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ORIGINAL PAPER

Genetic diversity of *Oxytropis* section *Xerobia* (Fabaceae) in one of the centres of speciation

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Abstract

The genetic diversity and phylogenetic relationships of *Oxytropis caespitosa*, *O. grandiflora*, *O. eriocarpa*, *O. mixotriche*, *O. nitens*, *O. peschkovae* and *O. triphylla*, section *Xerobia* subgenus *Oxytropis*, in one of the main speciation centres of the genus *Oxytropis* (Baikal Siberia and adjacent territories of Northeastern Mongolia) were studied based on sequence analysis of the *psbA–trnH*, *trnL–trnF* and *trnS–trnG* intergenic spacers of cpDNA, as well as the ITS nrDNA. Most populations are characterized by a high level of chloroplast genetic diversity (h varied from 0.327 to 1.000 and π from 0.0001 to 0.0090) due to the ancient origin for some species and to hybridization and polyploidy for others. 67 haplotypes were identified, of which six were shared. Phylogenetic relationships among species could not be satisfactorily resolved. Only the haplotypes of *O. triphylla* formed a group with rather high support. Probably, *O. caespitosa*, *O. grandiflora*, *O. mixotriche* and *O. nitens* constitute a single genetic complex. As regards the ITS nrDNA polymorphism, we detected only two ribotypes (RX1, RX2). Both were found in *O. caespitosa*, *O. eriocarpa*, *O. mixotriche* and *O. peschkovae*, while RX1 was present in *O. nitens* and *O. triphylla*, RX2 in *O. grandiflora*. The absence of diagnostic species-specific variants for the markers studied, together with the sharing of cpDNA haplotypes and nrDNA ribotypes between species, and the resulting polytomies on the phylogenetic trees, confirm the hypothesis on the hybrid origin of some of them. Obviously, the reproductive barriers within the sect. *Xerobia* are weak. However, morphological differences between the species of the sect. *Xerobia* are clearly pronounced, even when they grow in sympatry.

Keywords *Oxytropis* · Sect. *Xerobia* · Genetic diversity · CpDNA · ITS · Baikal Siberia

Introduction

The section *Xerobia* Bunge is one of the more specialized groups formed by montane xerophytes within the large genus *Oxytropis* DC. (Malyshev 2008). It contains 27 species (Zhu et al. 2010), with 20 species in Central Asia (Grubov 1998), 19 species in Asian Russia (Malyshev 2008; Pyak 2014), 17 species in Mongolia (Ulziykhutag 2003) and 22 species in China (Zhu et al. 2010). The species of sect. *Xerobia* are mostly cryoxerophytes associated with mountain-steppe territories. The plants are morphologically characterized by imparipinnate leaves with few leaflets (2–8 pairs) and racemes with one to eight flowers. The flowers are large, 20–30 mm long; legume sessile or with a stipe to 5 mm, body ovoid or cylindric, 5–27 mm, membranous or thickly leathery (Polozhii 1994; Malyshev 2008; Zhu et al. 2010).

Baikal Siberia is located in the southern part of Eastern Siberia, adjacent to the Lake Baikal, and covers the Baikal region, Western Transbaikalia, the eastern part of the Eastern Sayan and the Vitim Plateau (Tulokhonov 2009). It includes the territory of three federal subjects of the Russian Federation: the Irkutsk Region, the Republic of Buryatia and Trans-Baikal Territory. The Baikal centre of speciation is characterized by the uniqueness and richness of the flora and a high degree of endemism, which is due to the orography and climate of the region, as well as its buffer position between the North and Central Asia

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(Popov 1956; Malyshev and Peshkova 1984; Namzalov 2009). Here, 2858 species and subspecies of vascular plants, belonging to 723 genera and 140 families, are registered, accounting for 62.3% of the flora of Siberia (Flora of Baikal Siberia 2010). The origin of Baikal endemism is explained by hybridization processes associated with species migration (Popov 1956, 1957; Malyshev and Peshkova 1984).

Baikal Siberia is a one of the main secondary speciation centres of *Oxytropis* species (Polozhii 2003). Here, sect. *Xerobia* is represented by eight species: *O. caespitosa* (Pall.) Pers., *O. grandiflora* (Pall.) DC., *O. leptophylla* (Pall.) DC., *O. leucotricha* Turcz., *O. mixotriche* Bunge, *O. nitens* Turcz., *O. peschkovae* M. Popov subsect. *Ampulla* Vass. and *O. triphylla* (Pall.) Pers. subsect. *Stuppa* Vass. (Malyshev and Peshkova 1984; Polozhii 1994; Malyshev 2008). *Oxytropis peschkovae* and *O. triphylla* are narrow endemic species to the coasts of the Lake Baikal and Olkhon Island; the remaining species are subendemic and common in Baikal Siberia and adjacent territories of Northeastern Mongolia (Polozhii 1994; Peshkova 2001). With the exception of *O. leptophylla*, the remaining mentioned species of subsect. *Ampulla* are of common origin and the remains of the Miocene-Pliocene ancient Mediterranean xerophilous flora (Malyshev and Peshkova 1984). Based on the analysis of morphological characteristics, it was suggested that the primitive representatives of the sect. *Xerobia* had thickly leathery and almost one-locular legumes; the species closest to them are the species of the subsect. *Stuppa*: *O. eriocarpa* Bunge, endemic to the Yenisei River, glacial relict, one of the most ancient species in the modern flora (Polozhii 1965), as well as *O. triphylla*, which is a Miocene relict (Peshkova 2001). Previous karyotype studies have shown that more primitive species have smaller number of chromosomes. For *O. triphylla* (Krivenko et al. 2011; Konichenko et al. 2012) and *O. eriocarpa* [cited by Malyshev (2008)], 2n = 16; for *O. grandiflora* (Krivenko et al. 2013), *O. nitens* [Polozhii 1994; cited by Malyshev (2008)] and *O. peschkovae* (Krivenko et al. 2017a, b), 2n = 48; for *O. caespitosa*, 2n = 48 (Krivenko et al. 2017a, b) and 2n = 64 [cited by Malyshev (2008)].

Speciation within the genus *Oxytropis* is mainly produced by hybridization and polyploidization (Malyshev 2008). According to M.G. Popov (Popov 1956, 1957), *O. nitens* and *O. leucotricha* originated as a result of hybridization of *O. grandiflora* and *O. caespitosa*, *O. mixotriche* is a hybrid of *O. caespitosa* × *O. grandiflora*, *O. peschkovae* also has a hybrid origin; it is a jordanon (race, variety) of the linneon *O. caespitosa*. Currently, *O. triphylla* and *O. nitens* are listed in the Red Book of the Russian Federation (2008) as rare species. In addition, *O. eriocarpa* and *O. peschkovae* are also included in various regional Red Books as threatened species.

