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ORIGINAL PAPER

The species of *Oxytropis* DC. of section *Gloeocephala* Bunge (Fabaceae) from Northeast Asia: genetic diversity and relationships based on sequencing of the intergenic spacers of cpDNA and ITS nrDNA

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Abstract

Phylogenetic relationships within *Oxytropis* DC. sect. *Gloeocephala* Bunge from Northeast Asia were studied using plastid intergenic spacers (*psbA–trnH* + *trnL–trnF* + *trnS–trnG*) and ITS nrDNA. Populations of *O. anadyrensis* Vass., *O. borealis* DC., *O. middendorffii* Trautv., *O. trautvetteri* Meinsh., and *O. vasskovskyi* Jurtz. were monomorphic or characterised by a low level of chloroplast genetic diversity (*h* varied from 0.143 to 0.692, and π from 0.0001 to 0.0005). Presumably, the low genetic diversity was a result of the severe bottlenecks during Pleistocene glaciation-interglacial cycles. Twenty chlorotypes were identified; species studied had no shared chlorotypes. Chlorotypes of *O. anadyrensis*, *O. borealis*, and *O. middendorffii* formed two lineages each, while the chlorotypes of *O. trautvetteri* and *O. vasskovskyi* formed one separate lineage each in the phylogenetic network. There were specific diagnostic markers of cpDNA in each lineage, excluding *O. vasskovskyi*. The presence of a species-specific diagnostic marker in *O. trautvetteri* and specific markers in two lineages of *O. anadyrensis* support circumscribing these taxa as independent species. Regarding ITS nrDNA polymorphism, five ribotypes were detected. The differences revealed in plastid and nuclear genomes of *Oxytropis* sect. *Gloeocephala* confirmed that the Asian sector of Megaberingia was the main centre of diversification of arctic legumes.

Keywords Fabaceae · Arctic species · Phylogenetic relationships · Plastid and nuclear genomes · Population structure · Diversification

Introduction

The section *Gloeocephala* Bunge is one of the most taxonomically complicated sections within the genus *Oxytropis* DC., and it presumably derived from the section *Orobia* Bunge (Yurtsev 1986). Section *Gloeocephala* contains about 20 species and subspecies in North Asia and North America, most of which are arctic species (Malyshev 2008). They are acaulescent herbs with numerous gluey and odorous warty glands on different parts of the plant, including leaves, peduncles, and legumes (Pavlova 1989). Most *Oxytropis* sect. *Gloeocephala* show high polymorphism in morphological traits of leaves, flowers, and legumes; geographic isolation leads to allopatric speciation, and race formation is complicated by polyploidy (Yurtsev 1986, 1999). There are six species of the sect. *Gloeocephala* in Northeast Asia: *Oxytropis middendorffii* Trautv., arctic polymorphic species, also found in North Koryakia (2n = 48); *O. borealis* DC., distributed mainly in the north of Chukotka, in North Koryakia and on Karaginsky Island, as well as Alaska (2n = 48); *O. vasskovskyi* Jurtz., East Siberian hypoarctic montane species (2n = 16, 32); *O. anadyrensis* Vass., endemic to the Chukotka, Koryakia and Kamchatka (2n = 16, 48); *O. trautvetteri* Meinsh., endemic to the southern part of Okhotia and the north of Sakhalin Island (2n = 16); *O. kateninii* Jurtz., endemic to the Chukotka, poorly studied, and known only

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from "*locus classicus*" (Pavlova 1989). According to Yurtsev (1986), *O. borealis* replaces *O. middendorffii* in the eastern and south-eastern parts of the Chukchi Peninsula. Species distribution of sect. *Gloeocephala* is closely connected with mountainous regions of Northeast Asia (Sandanov et al. 2022). As rare species existing in isolated populations and confined to specific habitats, *O. middendorffii*, *O. borealis*, and *O. anadyrensis* are included in the Red Data Book of Kamchatskiy Krai (2018), and the latter species is also included in the Red Data Book of the Chukchi Autonomous District (2008).

