muricata*, *O. microphylla*, *O. pseudoglandulosa*, *O. varlakovii*, *O. glandulosa* (5–15 samples from the population), and *O. trichophysa* (one sample) of the section *Polyadena* were the material for this study (Table 1). The methods of DNA isolation, amplification, and sequencing of the *psbA–trnH, trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA were described in our previous works (Artyukova et al., 2004; Kholina et al., 2016, 2018a). Nucleotide sequences were determined on an ABI 3500 genetic analyzer (Applied Biosystems, United States) in the Joint-Use Center "Biotechnolohy and Genetic Engineering" of the Federal Scientific Center of East Asia Terrestrial Biodiversity (Far Eastern Branch, Russian Academy of Sciences). For each sample, the sequences of regions were aligned using the SeaView v. 4.7 program (Gouy et al., 2010). The haplotype (*h*) and nucleotide (π) diversity, genetic distances between populations/species (F_{ST}, pairwise values of fixation indices), interpopulation differentiation (Φ_{ST}, analysis of molecular variance, AMOVA), gene flow (*Nm*), and degree of divergence (D_{XY}) between populations/species/haplogroups were calculated using the programs Arlequin v. 3.5 (Excoffier and Lischer, 2010) and DnaSP v. 5.0 (Librado and Rozas, 2009). The genealogical relationships of haplotypes were analyzed by the median-joining (MJ) method in the program Network v. 5.0.1.1 (Bandelt et al., 1999), encoding each deletion or insertion (regardless of their size) as a single mutational event. Phylogenetic analysis of sequences was carried out by the maximum parsimony (MP) and neighbor-joining (NJ) methods using the PAUP v. 4.0b10 program package (Swofford, 2003), as well as Bayesian inference (BI) in the MrBayes 3.1.2 program (Huelsenbeck and Ronquist, 2001). The stability of the topology of the MP and NJ trees was estimated using bootstrap analysis of 1000 alternative trees (bootstrap percentage (BP), %). In BI analysis of the complete matrix, 10000000 generations of Markov chains were created, sampling every 1000 generations (that is, 10000 samples). Posterior probabilities (PP) were calculated using the trees selected during the stationary phase. The values BP < 50% and PP < 0.95 were not taken into account. The sample of *O. glabra* (Lam.) DC. section *Mesogaea* Bunge (GenBank accession numbers LT856572, LT856585, LT856598 (Kholina et al., 2018a)) was used as an outgroup.

RESULTS

The nucleotide sequences of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA were determined for 75 plants of *O. muricata*, *O. microphylla*, *O. pseudoglandulosa*, and *O. trichophysa* species. Previously obtained sequences of the same regions in 49 samples from four populations of *O. glandulosa* (Kholina et al., 2018b) and five samples of *O. varlakovii* (Kholina et al., 2018a) were included in the data matrix. The nucleotide sequences of each cpDNA region in 129 samples of the six species of the section *Polyadena* studied are characterized by relatively low nucleotide variability and different lengths due to the presence of short (4–16 nucleotides) insertions/deletions (indels) and mono- and dinucleotide repeats. In the *psbA–trnH* sequences, short indels (six nucleotides) and poly(A) motif (A_9–A_{13}) were found; there are no variable nucleotide substitutions. In *trnL–trnF*, three parsimony informative substitutions, deletion of 16 nucleotides, the poly(T) motif (T_8–T_{11}), and the dinucleotide TA motif (in which there are from 6 to 13 repeats) were detected. In the *trnS–trnG*,

Table 1. Populations studied of *Oxytropis* section *Polyadena* and parameters of genetic diversity according to polymorphism of *psbA–trnH, trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA

Location (number of samples)	Population code	Diversity (SD)		Haplotype
		haplotype	nucleotide	
O. muricata				
Irkutsk oblast				
near the Gyzgi-Nur Lake (9)	MUR1	0.417 (0.191)	0.0009 (0.0006)	P1–P3
near the village Sarma (8)	MUR2	0.250 (0.180)	0.0007 (0.0005)	P2, P4
near the Oto-Khusun Cape (13)	MUR3	0.692 (0.075)	0.0014 (0.0008)	P1, P2, P5
Total for the species (30)		0.533 (0.096)	0.0011 (0.0007)	P1–P5
O. microphylla				
Irkutsk oblast				
near the village Ozera (9)	MICR1	0.000 (0.000)	0.0000 (0.0000)	P6
near the Gyzgi-Nur Lake (6)	MICR2	0.600 (0.215)	0.0007 (0.0005)	P6, P7, P8
near the Gurbi-Nur Lake (11)	MICR3	0.182 (0.144)	0.0001 (0.0001)	P6, P7
near the Namish-Nur Lake (11)	MICR4	0.182 (0.144)	0.0001 (0.0001)	P6, P8
Total for the species (37)		0.203 (0.084)	0.0002 (0.0002)	P6–P8
O. pseudoglandulosa				
Mongolia, near the Ulan Bator (7)	PSEGLA	0.809 (0.130)	0.0011 (0.0007)	P9–P12
O. trichophysa				
Tyva, Mongun-Taiga Ridge, Mugur River valley (1)	TRICH	–	–	P13
O. varlakovii				
Transbaikalia, near the Nozhii Lake (5)	VARL	0.700 (0.218)	0.0003 (0.0003)	V1–V3*
O. glandulosa				
Buryatia				
near the village Argada (10)	GLAKUR	0.911 (0.077)**	0.0059 (0.0033)**	G1–G7**
near the village Urzhil (14)	GLABAR	0.703 (0.101)**	0.0036 (0.0020)**	G4, G6–G9**
near the village Shiringa (15)	GLASHIR	0.133 (0.112)**	0.0002 (0.0002)**	G10, G11**
near the village Garam (10)	GLAGAR	0.356 (0.159)**	0.0006 (0.0004)**	G10, G11**
Total for the species (49)		0.763 (0.053)	0.0081 (0.0040)	G1–G11

SD, standard deviation; *, haplotypes (Kholina et al., 2018a), **, values of genetic diversity and haplotypes (Kholina et al., 2018b).

poly(A) (A_{10}–A_{14}) and poly(T) (T_7–T_{11}) motifs, as well as eight parsimony informative nucleotide substitutions were found. The length of the combined matrix of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* sequences after the alignment was 2431 sites (1–463, 464–1239, and 1240–2431, respectively); out of them, there are 2359 monomorphic and 11 parsimony informative nucleotide substitutions in the positions 602, 1238, 1239, 1263, 1295, 1403, 1585, 1686, 1727, 2113, and 2199. All sequences of *O. muricata* samples are marked by two nucleotide substitutions (T in the position 1238 and A in the position 1239); *O. microphylla*, by a single substitution (C in the position 1686) and insertion of six nucleotides (TAAATA, positions 393–398); and *O. trichophysa*, by insertion of four nucleotides (TTTA, positions 765–768). There are no species-specific markers in *O. pseudoglandulosa*, *O. varlakovii*, and *O. glandulosa*.

Nucleotide substitutions and indel variations in 129 sequences of the combined matrix detected 27 haplotypes; out of them, 13 haplotypes of the species *O. muricata*, *O. microphylla*, *O. pseudoglandulosa*, and *O. trichophysa* were deposited in GenBank under the accession numbers MN199983–MN199995, MN199996–MN200008, and MN200009–MN200021 (*psbA–trnH*, *trnL–trnF*, and *trnS–trnG*, respectively); three haplotypes of *O. varlakovii* (V1–V3) and 11 haplotypes of *O. glandulosa* (G1–G11) previously determined and deposited in GenBank (Kholina et al., 2018a, 2018b). Five haplotypes (P1–P5)

belong to *O. muricata*; three (P6–P8), to *O. microphylla*; four (P9–P12), to *O. pseudoglandulosa*; and one (P13), to *O. trichophysa* (Table 1). No shared haplotypes in the species of section *Polyadena* were found.