A number of *Oxytropis* species have been used in traditional Tibetan and Mongolian medicine as well as in traditional medicine in Eastern and Western Siberia (Arkad'eva et al. 1966; Blinova and Sakanyan 1986). The chemical composition studies of some *Oxytropis* species, including members of the sect. *Xerobia*, show that they contain polyphenolic compounds (mainly flavonoids), which are biologically active substances with valuable medicinal properties (Blinova and Sakanyan 1986; Povyidyish et al. 2010). The extract of the aerial part of *O. grandiflora*, containing kempferol, quercetin, myricetin and others, has a hypotensive and vasodilating effect (Blinova and Sakanyan 1986; Povyidyish et al. 2010); the aqueous-alcoholic extract of the whole plant of *O. caespitosa* shows an inhibitory effect against the Sendai virus (Arkad'eva et al. 1966). Given the rarity and uniqueness of *Oxytropis* sect. *Xerobia* from Baikal Siberia as well as their value as potential drug sources, the need for genetic studies of this group becomes obvious.

Molecular markers of the nuclear and chloroplast genomes have been successfully used to investigate closely related *Oxytropis* species and the phylogeny of the genus *Oxytropis* (Archambault and Strömvik 2012; Dizkirici Tekpinar et al. 2016; Tekpinar et al. 2016; Shavvon et al. 2017). They have also been effectively used for many legumes, especially for *Astragalus* L. (Bartha et al. 2013; Bagheri et al. 2017; Amini et al. 2019; Khalili et al. 2020), which is a sister taxon of the genus *Oxytropis*. The present work continues the series of our publications devoted to the study of *Oxytropis* species (Kholina et al. 2016, 2018a, b, 2019, 2020; Kozyrenko et al. 2020). In this sense, analysis of polymorphism of nucleotide sequences of the *psbA–trnH*, *trnL–trnF* and *trnS–trnG* intergenic spacers (IGS) of chloroplast DNA (cpDNA) and the ITS region (ITS1–5.8S rRNA–ITS2) of the ribosomal nuclear DNA operon (nrDNA) allowed to evaluate the current state of endemic species populations (Kholina et al. 2018a, b) and to reconstruct phylogenetic relationships in some sections of the genus *Oxytropis*: *Verticillares* (Kholina et al. 2019), *Orobia* (Kozyrenko et al. 2020) and *Arctobia* (Kholina et al. 2020).

In this work, we studied the genetic diversity and population structure of *Oxytropis* sect. *Xerobia* and reconstructed of their phylogenetic relationships based on the analysis of the variability of the molecular markers of the chloroplast and nuclear genomes.

Material and methods

Taxon sampling

145 plants of *O. caespitosa*, *O. grandiflora*, *O. eriocarpa*, *O. mixotriche*, *O. nitens*, *O. peschkovae* and *O. triphylla*, sect. *Xerobia* subg. *Oxytropis*, from 23 different wild

populations in Southern Siberia and Mongolia (Fig. 1) were used to study cpDNA polymorphism. The ITS region of nrDNA was amplified for 47 samples: *O. caespitosa* (15), *O. eriocarpa* (3), *O. grandiflora* (5), *O. mixotriche* (7), *O. nitens* (3), *O. peschkovae* (2) and *O. triphylla* (12), representing most of cpDNA haplotypes identified in this work. The complete specimen list, including the sampling localities, sample size, geographic coordinates and codes for each population, is given in Table 1. The names of species, sections and subgenera are accepted according to Malyshev (2008). Taxonomic features of *Oxytropis* section *Xerobia* (according to Malyshev 2008) suitable for discriminating the studied species are given in Table 2.

DNA isolation, amplification and sequencing

The methods of DNA isolation, amplification and direct sequencing of three cpDNA IGS (*psbA–trnH*, *trnL–trnF* and *trnS–trnG*) and the ITS region of nrDNA were presented in our previous works (Artyukova et al. 2004; Kholina et al. 2016, 2018a; Kozyrenko et al. 2020). The cycle sequencing was accomplished on both strands and fragments were separated using a genetic analyzer ABI 3500 (Applied Biosystems, USA) in the Joint-Use Centre "Biotechnology and Genetic Engineering", Federal Scientific Centre of the East Asia Terrestrial Biodiversity (Vladivostok, Russia).

Data analysis

The sequences of four DNA regions were aligned with SeaView v. 4.7 (Gouy et al. 2010) using the CLUSTAL algorithm, and manually edited when necessary. (See Supplemental Materials 1–3, Figs. S1–S3 for the sequence alignments of each cpDNA region). We included in the dataset indels and length variation in mononucleotide and dinucleotide repeats because repeatability tests allowed us to exclude PCR errors.

The haplotypes were identified using DnaSP v. 5.0 (Librado and Rozas 2009). This program was also used to calculate the degree of divergence (D_{XY}) between cpDNA sequences based on nucleotide substitutions. Haplotype (h) and nucleotide (π) diversity of populations (for populations with five or more samples) were calculated in Arlequin v. 3.5 software package (Excoffier and Lischer 2010). An analysis of molecular variance (AMOVA; implemented in Arlequin) was performed to estimate the distribution of genetic variability within populations, between populations within