There are controversial opinions concerning the taxonomic status of *O. anadyrensis* and *O. trautvetteri*. The author of the first description of *O. anadyrensis*, Vassilchenko, accepts it as an independent species (Vassilchenko and Fedchenko 1948), and Pavlova (1989) and Yakubov (2014) agree, while other botanists (Yurtsev 1986; Polozhii 1994; Malyshev 2008) accept it as a subspecies of *O. middendorffii–O. middendorffii* Trautv. subsp. *anadyrensis* (Vass.) Jurtz. At the same time, Yurtsev noted (Yurtsev 1986) that the issue of the taxonomic status of *O. anadyrensis* has not been resolved, and in a later survey (Yurtsev 1999) emphasised that *O. middendorffii* subsp. *anadyrensis* with strongly glandular legumes and a calyx tube differed significantly from *O. middendorffii* and should perhaps be treated as a separate species. Considering the similarity between *O. anadyrensis* and *O. vasskovskyi*, which grow in sympatry on the Anyui Highlands, Yurtsev (1986) suggested that *O. anadyrensis* originated because of hybridization between one of the eastern forms of *O. middendorffii* and *O. vasskovskyi* (Yurtsev 1986). *O. trautvetteri* is considered in a similar manner; some authors (Vassilchenko and Fedchenko 1948; Voroshilov 1982; Pavlova 1989) consider it as an independent species, others authors (Yurtsev 1986; Polozhii 1994; Malyshev 2008) as a subspecies of *O. middendorffii–O. middendorffii* Trautv. subsp. *trautvetteri* (Meinsh.) Jurtz. The authors of the "Annotated Checklist of the Panarctic Flora (PAF) Vascular Plants" project (Elven and Murray 2011) accepted *O. trautvetteri* as a subspecies of *O. middendorffii* with strong doubts, and Yurtsev, one of the project participants, noted that it was possible to increase the taxonomic rank of *O. trautvetteri*, for which additional research was needed.

Until now, the studies of genetic diversity and phylogenetic relationships of *Oxytropis* sect. *Gloeocephala* have not been carried out. There are few genetic studies of *Oxytropis* species in which single members of the sect. *Gloeocephala* have been included: *O. borealis*, *O. borealis* var. *viscida* (Greene) S. L. Welsh, and *O. borealis* var. *hudsonica* (Greene) S. L. Welsh. So, the evolutionary relationships of the arctic *Oxytropis* species (Archambault and Strömvik 2012), as well as North American *Oxytropis* species (Meyers 2012), including *O. borealis* var. *viscida* were studied using molecular markers of the plastid and nuclear genomes. The proximity of *O. borealis* var. *viscida* to the *O. arctica* R.Br. var. *barnebyana* Welsh, *O. campestris* (L.) DC., and *O. maydelliana* Trautv. sect. *Orobia* Bunge was revealed based on the polymorphism of nucleotide sequences of the *CNGC5* of the nuclear DNA (nrDNA) and *matK* of chloroplast DNA (cpDNA) (Meyers 2012). Additionally, the proximity of *O. borealis* var. *viscida* to the *O. campestris* and *O. sericea* Torr. & A. Gray sect. *Orobia* was revealed based on the nucleotide polymorphisms in the ITS nrDNA (Archambault and Strömvik 2012). Later, the phylogenetic relationships of *Oxytropis* species, including *O. borealis*, *O. borealis* var. *viscida*, and *O. borealis* var. *hudsonica*, were studied using ITS nrDNA + *trnL–trnF* cpDNA (Shavvon et al. 2017); however, relationships among them were not resolved even at the section level.

In our own studies, *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* intergenic spacers (IGS) of cpDNA and ITS nrDNA were effective for the study of the genetic diversity of closely related and endemic *Oxytropis* species, for resolving of the controversial taxonomic issues, as well as for the reconstruction of the phylogenetic relationships within the sections *Orobia*, *Verticillares* DC., *Arctobia* Bunge, *Polyadena* Bunge, and *Xerobia* Bunge (Artyukova and Kozyrenko 2012; Kholina et al. 2016, 2018a, 2018b, 2019, 2020, 2021a, 2021b; Kozyrenko et al. 2020).

The main objectives of this study were to: (1) evaluate the genetic diversity of *Oxytropis anadyrensis*, *O. borealis*, *O. middendorffii*, *O. trautvetteri*, and *O. vasskovskyi* sect. *Gloeocephala* from Northeast Asia; (2) reconstruct their phylogenetic relationships; and (3) clarify the taxonomic status of *O. anadyrensis* and *O. trautvetteri*, based on sequencing of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* IGS cpDNA and ITS nrDNA.