Haplotype and nucleotide diversity in the populations vary from 0.133 to 0.911 and from 0.0001 to 0.0059, respectively; one *O. microphylla* population (MICR1) was monomorphic (Table 1). The species are characterized by a low level of nucleotide diversity (except for *O. glandulosa*) and different levels of haplotype diversity: low in *O. microphylla*, moderate in *O. muricata*, high in *O. pseudoglandulosa*, *O. varlakovii*, and *O. glandulosa* (Table 1). In *O. muricata*, the pairwise genetic distances (F_{ST}) between the populations MUR1–MUR2, MUR1–MUR3, and MUR2–MUR3 are very small (0.02591, 0.03291, and 0.15937, respectively) and statistically significant only for the last pair ($P < 0.028$); the gene flow (Nm) was ~3 migrants per generation. In *O. microphylla*, F_{ST} values between populations of all pairs were also very small and all statistically insignificant (data not shown). The analysis of the distribution of variability (AMOVA) demonstrated that significant genetic differentiation between geographically isolated populations of *O. muricata* and between populations in *O. microphylla* is absent ($\Phi_{ST} = 0.08395$, $P > 0.130$ and $\Phi_{ST} = 0.02361$, $P > 0.263$, respectively). The divergence of nucleotide sequences (D_{XY}), one of the parameters showing the degree of genetic disunity between the populations, is absent between the populations of each of these species. Previously, it was established (Kholina et al., 2018b) that >75% variability in *O. glandulosa* falls on the interpopulation component and <25% on the intrapopulation one ($\Phi_{ST} = 0.75783$, $P < 0.0001$), $Nm = 0.25$. Such high interpopulation differentiation is associated with the presence of three evolutionary (phyletic) lineages of the chloroplast genome in this species (Kholina et al., 2018b).

To calculate genetic distances and to reconstruct phylogenetic relationships with closely related species, previously obtained sequences of haplotypes L1–L18 of *O. lanata* (LT994841–LT994858, LT994895–LT994912, LT994949–LT994966) (Kholina et al., 2019) and H23–H27 of *O. interposita* (LT856489–LT856493, LT856522–LT856526, LT856555–LT856559) (Kholina et al., 2018a) (section *Verticillares*) were added to the combined matrix of haplotype nucleotide sequences of the species from the section *Polyadena*. The matrix length after the alignment was 2435 sites, 2347 of which are monomorphic, and 16 nucleotide substitutions were parsimony informative.

The genetic distances between species/lineages of the section *Polyadena* varied significantly (Table 2). High and statistically significant F_{ST} values were determined between *O. lanata* and all species of the section *Polyadena* and *O. interposita* of the section *Verticillares*. The pairwise genetic distances between *O. interposita*, *O. muricata*, *O. pseudoglandulosa*, and *O. varlakovii* were very low and statistically insignificant. The values of nucleotide sequence divergence between the pairs formed by each of the species *O. lanata* and *O. interposita* with all other species of the section *Polyadena* are in agreement with the F_{ST} values. It should be noted that the mean number of nucleotide substitutions per site between *O. interposita* and *O. pseudoglandulosa* is zero.

Phylogenetic trees constructed by different methods (MP, NJ, BI) almost do not differ in topology. The MP-tree (consensus of 78 trees: length of 34 steps, CI = 0.9706, HI = 0.0294, RI = 0.9923), in which haplotypes of the species from two sections formed two clades, is presented in Fig. 1. All *O. lanata* haplotypes formed clade I with a low support in the MP- and NJ-analyses (74 and 67%, respectively) but not supported in the BI-analysis, while haplotypes of all species of the section *Polyadena* and *O. interposita* section *Verticillares* formed monophyletic clade II weakly supported in the MP- and NJ-analyses (63 and 62%, respectively) and highly supported in the BI-analysis (PP = 1.00), in which three supported clades of the second order can be distinguished (Fig. 1). Clade 1 is formed by the *O. glandulosa* haplotypes (lineage 2); clade 2, by *O. microphylla*, *O. trichophysa*, and *O. glandulosa* (lineage 3); clade 3, by *O. muricata*, *O. glandulosa* (lineage 1), *O. pseudoglandulosa*, *O. varlakovii*, and *O. interposita* haplotypes. The relationship of the last three species remains unresolved.