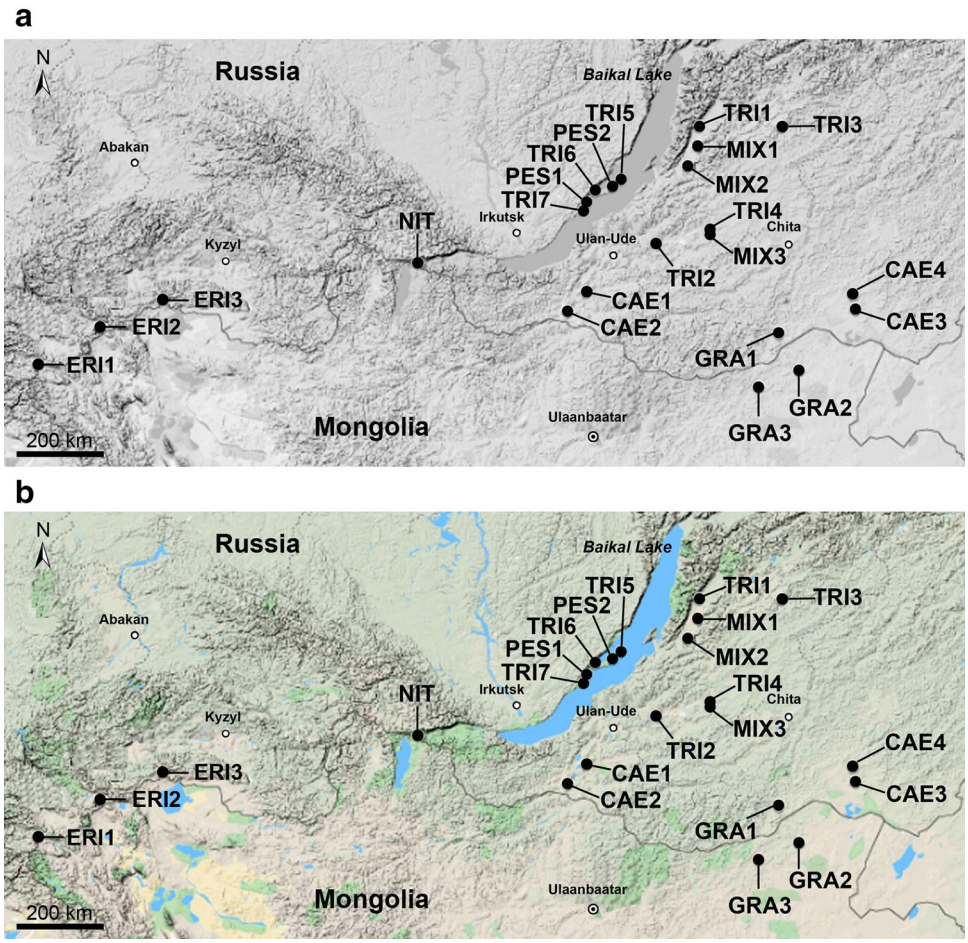

Fig. 1 Map of sample sites for natural populations of *Oxytropis* section *Xerobia* (23 populations, black circles). Population codes correspond to those in Table 1

Table 1 Sampling site locations, sample size, codes, haplotypes and genetic diversity within populations of *Oxytropis* section *Xerobia* according to cpDNA data

Species, the location of the population (no. of samples)	Vaucher number (herbarium code)	Latitude, longitude	Code	Haplotype	Genetic diversity (SD)	
					Haplotype diversity	Nucleotide diversity
O. caespitosa						
Russia, Buryatia, near the village Novoselenginsk (11)	016269 (UUH)	51.08°, 106.60°	CAE1	X1, X2	0.327 (0.153)	0.0001 (0.0001)
Russia, Buryatia, near the village Dyrestuy (9)	009765 (UUH)	50.64°, 106.01°	CAE2	X1	0.000 (0.000)	0.0000 (0.0000)
Russia, Transbaikalia, near the village Kusocha (5)	018797 (UUH)	50.69°, 115.70°	CAE3	X1–X5	0.700 (0.218)	0.0030 (0.0020)
Russia, Transbaikalia, near the village Tsugol (5)	018798 (UUH)	51.02°, 115.60°	CAE4	X6–X8	0.700 (0.218)	0.0017 (0.0012)
O. eriocarpa						
Russia, Altai Mts., Ukok Plateau, Zhumaly River (8)	OER220716 (NS)	49.29°, 088.07°	ERI1	X9–X14	0.893 (0.111)	0.0090 (0.0051)
Russia, Tyva, Mongun-Tayga Mt., Mugur River (1)	OER200714 (NS)	50.19°, 090.13°	ERI2	X22	–	–
Russia, Tyva, Tannu-Ola Range (1)	OER180714 (NS)	50.54°, 092.19°	ERI3	X23	–	–
O. grandiflora						
Russia, Transbaikalia, near the village Bytev (5)	018799 (UUH)	50.17°, 113.11°	GRA1	X6, X15–X17	0.900 (0.161)	0.0046 (0.0029)
Mongolia, Dornod Province, near the Bayandun sum (7)	018801 (UUH)	49.37°, 113.81°	GRA2	X3, X6, X16, X18, X19	0.857 (0.137)	0.0035 (0.0021)
Mongolia, Dornod Province, near the Bayan-Uul sum (9)	018800 (UUH)	49.01°, 112.45°	GRA3	X16, X20, X21	0.667 (0.132)	0.0043 (0.0024)
O. mixotriche						
Russia, Buryatia, near the village Urzhil (5)	015758 (UUH)	54.07°, 110.39°	MIX1	X24, X25	0.400 (0.237)	0.0002 (0.0002)
Russia, Buryatia, near the village Suvo (7)	015754 (UUH)	53.65°, 110.02°	MIX2	X8, X24, X26–X28	0.851 (0.137)	0.0053 (0.0031)
Russia, Buryatia, near the village Mozhayka (5)	018475 (UUH)	52.35°, 110.80°	MIX3	X26, X29–X32	1.000 (0.126)	0.0019 (0.0013)
O. nitens						
Russia, Buryatia, near the village Mondy, Irkut River (3)	017232 (UUH)	51.40°, 100.57°	NIT	X20, X33	–	–
O. peschkovae						
Russia, Irkutsk Region, near Lake Gyzgi-Nur (9)	018791 (UUH)	52.91°, 106.63°	PES1	X34–X41	0.972 (0.064)	0.0082 (0.0046)
Russia, Irkutsk Region, Olkhon Island, near the village Khuzhir (11)	018792 (UUH)	53.25°, 107.49°	PES2	X42–X44	0.345 (0.172)	0.0016 (0.0009)

Table 1 (continued)

Species, the location of the population (no. of samples)	Vaucher number (herbarium code)	Latitude, longitude	Code	Haplotype	Genetic diversity (SD)	
					Haplotype diversity	Nucleotide diversity
O. triphylla						
Russia, Buryatia, near the village Sakhuli (12)	018532 (UUH)	54.45°, 110.45°	TRI1	X38, X45–X51	0.924 (0.057)	0.0018 (0.0011)
Russia, Buryatia, near the village Udinsk (6)	016342 (UUH)	52.08°, 108.98°	TRI2	X38, X49, X52, X53	0.800 (0.172)	0.0022 (0.0014)
Russia, Buryatia, near the village Bagdarin (10)	018413 (UUH)	54.44°, 113.22°	TRI3	X50, X54–X58	0.844 (0.103)	0.0021 (0.0013)
Russia, Buryatia, near the village Mozhayka (2)	018796 (UUH)	52.36°, 110.80°	TRI4	X38, X46	–	–
Russia, Irkutsk Region, Olkhon Island, Khoboy Cape (4)	018795 (UUH)	53.38°, 107.78°	TRI5	X50, X59–X61	–	–
Russia, Irkutsk Region, Cape Hadarta (4)	018793 (UUH)	53.16°, 106.93°	TRI6	X54, X56, X62, X63	–	–
Russia, Irkutsk Region, Begul Bay (6)	018794 (UUH)	52.74°, 106.54°	TRI7	X35, X63–X67	1.000 (0.096)	0.0025 (0.0016)