Materials and methods

Taxon sampling

Seventy-five plants of *Oxytropis anadyrensis* (26 samples), *O. borealis* (10), *O. middendorffii* (10), *O. trautvetteri* (10), and *O. vasskovskyi* (19) sect. *Gloeocephala* from Northeast Asia (Fig. 1) were used to study cpDNA polymorphism. Plant samples were taken from natural populations (randomly chosen plants located about 100 m apart) or herbarium specimens from Vascular Plants Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (LE); the Herbarium of the Institute of Biological Problems of the North, Far Eastern Branch of the Russian Academy of Sciences, Magadan, Russia (MAG); Moscow State University Herbarium (MW); the Herbarium of the Institute of General and Experimental

Fig. 1 (*Grayscale and RGB Color*) Map of sample sites for natural populations of *Oxytropis* sect. *Gloeocephalla* (16 populations, black circles). Population codes correspond to those in Table 1

Biology, Siberian Branch of the Russian Academy of Sciences, Ulan-Ude, Russia (UUH). The ITS region of nrDNA was amplified for 21 samples: *O. anadyrensis* (5), *O. borealis* (3), *O. middendorffii* (4), *O. trautvetteri* (2), and *O. vasskovskyi* (7), representing most of cpDNA haplotypes identified in this work. The sampling localities, sample size, geographic coordinates, and codes for each population are given in Table 1. The complete list of samples, vouchers information, sample numbers for DNA extraction, chlorotypes, and ribotypes is provided in Supplemental Material 1, Table S1.

The names of species and sections are accepted according to Pavlova (1989). Taxonomic features of *Oxytropis* sect. *Gloeocephala* (according to Yurtzev 1986; Pavlova 1989; Malyshev 2008) suitable for discriminating the studied species are given in Table 2.

DNA isolation, amplification and sequencing

Total DNA was extracted from dried leaves. The extraction buffer contained 100 mM Tris–HCl (pH 8.0), 0.7 M NaCl, 40 mM EDTA, 1% CTAB (hexadecyltrimethylammonium bromide), and 10 mL/L β-mercaptoethanol. The extract was incubated at 65 °C for 40 min. The DNA was deproteinized with chloroform:octanol (24:1) and precipitated with equal volume of isopropanol in the presence of 0.3 M sodium acetate. DNA pellets was washed with 75% ethanol and dissolved in the buffer containing 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA. Amplification of the *trnH–psbA*, *trnL–trnF*, and *trnS–trnG* intergenic spacer regions of cpDNA was performed with the use of universal primers, reaction conditions, and temperature regimes recommended for these regions (Taberlet et al. 1991; Shaw et al. 2005). The ITS region of nrDNA was amplified with the ITS1 and ITS4 primers under the reaction conditions and temperature described in (Mir et al. 2010). 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

Complete sequences were assembled using the Staden Package v. 1.5 (Bonfeld et al. 1995). The sequences of four DNA regions were aligned with SeaView v. 4.7 (Gouy et al. 2010) using the CLUSTAL algorithm, manually edited when necessary, and concatenated for each specimen. 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})