The haplotypes of eight species of two sections form six divergent haplogroups (I–VI) in the median network of genealogical relationships of haplotypes (Fig. 2). The haplogroup I included all *O. lanata* haplotypes (L1–L18); II, G4, G6–G9 *O. glandulosa* haplotypes (lineage 2); III, P13 *O. trichophysa* haplotype; IV, all *O. microphylla* haplotypes (P6–P8); V, all *O. muricata* (P1–P5), *O. pseudoglandulosa* (P9–P12), *O. varlakovii* (V1–V3), and *O. interposita* (H23–H27) haplotypes, and G1–G3, G5 *O. glandulosa* haplotypes (lineage 1); and VI, G10 and G11 *O. glandulosa* haplotypes (lineage 3). It should be noted that the distribution of haplotypes in the haplogroup V does not correspond to either population or taxonomic affiliation. Thus, the *O. muricata* haplotypes are in three different branches, as are the *O. glandulosa* haplotypes (lineage 1). The haplotypes V3 and V2 of *O. varlakovii* are connected through haplotype H24 of *O. interposita*, and, in addition, the haplotypes V2 of *O. varlakovii* and H26 of *O. interposita* were identical (shared haplotype), although these species refer to different sections (Fig. 2). Marker nucleotide substitutions and indels were detected in all haplogroups: in haplogroup I, A in the positions 1172 and 1462, C in the position 2144; II, C and T in the positions 602 and 1730, respectively; III, insertion of four nucleotides (TTTA, positions 765–768); IV, insertion of six nucleotides (TAAATA, positions 393–398) and C in the position 1689; V, T in the position 1588; VI, deletion of six nucleotides (TATTTT, positions 162–167). The genetic distances

Table 2. Nucleotide divergence and genetic distances between *O. glandulosa* (lines 1–3), *O. muricata* (4), *O. microphylla* (5), *O. pseudoglandulosa* (6), *O. varlakovii* (7), *O. trichophysa* (8), *O. lanata* (9), and *O. interposita* (10) according to polymorphism of *psbA–trnH, trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA

Species	1	2	3	4	5	6	7	8	9	10
					D_{XY}					
1	–	4.000 (4)	5.000 (5)	4.000 (4)	6.000 (6)	2.000 (2)	1.333 (1)	3.000 (3)	5.889 (5)	2.000 (2)
2	0.00168	–	5.000 (5)	6.000 (6)	6.000 (6)	4.000 (4)	3.333 (3)	3.000 (3)	5.889 (5)	4.000 (4)
3	0.00209	0.00211	–	7.000 (7)	1.000 (1)	5.000 (5)	4.333 (4)	2.000 (2)	6.889 (6)	5.000 (5)
4	0.00167	0.00253	0.00293	–	8.000 (8)	2.000 (2)	2.667 (2)	5.000 (5)	7.889 (7)	2.000 (2)
5	0.00251	0.00253	0.00042	0.00334	–	6.000 (6)	5.333 (5)	3.000 (3)	7.889 (7)	6.000 (6)
6	0.00083	0.00168	0.00209	0.00084	0.00251	–	0.667 (0)	3.000 (3)	5.889 (5)	0.000 (0)
7	0.00056	0.00140	0.00181	0.00111	0.00223	0.00028	–	2.333 (2)	5.222 (4)	0.667 (0)
8	0.00125	0.00126	0.00084	0.00209	0.00125	0.00125	0.00097	–	4.889 (4)	3.000 (3)
9	0.00246	0.00248	0.00289	0.00330	0.00330	0.00246	0.00218	0.00204	–	5.889 (5)
10	0.00084	0.00168	0.00209	0.00084	0.00251	0.00000	0.00028	0.00124	0.00246	–
					F_{ST}					
1	0.00000									
2	0.54912**	0.00000								
3	0.82786**	0.67449**	0.00000							
4	0.43911**	0.57627*	0.80583***	0.00000						
5	0.79923**	0.68848**	0.85564***	0.78502**	0.00000					
6	0.36905**	0.59045**	0.85772***	0.33892**	0.81150**	0.00000				
7	0.33956**	0.54812**	0.91058***	0.37138**	0.86792***	0.16049***	0.00000			
8	0.67742***	0.38462***	0.84615***	0.65432***	0.81481***	0.73099***	0.87879***	0.00000		
9	0.68718*	0.72446*	0.86189**	0.69489*	0.80840*	0.67706*	0.66002*	0.70918**	0.00000	
10	0.35706*	0.59282**	0.83765**	0.25758***	0.79850**	0.05224***	0.00319***	0.68182***	0.65659*	0.00000