Table 2 Taxonomic characteristics of *Oxytropis* section *Xerobia* (according to Malyshev 2008)

Subsection	*Ampulla* Vass					*Stuppa* Vass	
Series	*Villosae* Vass		*Glabratae* Vass			*Triphyllae* Vass	
Taxon	*O. nitens*	*O. grandiflora*	*O. caespitosa*	*O. peschkovae*	*O. michotriche*	*O. triphylla*	*O. eriocarpa*
No. of pairs of leaflets	3–8	5–11	5–7	4–7	4–8	1, rare 2	2–5
Stipule	Ciliate, with many veins	Ciliate, with one vein	Ciliate, pilose	Pilose	Ciliate, with cephalic hairs	Pilose, with one vein	Ciliate
No. of flowers per raceme	Many flowers	Many flowers	2–3	4–6	3–8	2–3	2–4
Ratio length peduncles/ leaves	Peduncles equal to leaves	Peduncles equal to leaves or longer	Peduncles shorter than leaves	Peduncles equal to leaves or longer	Peduncles equal to leaves or longer	Peduncles equal to leaves	Peduncles equal to leaves
Corolla color	Purple	Crimson	White with a purple spot on the keel	White	Purple	Purple	Dark-crimson
Corolla: keel length, mm	1.5–2	3–4	2–3	2–3	2–3	3–4	2–2.5
Pod: texture	Leathery	Leathery	Membranous	Membranous	Membranous	Leathery	Leathery

groups, and among groups. The statistical significance (*P*) of the variance components was evaluated based on 1023 permutations.

Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP), and neighbour-joining (NJ) methods as implemented in PAUP v. 4.0b10 (Swofford 2003). Bayesian inference (BI) was conducted using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003) on the CIPRES portal (http://www.phylo.org/; Miller et al. 2010). For the MP analysis, gaps were coded according to Simmons and Ochoterena (2000), as implemented in the program FastGap v. 1.2 (Borchsenius 2009). Optimal trees were found using a heuristic search with 1000 random addition sequence replicates, starting trees obtained via stepwise addition, tree bisection and reconnection (TBR) branch swapping and the MulTrees option in effect. For ML and BI analyses, GTR + G + I model was selected according to the Akaike information criterion (AIC) using Modeltest v. 3.6

(Posada and Crandall 1998). ML heuristic searches were done using the resulting model settings, 100 replicates of random sequence addition, TBR branch swapping and MULTrees option on. In BI, using the default prior settings, two parallel MCMC runs were carried out for ten million generations, sampling every 1000 generations for a total of 10,000 samples. Convergence of the two chains was assessed, and the posterior probabilities (PP) were calculated from the trees sampled during the stationary phase. The robustness of nodes in ML and MP trees was tested using bootstrap with 1000 replicates (bootstrap percentage, BP). BP < 50% and PP < 0.95 were not taken into account. A haplotype network was built using Network v. 5.0 (Bandelt et al. 1999), treating each deletion/insertion, regardless of size as a single mutational event and using the median joining (MJ) algorithm with default settings.

Results

Genetic diversity and divergence

Sequences of three cpDNA regions were obtained for 145 specimens. Sequence polymorphism analysis showed that the length of each region in the studied samples was different because of the presence of mononucleotide repeats (poly-A and/or poly-T) and dinucleotide repeat (TA motif), short (1–3 bp) and multi-base (6–12 bp) indels. The single longest insert (30 bp) was found in one sample, *O. eriocarpa*, from the Altai Mountains. The aligned length of the *psbA–trnH*, *trnL–trnF* and *trnS–trnG* IGS was 509, 780 and 1,197 sites, respectively; of those, 4, 3 and 6 nucleotide substitutions, respectively, were parsimony informative. The total length of the combined sequences of three IGS was 2486 sites, of which 2355 were monomorphic, 109 were indels and 22 were variable, 13 of which were parsimony informative.

Nucleotide substitutions and indel variations in 145 sequences of the combined matrix identified 67 (X1–X67) haplotypes (Table 1). Sequences of these haplotypes were deposited in the European Nucleotide Archive (ENA) database; their accession numbers are available in Table 3. 42 haplotypes were found in only single specimens (unique), and six haplotypes (X3, X6, X8, X20, X35 and X38) were shared by several species (Table 1). Specimens of populations CAE3, CAE4 of *O. caespitosa* from Transbaikalia and GRA1, GRA2 of *O. grandiflora* from Transbaikalia and Mongolia shared haplotypes X3 and X6, while populations CAE4 of *O. caespitosa* (Transbaikalia) and MIX2 of *O. mixotriche* (Buryatia) shared haplotype X8. Specimens from populations GRA3 of *O. grandiflora* (Mongolia) and NIT of *O. nitens* (Buryatia) shared haplotype X20. The populations PES1 of *O. peschkovae* (Irkutsk Region) and TRI1, TRI2, TRI4 and TRI7 of *O. triphylla* (Irkutsk Region and Buryatia) shared haplotypes X35 and X38 (Table 1). No species-specific molecular markers were found, but it should be noted that a marker nucleotide substitution (G at position 2368) was revealed for two *O. eriocarpa* specimens from Tyva.

The parameters of genetic variability of populations are presented in Table 1. In the populations studied, haplotype diversity (h) varies from 0.327 to 1.000, and nucleotide diversity (π) varies from 0.0001 to 0.0090, and only the CAE2 population of *O. caespitosa* was monomorphic.

According to AMOVA (Table 4), more than 88% of the total genetic variability belonged to the interpopulation component for *O. caespitosa*. For *O. mixotriche*, the genetic variability was almost equally distributed between and within populations. For *O. peschkovae*, most of the genetic variation (about 69% of the total) was concentrated within populations. The populations of each of the species *O. eriocarpa*, *O. grandiflora* and *O. triphylla* were weakly differentiated. It should be noted that population differentiation is found to be statistically significant for all species except *O. eriocarpa.* Hierarchical AMOVA showed that the differences among the seven species accounted for about 40% of the total variance (Table 4).