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

Species	Population code	The location of the population (No. of samples)	Herbarium acronym[a]	Haplotype (No. of samples)	Genetic diversity (SD)	
					Haplotype diversity	Nucleotide diversity
O. anadyrensis Vass.	ANAD1	KK, Ust-Kamchatskiy District, Sopka Ploskaya Volcano (10)	Yakubov, personal collection	GL1 (10)	0.000 (0.000)	0.0000 (0.0000)
	ANAD2	ChAD, near the Beringovsky Urban Settlement, Alkatvaam River (14)	MAG	GL2 (13), GL3 (1)	0.143 (0.119)	0.0001 (0.0001)
	ANAD3	ChAD, Amguema River (1)	LE	GL4 (1)	–	–
	ANAD4	ChAD, Emeem River (1)	MAG	GL5 (1)	–	–
O. borealis DC.	BOR1	ChAD, Chukotsky District, near the Lavrentiya Rural Settlement (7)	MAG	GL6 (7)	0.000 (0.000)	0.0000 (0.0000)
	BOR2	ChAD, Chukotsky District, Chegitun River (1)	MAG	GL7 (1)	–	–
	BOR3	ChAD, Providensky District, Kurupka River (2)	MAG	GL8 (2)	–	–
O. middendorffii Trautv.	MID1	KK, Olyutorsky District, Govena Peninsula, Govena Cap (5)	Yakubov, personal collection	GL9 (5)	0.000 (0.000)	0.0000 (0.0000)
	MID2	TP, Kotuy River, near the estuary of Medvezhye River (1)	MW	GL10 (1)	–	–
	MID3	TP, Taimyr Lake, south foothills of Byrranga Mountains (1)	LE	GL11 (1)	–	–
	MID4	TP, Nizhnyaya Taymyra River, Byrranga Mountains (1)	LE	GL12 (1)	–	–
	MID5	TP, Pyasina River, near the village Tareya (2)	LE	GL13 (2)	–	–
O. trautvetteri Meinsh.	TRA	MO, Olsky District, Zavyalov Island (10)	MAG	GL14 (10)	0.000 (0.000)	0.0000 (0.0000)
O. vasskovskyi Jurtz.	VAS1	MO, Susumansky District, near the town Susuman, Byoryolyokh River (13)	UUH	GL15 (1), GL16 (7), GL17 (3), GL18 (1), GL19 (1)	0.692 (0.119)	0.0005 (0.0004)
	VAS2	MO, Tenkinskaya Trassa, Arga-Jurjach River (4)	UUH	GL19 (3), GL20 (1)	0.500 (0.265)	0.0002 (0.0002)
	VAS3	MO, Yagodninsky District, Kolyma River (2)	MAG	GL16 (1)	–	–

ChAD Chukchi Autonomous District; *KK* Kamchatskiy Krai; *MO* Magadan Oblast; *TP* Taimyr Peninsula

[a]Herbarium acronym: *LE* Vascular Plants Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg, Russia; *MAG* Herbarium of the Institute of Biological Problems of the North, Far Eastern Branch of the Russian Academy of Sciences, Magadan, Russia; *MW* Moscow State University Herbarium, Moscow, Russia; *UUH* The Herbarium of the Institute of General and Experimental Biology, Siberian Branch of the Russian Academy of Sciences, Ulan-Ude, Russia

Table 2 Taxonomic characteristics of *Oxytropis* sect. *Gloeocephala*(according to Yurtzev 1986; Pavlova 1989; Malyshev 2008)

Feature\Taxon	*O. borealis*	*O. vasskovskyi*	*O. middendorffii*	*O. anadyrensis*	*O. trautvetteri*
Raceme	Compact, capitate	Rather lax, capitate or elongate	Rather lax, capitate	Rather lax, capitate or umbellate	Rather lax, capitate
Peduncle	Pilose, glandular-verrucose (rather big glands)	Glabrous or glabrate, glandular (small glands)	Pilose, sparsely glandular	Sparsely pilose, profusely glandular-verrucose (small glands)	Glabrate, glandular-verrucose (rather big glands)
Corolla color	Reddish- purple	Pinkish-purple	Reddish- purple	Pale purple	Purple
Corolla length, mm	15-17	14-17	20-25	15-20	20-25
Keel beak length, mm	0.7	0.4	1	1	0.5
Calyx tube	Tubular-campanulate	Tubular	Tubular-campanulate	Tubular-campanulate	Tubular
Calyx tube: pubescence	Densely black- or dark-pubescent with longer white hairs, sparsely glandular	Sparsely black- pubescent, glandular	Densely black- or dark-pubescent with longer white hairs, almost non-glandular	Sparsely black- and white-pubescent, glandular	Densely black-pubescent, almost non-glandular
Calyx teeth	Linear, about half as long as tube	Triangular, 3-7 times shorter than tube	Linear, more than half of tube	Linear, less than half of tube	Linear, less than half of tube
Pod: pubescence	Black-pubescent, with glands on beak	Black-pubescent, glandular	Black-pubescent, with glands on beak	Sparsely pubescent, glandular	Glabrous or sparsely pubescent, profusely glandular-verrucose
Pod: length/width, mm	11-12/6	15/5	15-20/6-8	15/5	20-25/5
Pod: beak length, mm	5	10	10	5-7	10

between cpDNA sequences based on nucleotide substitutions. Haplotype (h) and nucleotide (π) diversity of populations (for populations with four 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 groups, and among groups. The statistical significance (P) of the variance components was evaluated based on 1023 permutations.