D_{XY}, nucleotide divergence; above the diagonal, the mean number of nucleotide differences (number of fixed differences); below the diagonal, the mean number of nucleotide substitutions per site; F_{ST}, pairwise genetic distances. * $P < 0.0001$; ** $0.01 < P < 0.05$; *** $P > 0.05$. The level of significance was determined based on 1023 permutations; for Tables 2 and 3.

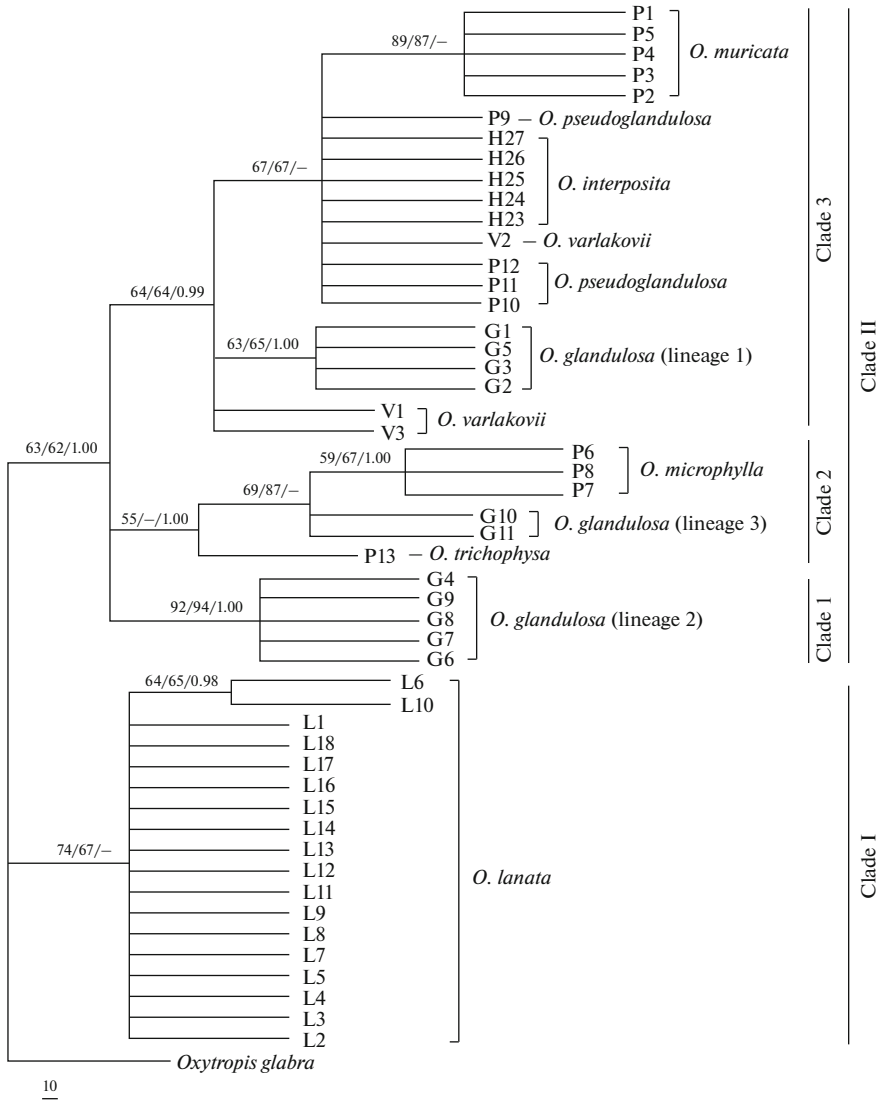

Fig. 1. MP-tree of genetic relationships of *Oxytropis* section *Polyadena* and species of the section *Verticillares* closely related to them. Numbers in nodes, bootstrap values for the MP- and NJ-methods (>50%) and Bayesian posterior probabilities for BI analysis (>0.95). Outgroup, *O. glabra*.

between haplogroups are high (Table 3), except for the pair of haplogroups II–III, which unites haplotypes of the *O. glandulosa* (lineage 2) and *O. trichophysa*, respectively. The lowest nucleotide divergence was determined in pairs of haplogroups III–VI and IV–VI, that is, *O. glandulosa* (lineage 3)–*O. trichophysa* and *O. glandulosa* (lineage 3)–*O. microphylla* (Table 3).