Table 3 Haplotypes of *Oxytropis* section *Xerobia* and ENA accession numbers of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacer regions of cpDNA

Species	Haplotype	Accession number		
		psbA–trnH	*trnL–trnF*	*trnS–trnG*
O. caespitosa	X1–X8	LR861115–LR861122	LR828424–LR828431	LR828468–LR828475
O. eriocarpa	X9–X14, X22, X23	LR861754–LR861759, LR884431, LR884432	LR861762–LR861767, LR884433, LR884434	LR861770–LR861775, LR884435, LR884436
O. grandiflora	X3, X6, X15–X21	LR861123, LR861124, LR861125–LR861131	LR828432, LR828433, LR828434–LR828440	LR828476, LR828477, LR828478–LR828484
O. mixotriche	X8, X24–X32	LR861724, LR861725–LR861733	LR861734, LR861735–LR861743	LR861744, LR861745–LR861753
O. nitens	X20, X33	LR861760, LR861761	LR861768, LR861769	LR861776, LR861777
O. peschkovae	X34–X44	LR861691–LR861701	LR861702–LR861712	LR861713–LR861723
O. triphylla	X35, X38, X45–X67	LR861134, LR861135, LR861136–LR861158	LR828443, LR828444, LR828445–LR828467	LR828487, LR828488, LR828489–LR828511

Table 4 The results of AMOVA for distribution of the total genetic variability between groups of *Oxytropis* section *Xerobia* according to cpDNA data

Species	Source of variation	D.f	Variation (%)	Fixation index
O. caespitosa	Between populations	3	88.43	$\Phi_{ST}=0.88426$*
	Within populations	26	11.57	
O. eriocarpa	Between populations	2	16.48	$\Phi_{ST}=0.16484$ ns
	Within populations	7	83.52	
O. grandiflora	Between populations	2	18.80	$\Phi_{ST}=0.18804$**
	Within populations	18	81.20	
O. mixotriche	Between populations	2	57.13	$\Phi_{ST}=0.57127$*
	Within populations	14	42.87	
O. peschkovae	Between populations	1	31.55	$\Phi_{ST}=0.31551$**
	Within populations	18	68.45	
O. triphylla	Between populations	6	12.17	$\Phi_{ST}=0.12169$**
	Within populations	37	87.83	
O. caespitosa vs *O. grandiflora* vs *O. eriocarpa* vs *O. mixotriche* vs *O. nitens* vs *O. peschkovae* vs *O. triphylla****	Between species	6	39.99	$\Phi_{CT}=0.39990$*
	Between populations within species	16	26.57	$\Phi_{SC}=0.44271$*
	Within populations	122	33.44	$\Phi_{ST}=0.66557$*

D.f. is degrees of freedom; Φ_{ST}, correlation within populations relative to the total; Φ_{CT}, correlation of individuals within groups relative to the total; Φ_{SC}, correlation within populations relative to groups; ns, not significant

*P < 0.0001; **P < 0.05 (1023 permutations); ***Seven groups, where one species is one group

The nucleotide divergence (D_{XY}, specifically the average number of nucleotide substitutions per site) between populations of each of the species *O. caespitosa*, *O. eriocarpa*, *O. grandiflora*, *O. mixotriche* and *O. peschkovae* ranged from 0.00053 to 0.00319 (Supplemental Material 4, Table S1). The highest divergence was found between ERI1–ERI2 and ERI1–ERI3 populations of *O. eriocarpa*. There was no divergence between most populations of *O. triphylla*. The pairwise divergences of nucleotide sequences between species are given in Table 5. The highest *D*xy values were determined between *O. eriocarpa* and each of the species *O. grandiflora*, *O. mixotriche* and *O. nitens*; the lowest values were determined between *O. grandiflora* and *O. nitens*.

The ITS nrDNA nucleotide sequences of 47 *Oxytropis* plants had the same length (603 bp), and only one parsimony nucleotide substitution (T ↔ G at position 119 of the ITS1 region). Two ribotypes (RX1 and RX2) were identified, the sequences of which were deposited in GenBank (MW015134–MW015144). The RX1 ribotype (G at position 119) was detected in *O. nitens* and *O. triphylla*, RX2 (T at position 119) was detected in *O. grandiflora* and both ribotypes were found in the species *O. caespitosa*, *O. eriocarpa*, *O. mixotriche* and *O. peschkovae*.

Phylogenetic relationships

The phylogenetic reconstruction methods (MP, NJ, ML and BI) all resulted in similar topologies, with few differences in statistical support. The MP analysis produced 72 equally parsimonious trees of 27 steps in length (CI = 0.8519,

Table 5 Nucleotide divergence between the species *Oxytropis* of section *Xerobia* according to cpDNA data

Species	*O. caespitosa*	*O. eriocarpa*	*O. grandiflora*	*O. mixotriche*	*O. nitens*	*O. peschkovae*	*O. triphylla*
O. caespitosa	–	4.807 (0)	2.832 (0)	2.784 (0)	2.933 (0)	3.167 (0)	4.089 (2)
O. eriocarpa	0.00202	–	5.243 (0)	4.935 (0)	5.300 (2)	4.090 (0)	3.323 (0)
O. grandiflora	0.00119	0.00220	–	2.294 (0)	0.857 (0)	3.960 (0)	4.118 (2)
O. mixotriche	0.00117	0.00207	0.00096	–	2.176 (0)	3.415 (0)	4.082 (2)
O. nitens	0.00123	0.00222	0.00036	0.00091	–	4.150 (2)	4.023 (4)
O. peschkovae	0.00133	0.00171	0.00166	0.00143	0.00174	–	3.373 (0)
O. triphylla	0.00172	0.00139	0.00173	0.00171	0.00169	0.00141	–

Above the diagonal is the mean number of nucleotide differences between species (the number of fixed differences), below the diagonal is the mean number of nucleotide substitutions per one site (D_{XY})

RI = 0.9730). The strict consensus of all maximum parsimony trees is shown in Fig. 2. Figures derived from the NJ, ML and BI analyses are presented in the Supplemental Material 4, Fig. S4. Tree topology showed poor resolution among species, and only some relationships were significantly supported. Thus, all haplotypes of *O. triphylla*, four haplotypes of the PES1 population of *O. peschkovae* and three haplotypes of the ERI1 population of *O. eriocarpa* grouped in a single clade, whose support was weak in MP and NJ analyses (BP 66 and 65%, respectively), and moderate and high in ML and BI analyses (BP 79% and PP 0.99). Clade II comprised most of the haplotypes of *O. caespitosa*, *O. grandiflora*, *O. mixotriche* and two haplotypes of *O. nitens*, but the statistical support for this topology was even lower (Fig. 2). Other haplotypes of the PES1 population and one haplotype of the PES2 population of *O. peschkovae* formed a highly supported group in BI (PP 0.99) analysis, and a weakly supported group in MP, NJ and ML analyses (BP 64, 60, and 60%, respectively).