The genealogical relationships of cpDNA haplotypes were determined using the median joining (MJ) method as implemented in the Network v. 5.0.1.1 (Bandelt et al. 1999). Each insertion or deletion, regardless of their size, was coded as a single mutational event. The nucleotide sequences of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* IGS cpDNA previously obtained for *O. glabra* (Lam.) DC. sect. *Mesogaea* Bunge (accession numbers in GenBank LT856572, LT856585, and LT856598, respectively) (Kholina et al. 2018a) were used as an outgroup.

Phylogenetic analyses of ITS ribotypes 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). 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. The nucleotide sequence of the ITS of *O. glabra* (accession number in GenBank LC213354) was used as an outgroup.

Results

The nucleotide sequences of the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* IGS cpDNA were obtained for 75 specimens of *O. anadyrensis*, *O. borealis*, *O. middendorffii*, *O.*

trautvetteri, and *O. vasskovskyi* sect. *Gloeocephala*. They were characterised by low nucleotide variability and different lengths due to the presence of short (5–9 nucleotides) indels, as well as mono- and dinucleotide repeats. The total length of the combined sequences of three IGS was 2483 sites. Eight sites were variable, of which three sites were parsimony informative. The only species-specific molecular marker was revealed for *O. trautvetteri*: an insertion of nine bp in the *trnS–trnG* (positions 2047–2055 of the combined matrix).

Of the seven populations with four or more samples, four populations (ANAD1 *O. anadyrensis*, BOR1 *O. borealis*, MID1 *O. middendorffii*, and TRA *O. trautvetteri*) were monomorphic. In the remaining three populations, haplotype diversity (h) varies from 0.143 to 0.692, and nucleotide diversity (π) varies from 0.0001 to 0.0005 (Table 1). According to AMOVA (Table 3), more than 99% of the total genetic variability belonged to the interpopulation component for *O. anadyrensis*. For *O. borealis* and *O. middendorffii*, the interpopulation component of the total genetic variability was 100%. For *O. vasskovskyi*, the genetic variability was almost equally distributed between and within populations. The divergence of nucleotide sequences allows the evaluation of the degree of genetic disunity of the populations/species. For *O. anadyrensis*, the average number of nucleotide substitutions per site between ANAD1 and the remaining populations was 0.00125, while there was no divergence between ANAD2, ANAD3, and ANAD4 populations. For *O. borealis*, BOR2 was significantly diverged from two other populations (Dxy = 0.00125), and Dxy between BOR1 and BOR3 was 0.00041. For *O. middendorffii*, the average number of nucleotide substitutions per site between MID1 and the remaining populations was 0.00083, while there was no divergence between MID2, MID3, MID4, and MID5 populations. For *O. vasskovskyi*, Dxy between populations ranged from 0.000 to 0.00003. The divergence of nucleotide sequences between *Oxytropis* sect. *Gloeocephala* (Table 4) was lower than that between some populations, and ranged from 0.00002 to 0.00104. Hierarchical AMOVA showed (Table 3) that the differences among species accounted for only 17.4% of the total variation, but this value was not significant; about 81% of the variability was due to the interpopulation component within the species, and about 1% was due to the intrapopulation component.

Analysis of 75 sequences of the combined matrix identified 20 chlorotypes (GL1–GL20). The sequences of these chlorotypes for each the *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* IGS cpDNA were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession numbers OD978281–OD978300, OD982670–OD982689, and OD982967–OD982986, respectively. For *O. vasskovskyi*, six chlorotypes were identified (GL15–GL20), and five chlorotypes were revealed in *O. anadyrensis* and in *O. middendorffii* (GL1–GL5 and GL9–GL13, respectively). Three

Table 3 The results of amova for distribution of the total genetic variability between groups of *Oxytropis* sect. *Gloeocephala* according to cpDNA data