DISCUSSION

The study of *Oxytropis* section *Polyadena* according to nucleotide polymorphism of *psbA–trnH, trnL–trnF*, and *trnS–trnG* intergenic spacers of cpDNA showed that the level of nucleotide diversity is low in all species, while the level of haplotype diversity varies from low to high. Three *O. muricata* populations studied and three of four *O. microphylla* populations are characterized by low levels of genetic diversity, which is usually associated with the passage of the population through a "bottleneck," that is, a sharp reduction in the number with its subsequent recovery (Abramson, 2007). Monomorphism of MICR1 population of *O. microphylla* can indicate its relatively recent origin from a small group of closely related plants. The absence of significant intraspecific interpopulation differentiation in *O. muricata* and in *O. microphylla* (as well as in *O. bargusinensis* (Kholina et al., 2018a) and in *O. myriophylla* (Kholina et al., 2019)) is probably associated with a constant gene flow through a chain of intermediate habitats, while the absence of nucleotide divergence could indicate that the local populations of each species studied represent a regional meta-

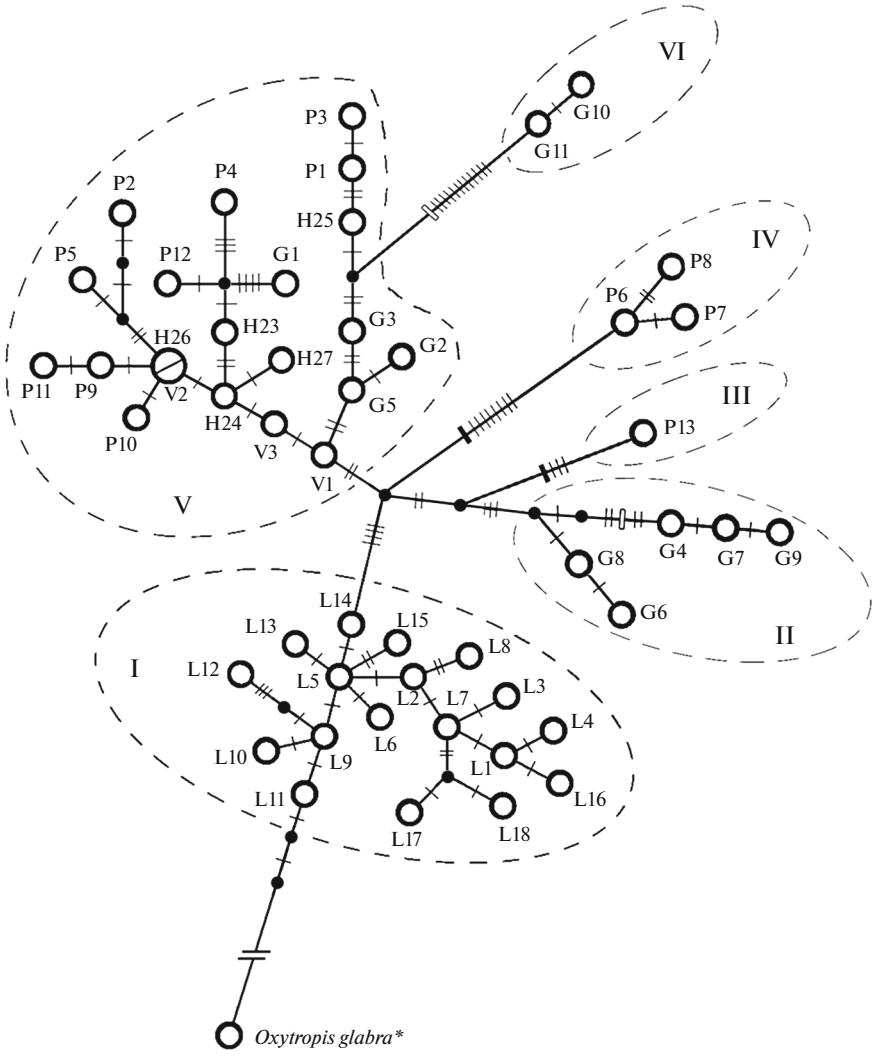

Fig. 2. Median network of haplotypes of *Oxytropis* section *Polyadena* and species of the section *Verticillares* closely related to them. The size of circles reflects the frequency of haplotypes; small black circles, hypothetical haplotypes; transverse thin bars on the branches, mutational events; black thick bars, nucleotide insertion; white thick bars, nucleotide deletion; haplogroups I–VI are circled by dashed lines. See Table 1 for the haplotype code. *, mutations for *O. glabra* used as an outgroup are not indicated and not considered.

population. The genetic diversity in *O. pseudoglandulosa* and *O. varlakovii* is comparable with those in endemic species *O. chankaensis* ($h = 0.718$, $\pi = 0.0005$) (Artyukova et al., 2011), *O. bargusinensis* ($h = 0.844$, $\pi = 0.0013$), *O. interposita* ($h = 0.769$, $\pi = 0.0015$), and *O. triphylla* ($h = 0.915$, $\pi = 0.0020$) (Kholina et al., 2018a).