The MJ network revealed two main haplogroups (Fig. 3) that were broadly consistent with the MP tree topology (Fig. 2). Within haplogroup I, most haplotypes of

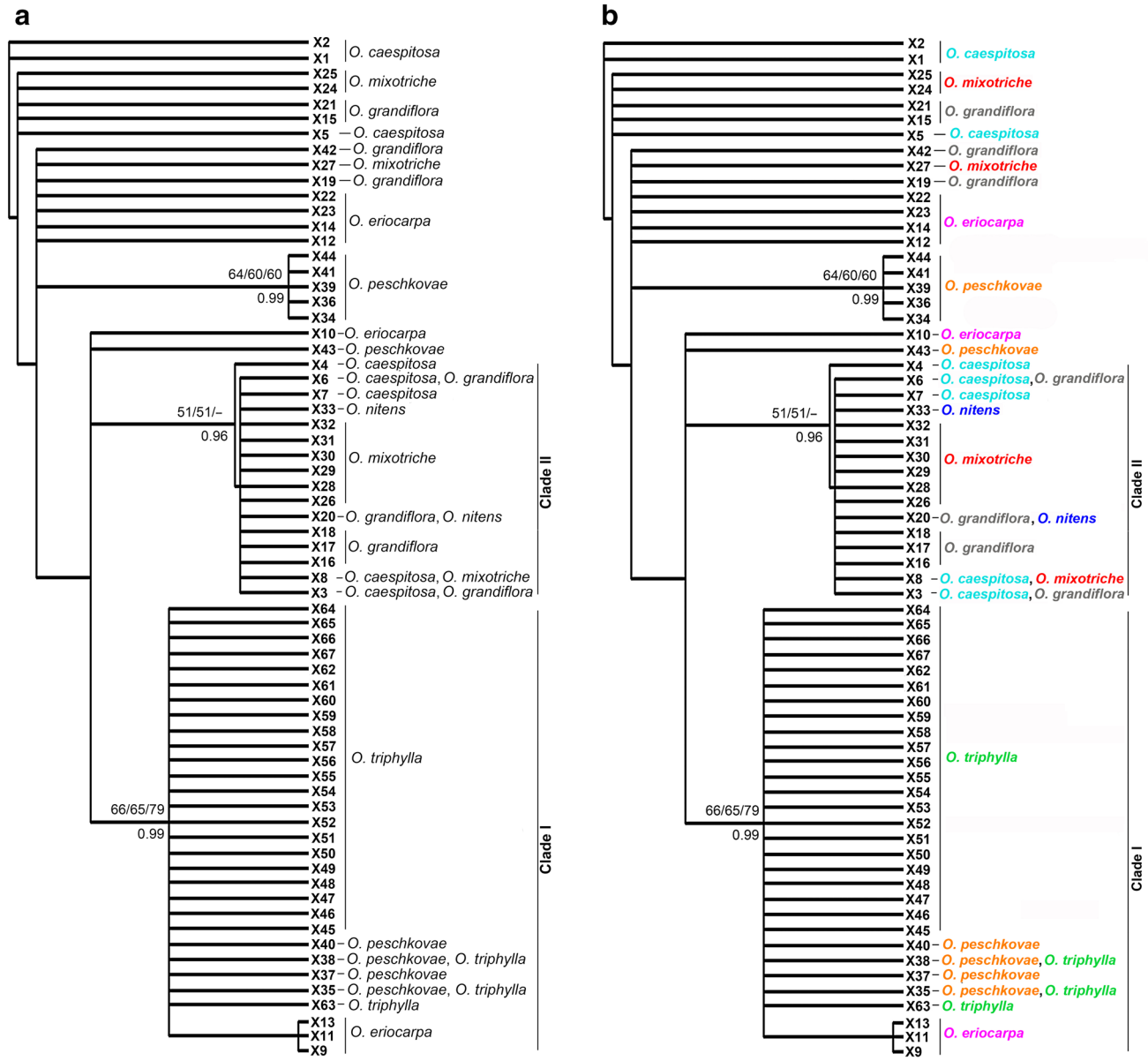

Fig. 2 Phylogenetic consensus MP tree (Tree length of 35 steps, CI = 0.6571, RI = 0.3429) of *Oxytropis* section *Xerobia* haplotypes based on concatenated matrix (*psbA–trnH* + *trnL–trnF* + *trnS–trnG*). The numbers above and below branches indicate bootstrap values (> 50%) for MP/NJ/ML analyses and Bayesian posterior probabilities (> 0.95) for BI analysis, respectively. Each species is written in a specific coloured font. Haplotypes codes correspond to those in Table 1