Source of variation	Genetic differences (%) between		
	Groups	Populations within groups	Individuals in population
Populations of *Oxytropis* species			
One group: (all populations of *O. anadyrensis*)	–	99.79*	0.21
One group: (all populations of *O. borealis*)	–	100.00**	0.00
One group: (all populations of *O. vasskovskyi*)	–	56.29**	43.71
One group: (all populations of *O. middendorffii*)	–	100.00**	0.00
Five groups: (all populations of *O. anadyrensis*), (all populations of *O. borealis*), (all populations of *O. middendorffii*), (population of *O. trautvetteri*), (all populations of *O. vasskovskyi*)	17.40 ns	81.64*	0.96*
Haplogroups identified in network analysis			
Two groups of *O. anadyrensis*: (haplogroup V) и (haplogroup VI)	92.08 ns	7.74**	0.18*
Two groups of *O. borealis*: (haplogroup III) и (haplogroup IV)	92.65 ns	7.35**	0.00**
Two groups of *O. middendorffii*: (haplogroup VII) и (haplogroup VIII)	77.24 ns	22.76ns	0.00**
Eight haplogroups: (*O. trautvetteri*, haplogroup I), (*O. vasskovskyi*, haplogroup II), (*O. borealis*, haplogroup III), (*O. borealis*, haplogroup IV), (*O. anadyrensis*, haplogroup V), (*O. anadyrensis*, haplogroup VI), (*O. middendorffii*, haplogroup VII), (*O. middendorffii*, haplogroup VIII)	89.18*	9.89*	0.93*

*$P = 0.0000$; **$0.0009 < P < 0.05$; *ns* not significant. The statistical significance (P) was evaluated based on 1023 permutations

Table 4 Nucleotide divergence between *Oxytropis* sect. *Gloeocephala* according to cpDNA data

Species	*O. anadyrensis*	*O. borealis*	*O. middendorffii*	*O. trautvetteri*	*O. vasskovskyi*
O. anadyrensis	–	2.485 (0)	1.769 (0)	1.385 (0)	1.437 (0)
O. borealis	0.00104	–	2.100 (0)	1.100 (0)	1.153 (0)
O. middendorffii	0.00074	0.00088	–	1.000 (0)	1.053 (0)
O. trautvetteri	0.00058	0.00046	0.00042	–	0.053 (0)
O. vasskovskyi	0.00060	0.00048	0.00044	0.00002	–

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 site (D_{XY})

chlorotypes were revealed in *O. borealis* (GL6–GL8), and *O. trautvetteri* was represented by only one chlorotype (GL14). No shared chlorotypes were found (Table 1).

The MJ network revealed eight haplogroups (I–VIII) (Fig. 2) according to species affiliation: *O. trautvetteri*, haplogroup I; *O. vasskovskyi*, haplogroup II; *O. borealis*, haplogroups III and IV; *O. anadyrensis*, haplogroups V and VI; *O. middendorffii*, haplogroups VII and VIII. All haplogroups, except haplogroup II, had their own specific marker substitutions/indels. Hierarchical AMOVA showed (Table 3) that the differences among haplogroups of the same species were rather high, but they were not statistically significant. At the same time, the differences between all identified haplogroups accounted for more than 89% of the total variation, and these differences were statistically significant (Table 3). Thereby, genealogical analysis revealed a clear division of cpDNA chlorotypes of five *Oxytropis* sect. *Gloeocephala* from Northeast Asia into eight evolutionary branches: (1) *O. trautvetteri*; (2) *O. vasskovskyi*; (3) *O. borealis*, lineage 1, populations from Chukchi Autonomous District (BOR1 and BOR3); (4) *O. borealis*, lineage 2, population from Chukchi Autonomous District (BOR2); (5) *O. anadyrensis*, lineage 1, population from Kamchatskiy Krai (ANAD1); (6) *O. anadyrensis*, lineage 2, populations from Chukchi Autonomous District (ANAD2–ANAD4); (7) *O. middendorffii*, lineage 1, population from Kamchatskiy Krai (MID1); and (8) *O. middendorffii*, lineage 2, populations from Taimyr Peninsula (MID2–MID5).