High values of genetic distances between *O. muricata, O. microphylla*, *O. trichophysa*, *O. glandulosa* (lineage 2), and *O. glandulosa* (lineage 3) (Table 2), haplotypes of which in phylogenetic analyses are in different haplogroups (Fig. 2), as well as detected marker nucleotide substitutions and indels, indicate a significant differentiation of their chloroplast genomes. Taking into account the existing opinions of botanists (Siplivinskii, 1966; Malyshev, 2008) about the origin of some species of the section *Polyadena* and their similarity with the members of the section *Verticillares*, we included closely related species into phylogenetic analyses. The results of the study did not confirm the assumption that *O. microphylla* originated as a result of intersectional hybridization of *O. muricata* and *O. lanata* (Malyshev, 2008). Thus, no shared haplotypes were found in these three species, each of them has species-specific markers. The species in phylogenetic reconstructions are positioned in different clades/haplogroups (Figs. 1, 2), the values of genetic distances between which are high (Table 3).

The association of all haplotypes of *O. muricata*, *O. varlakovii*, *O. pseudoglandulosa*, and *O. glandulosa* (lineage 1) of the section *Polyadena* and *O. interposita* of the section *Verticillares* into a single clade/hap-

Table 3. Nucleotide divergence and genetic distances between haplogroups I–VI (Fig. 2) formed by cpDNA haplotypes of *Oxytropis muricata*, *O. microphylla*, *O. pseudoglandulosa*, *O. trichophysa*, *O. varlakovii*, *O. glandulosa*, *O. lanata*, and *O. interposita*

Haplogroup	I	II	III	IV	V	VI
			D_{XY}			
I	–	5.889 (5)	4.889 (4)	7.889 (7)	6.270 (4)	6.889 (6)
II	0.00248	–	3.000 (3)	6.000 (6)	4.381 (3)	5.000 (5)
III	0.00204	0.00126	–	3.000 (3)	3.381 (2)	2.000 (2)
IV	0.00330	0.00253	0.00125	–	6.381 (5)	1.000 (1)
V	0.00262	0.00184	0.00141	0.00267	–	5.381 (4)
VI	0.00289	0.00211	0.00084	0.00042	0.00225	–
			F_{ST}			
I	0.00000					
II	0.72446*	0.00000				
III	0.70918***	0.38462***	0.00000			
IV	0.80840*	0.68848***	0.81481***	0.00000		
V	0.60677*	0.64769*	0.58729***	0.72947*	0.00000	
VI	0.86189*	0.67449***	0.84615***	0.85564**	0.78510**	0.00000