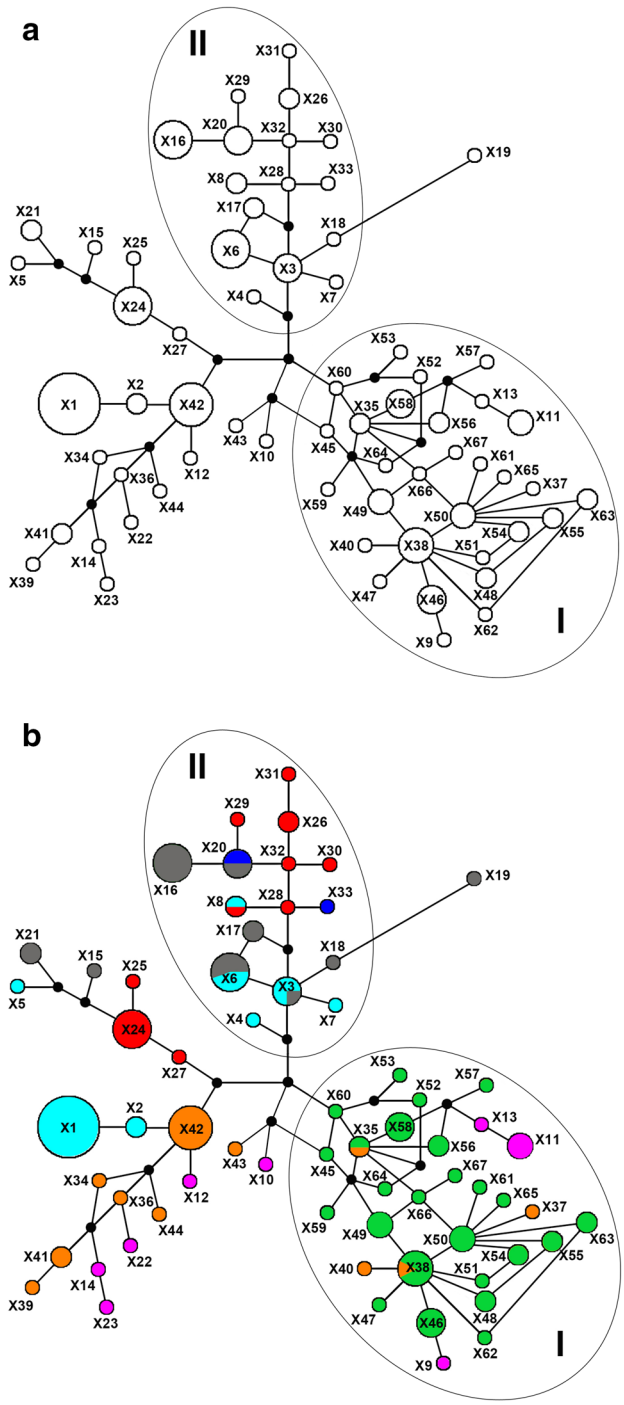

Fig. 3 Median-joining network of cpDNA haplotypes (X1–X67) for *Oxytropis* section *Xerobia*. The size of the circles reflects the frequency of occurrence of haplotypes. Black circles represent missing haplotypes (extinct or not sampled). The color of the haplotype corresponds to the colour font of species to those in Fig. 2. Haplotypes of I and II haplogroups are encircled with thin lines. Haplotypes codes correspond to those in Table 1

O. triphylla were separated from the neighbouring ones by a single mutational step and not distributed according to their population affiliation. The haplotypes form a number of star-like structures, the centres of which are occupied by the shared haplotypes X35 and X38 of *O. triphylla* and *O. peschkovae* and the haplotype X50 found in *O. triphylla* populations from Irkutsk Region and Buryatia. Haplogroup II (Fig. 3) was formed by the same haplotypes that made up clade II on the MP tree (Fig. 2). In this haplogroup, the haplotypes also form star-like structures, the centres of which are occupied by haplotypes X3 (shared for *O. caespitosa* population from Transbaikalia and *O. grandiflora* population from Mongolia) and X20 (shared for the *O. grandiflora* population from Mongolia and *O. nitens* from Buryatia). Alternative connections (loop structures) between haplotypes point to homoplasy, which hampers the identification of genetic relationships between them. At the centre of the network haplotypes, connecting all haplotypes of *Oxytropis* sect. *Xerobia* in a single network, are missing (extinct or not sampled). Thus, the phylogenetic relationships of *Oxytropis* sect. *Xerobia*, both in phylogenetic reconstructions and in the median network of genealogical relationships, remain unresolved.

Discussion

In the current research, most of the studied populations of *Oxytropis* sect. *Xerobia* had high levels of haplotype diversity, three populations had moderate levels, while nucleotide diversity demonstrated a great range in values among populations; one population of *O. caespitosa* was monomorphic (Table 1).

The high genetic diversity in most populations of the relict species *O. triphylla* and the ERI1 population of *O. eriocarpa* is probably associated with their long-term independent evolution, sufficient for the accumulation of many genetic variations. Thus, in the long-lived relict species *Linum flavum* L., the greatest genetic diversity was revealed in the populations on the western edge of the range ($h = 1.000$, $\pi = 0.0014$), according to cpDNA data, which indicates a long-term persistence (relict status) of populations in this region (Plenk et al. 2017). Also, more ancient diploid populations of *Astragalus onobrychis* L. are characterized by high levels of haplotype diversity (h varied from 0.833 to 1.000), possibly because these populations were stable for a long time, and ancestral genetic variation was maintained (Plenk et al. 2020). Haplotype and nucleotide diversity in different populations of the relict *Gymnocarpos przewalskii* Maxim. ranged from 0.200 to 0.889, and from 0.001 to 0.007, respectively (Ma and Zhang 2012). According to the authors, the high diversity in some populations of this species should mainly be attributed to its

antiquity. In addition, a high level of polymorphism is often found in polyploid species (Weiss-Schneeweiss et al. 2013). For example, tetraploid *Solidago arenicola* Keener & Kral, as well as tetra- and hexaploid varieties of *S. simplex* Kunth subsp. *randii*, are characterized by a high level of haplotype diversity ($h = 0.810$ and h varied from 0.700 to 0.840, respectively) (Peirson et al. 2013). The populations PES1 of *O. peschkovae*, CAE3 and CAE4 of *O. caespitosa*, and all populations of *O. grandiflora*, are characterized by a similar high genetic diversity (Table 1), which is possibly caused by the polyploid origin of these species.

The level of genetic diversity in the studied populations of *Oxytropis* sect. *Xerobia* is comparable to that of the species *O. bargusinensis* Peschkova, *O. interposita* Sipl. and *O. glandulosa* Turcz., which are endemic to Baikal Siberia (Kholina et al. 2018a, b); they are similar or even higher than those of *O. lanata* (Pall.) DC., *O. myriophylla* (Pall.) DC. and *O. oxyphylla* (Pall.) DC., which are widespread species in this region (Kholina et al. 2019). It can be assumed that Baikal Siberia provides a climatic optimum for the studied *Oxytropis* sect. *Xerobia*. It is one of the speciation centres of *Oxytropis* species (Malyshev and Peshkova 1984; Polozhii 2003) and a number of other species (Namzalov 2009). Our data on the genetic diversity of *Oxytropis* sect. *Xerobia* indirectly confirm the hypothesis that Baikal Siberia is one of the main centres of biodiversity in North and Central Asia.

The high population differentiation ($\Phi_{ST} = 0.88426$, Table 4) for *O. caespitosa* as well as the nucleotide divergence between populations of this species from Buryatia and Transbaikalia ($D_{XY} = 0.00176$, see text above), exceeding interspecific values (Table 5), are most likely due to the extremely low gene flow between populations located on the western and eastern borders of the range (isolation by distance). A similar value of nucleotide divergence was obtained between MIX1 and MIX2 populations of *O. mixotriche* located at a large dispersal distance from each other (Fig. 1). For these populations, the interpopulation variability was about 60% (Table 4), indicating a rather weak gene flow. The similarly high values of nucleotide divergence were determined between the populations of *O. ochotensis* Bunge from Magadan Region and Kamchatka ($D_{XY} = 0.00167$), with a distance between them of about 800 km; in addition, similar D_{XY} values (from 0.00188 to 0.00206) were determined between the major cpDNA lineages of *O. ruthenica* Vass. (Kozyrenko et al. 2020). High levels of the population differentiation and nucleotide divergence for *O. caespitosa* and *O. mixotriche* may indicate actively ongoing speciation processes. The low level of interpopulation differentiation for *O. peschkovae* and *O. grandiflora* ($\Phi_{ST} = 0.31551$ and $\Phi_{ST} = 0.18804$, respectively, Table 4) is probably caused by the fact that most populations of these species are located quite close to each other (Fig. 1). The absence of nucleotide divergence between the populations of *O. triphylla* and the low interpopulation differentiation ($\Phi_{ST} = 0.12169$, Table 4) may indicate the following: (1) continuous gene exchange between the populations; (2) conservation of ancestral polymorphism; (3) local populations are the constituent parts of a single metapopulation. Absence of significant interpopulation differentiation was shown for *O. bargusinensis* ($\Phi_{ST} = 0.027$, $P > 0.20$, Kholina et al. 2018a) and for *O. myriophylla* ($\Phi_{ST} = 0.008$, $P > 0.37$, Kholina et al. 2019) from Baikal Siberia.