logroup (Figs. 1, 2) is not random. The genetic proximity of *O. interposita* to the species of the section *Polyadena* was demonstrated when studying the endemic species *Oxytropis* (Kholina et al., 2018a) and 11 species of the section *Verticillares* (Kholina et al., 2019). Thus, the presence of species-specific nucleotide substitutions in *O. interposita*, the absence of shared haplotypes with other species of the section *Verticillares*, and the high statistically significant genetic distances (from 0.67524 to 0.89490) between *O. interposita* and other species confirm the significant divergence of the *O. interposita* chloroplast genome (Kholina et al., 2019). Low statistically insignificant values of the genetic distances between *O. interposita*–*O. muricata* and *O. interposita*–*O. varlakovii* (0.25758 and 0.00319, respectively), the presence of a shared haplotype in *O. interposita* and *O. varlakovii* (Table 1), and the absence of species-specific differentiating markers confirm the genetic proximity of these species. In addition, we compared the morphological traits given earlier (Malyshev, 2008) for *O. interposita*, *O. varlakovii*, and *O. muricata* and for the species of the section *Verticillares O. myriophylla,* and *O. oxyphylla* closest to the *O. interposita,* and found a complete coincidence by a number of diagnostic traits in *O. interposita* and in the members of the section *Polyadena* (unlike the section *Verticillares*). Among these traits, semi-verticillate or spaced (except for verticillate) leaflets, large flower with a length of 25–30 mm (unlike the flower with a length of 15–20 mm in the members of the section *Verticillares*), calyx tubular-campanulate in the *O. interposita* and *Polyadena* species, but tubular in the *Verticillares* species, almost two-locular legumes with a wide ventral septum (unlike almost one-locular ones in the *Verticillares* species), and some others were noted. Thus, our genetic data and the similarity of morphological traits support the assumption (Kholina et al., 2018a) of the likely origin of *O. interposita* from a member of the section *Polyadena*, but further studies are needed to clarify the sectional affiliation of *O. interposita*.

The absence of significant genetic differences between *O. muricata*, *O. varlakovii*, *O. pseudoglandulosa, O. glandulosa* (lineage 1), and *O. interposita*, combining their haplotypes into a single haplogroup, and the mixed distribution of haplotypes (not corresponding to either population or taxonomic affiliation) indicate a polyphily of this group. The most general reasons for this include a common origin, relatively recent divergence, and rapid radiation of young species, which is accompanied by incomplete lineage sorting (Abramson, 2007; Shantser, 2013). Based on the analysis of nuclear and chloroplast markers, it was demonstrated that rapid radiation is typical for species of the genus *Oxytropis* (Shavvon et al., 2017), as well as for a number of large genera of the family Fabaceae, including *Astragalus* L. (Bagheri et al., 2017), *Lupinus* L. (Drummond et al., 2012), etc. These processes are often complicated by introgression (Abramson, 2007; Shantser, 2013), and also by polyploidy in the case of the *Oxytropis* (Malyshev, 2008). All this is true for the group of species that we study. Thus, *O. interposita* from the only known population is tetraploid ($2n = 32$), and *O. varlakovii* from Nozhii Lake is triploid ($2n = 24$) (Konichenko and Selyutina, 2013), while diploid and tetraploid races ($2n = 16$ and 32, respectively) are known for *O. muricata* (Malyshev, 2008).

CONCLUSIONS

The study of genetic diversity of *Oxytropis* section *Polyadena* from Asian Russia detected an extremely low nucleotide diversity in the populations (0.0001–0.0014), except for two *O. glandulosa* populations. The chloroplast genomes of the species *O. muricata*, *O. microphylla*, *O. trichophysa*, and *O. glandulosa* (lineages 2, 3) are significantly differentiated. The group of the species *O. muricata*, *O. varlakovii*, *O. pseudoglandulosa*, and *O. glandulosa* (lineage 1) form a single complex, which is probably caused by their relatively recent divergence and the incomplete lineage sorting. Low level of polymorphism does not provide the adaptive potential of species and increases the risk of their extintion. Therefore, it is necessary to search for new locations of species, to study the demographic structure, vitality, and genetic diversity of populations, and to monitor their state regularly.

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COMPLIANCE WITH ETHICAL STANDARDS

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

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