The high nucleotide divergence between the populations of *O. eriocarpa* as well as between *O. eriocarpa* and other species (Table 5) is likely due to geographic disjunction, in the first case, between the populations of *O. eriocarpa* from the Altai Mountains and Tyva, and, in the second case, between the species from the Altai Mountains, Tyva and Baikal Siberia. The exception is a pair of species, *O. triphylla* and *O. eriocarpa*, with low D_{XY} indices (Table 5); the genetic affinity of these ancient diploid species from subsect. *Stuppa* can be partly explained by the wide distribution of the ancestor of *O. triphylla* in Southern Siberia in the past (Peshkova 2001). The absence of species-specific markers for the studied species *Oxytropis* sect. *Xerobia* and the presence of shared haplotypes for *O. caespitosa* and *O. grandiflora*, for *O. caespitosa* and *O. mixotriche*, for *O. grandiflora* and *O. nitens*, for *O. triphylla* and *O. peschkovae* (Table 1) indicate their close relationships.

Shared haplotypes are characteristic for groups of closely related species. For example, shared haplotypes were found in *O. bargusinensis*, *O. oxyphylla*, *O. selengensis* Bunge and *O. stukovii* Palib. sect. *Verticillares* from Baikal Siberia (Kholina et al. 2019) as well as in *Sophora* species from New Zealand (Shepherd et al. 2017), in *Solidago* species (Peirson et al. 2013), in *Petunia* species (Segatto et al. 2017) and in *Atraphaxis* species (Xu et al. 2016). Shared haplotypes can be a manifestation of the ancestral polymorphism, incomplete lineage sorting, homoplasy, hybridization and introgression, or of a combination of several of them (Peirson et al. 2013; Xu et al., 2016; Segatto et al. 2017; Shepherd et al. 2017). Apparently, the shared haplotype for *O. nitens* and *O. grandiflora* (Table 1), whose populations are more than 1500 km away from each other, can be explained by ancestral polymorphism and incomplete lineage sorting. At the same time, the shared haplotypes for other pairs of species (Table 1) found in closely located populations may be the result of intensive hybridization processes.

Phylogenetic relationships based on cpDNA data of *Oxytropis* sect. *Xerobia* from Baikal Siberia remain unresolved. The distribution of haplotypes both in the MP tree and in the median network (Figs. 2 and 3) does neither correspond to the division of the section into subsections nor to the population and species affiliation. Only the haplotypes of *O. triphylla* formed the clade I/haplogroup I with rather high support. The isolated position inferred by plastid data agrees

with the morphology, as *O. triphylla* differs from the other species by a number of morphological features, for example, reduction of the leaflets (only one pair) (Table 2). The haplotypes of *O. eriocarpa* and *O. peschkovae* belong to the same clade/haplogroup. We assume that the affinity of *O. triphylla* and *O. eriocarpa* is due to ancestral polymorphism, and the affinity of *O. triphylla* and *O. peschkovae* is due to the origin of the latter. It is speculated (Popov 1956, 1957) that the polyploid *O. peschkovae* is a species of hybrid origin, and one of its parents is *O. caespitosa*. This is confirmed by our results, which revealed that the widespread X42 haplotype of *O. peschkovae* differs from the X1 haplotype of *O. caespitosa* by one substitution, a 6-bp indel and the length of mono- and dinucleotide repeats (Fig. 3). The shared haplotypes for *O. peschkovae* and *O. triphylla*, the proximity of the haplotypes of *O. peschkovae* to the haplotypes of *O. eriocarpa* as well as the presence of star-like structures in the network (Fig. 3) revealed in our study indicate a second potential parent of the hybrid *O. peschkovae*. Since *O. peschkovae* and *O. triphylla* have overlapping ranges, it can be assumed that the second parent is *O. triphylla*, but in any case, it is most likely a member of the subsect. *Stuppa*. Most haplotypes of *O. caespitosa*, *O. grandiflora*, *O. mixotriche*, and *O. nitens* subsect. *Ampulla* are grouped in clade II/haplogroup II (Figs. 2 and 3), in which four shared haplotypes are present. This indicates a common origin (ancestral polymorphism and incomplete lineage sorting), a relatively recent divergence of species or intensive processes of introgression and hybridization in the zone of sympatry, leading to the appearance of closely related allopolyploids. Taking into account that the segregation of the branch of the legume genera, which includes *Astragalus* and *Oxytropis*, took place about 39 million years ago (Lavin et al. 2005), and separation of the genus *Oxytropis* from *Astragalus* took place at the Miocene-Pliocene boundary about 5.6 million years ago (Shavvon et al. 2017), the splitting of the *Oxytropis* species is probably relatively recent. Our data are consistent with the earlier expressed opinion (Popov 1956, 1957) about the hybrid origin of *O. mixotriche* and *O. nitens* from the parental pair of *O. caespitosa* and *O. grandiflora*. Probably, these species form a single genetic complex in Baikal Siberia and the adjacent territory of Northern Mongolia. We assume that the processes of introgression are currently actively taking place in this group of species. A similar genetic complex is formed by the species *O. bargusinensis*, *O. oxyphylla*, *O. selengensis* and *O. stukovii* sect. *Verticillares* from Baikal Siberia (Kholina et al. 2019). The interaction of introgression and polyploidization processes leads to the reticular evolution of the sect. *Xerobia*, which is characteristic for the whole genus *Oxytropis* (Malyshev 2008) and for the closely related genus *Astragalus* (Bartha et al. 2013).

Thus, a fairly high level of genetic polymorphism was revealed for *Oxytropis* sect. *Xerobia* from Baikal Siberia, mainly due to the ancient origin for some of them and hybridization and polyploidy for others, as well as to the climatic conditions of these habitats being close to the optimum for these *Oxytropis* species. The absence of diagnostic species-specific variants for the markers studied, together with the sharing of cpDNA haplotypes and nrDNA ribotypes between species, and the resulting polytomies on the phylogenetic trees, confirm the hypothesis on the hybrid origin of some of them. Obviously, the reproductive barriers within the sect. *Xerobia* are weak. However, morphological differences between the species of the sect. *Xerobia* are clearly pronounced, even when they grow in sympatry.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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