The ITS nrDNA nucleotide sequences of *Oxytropis* plants had the same length (603 bp); of the 603 sites, six were variable and parsimony informative (positions 68, 119, 122, 227, 466, and 564). Five ribotypes (RGL1–RGL5) were identified, the sequences of which were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI for each species under accession numbers OD983284–OD983291. Two ribotypes were detected in *O. anadyrensis* (RGL1 and RGL2), *O. borealis* (RGL3 and RGL4), and *O. middendorffii* (RGL1 and RGL5), and one ribotype was detected in *O. trautvetteri* (RGL1) and *O. vasskovskyi* (RGL2). Thus, ribotype RGL1 was shared by *O. anadyrensis*, *O.*

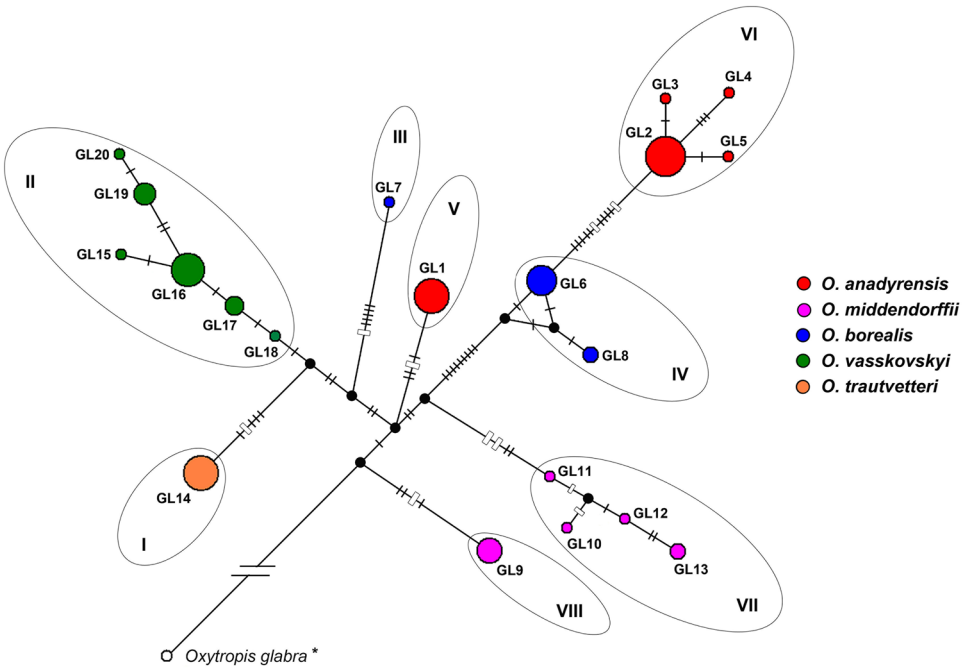

Fig. 2 (*Grayscale and RGB Color*) Genealogical network of cpDNA chlorotypes (GL1–GL20) for *Oxytropis* sect. *Gloeocephalla* constructed by the MJ method. The size of the circles reflects the frequency of occurrence of chlorotypes. Black circles represent the median vectors. Thin transverse lines on the branches correspond to the mutational events; white thick lines indicate the indels. Haplogroups I–VIII are circled by solid lines. *Mutations for *O. glabra*, used as an outgroup, are not shown and have not been considered

middendorffii and *O. trautvetteri*, and ribotype RGL2 was shared by *O. anadyrensis* and *O. vasskovskyi*.

The phylogenetic reconstruction methods (MP, NJ, ML, and BI) resulted in similar topologies with few differences in statistical support. The MP analysis yielded the single most parsimonious tree, as shown in Fig. 3 (tree length of 12 steps, CI = 1.0000, RI = 1.0000). Tree topology showed poor resolution, and only some relationships were significantly supported. Thus, the samples with ribotype RGL1 of *O. anadyrensis*, *O. middendorffii*, and *O. trautvetteri* formed a distinct group, whose support was weak in MP, NJ, and ML analyses (BP 62, 60, and 68%, respectively) and high in BI analysis (PP 0.99). The samples with ribotypes RGL2, RGL3, and RGL4 formed another group that included other samples of *O. anadyrensis*, as well as all samples of *O. vasskovskyi* and *O. borealis*; this group was highly supported in MP, NJ, ML, and BI analyses (BP 87, 85, and 88%, PP 1.0). The positions of *O. middendorffii* samples with ribotype RGL5 in the phylogenetic tree remained unresolved. Phylogenetic analysis of the combined ITS and plastid DNA sequences obtained for 21 samples, performed by different methods (ML, MP and NJ), did not give a better relationship of the resolution. The topology of the tree turned out to be similar to that shown in Fig. 3 and only one clade received weak bootstrap support. Therefore, we did not present the results of the combined matrix of two genomes.

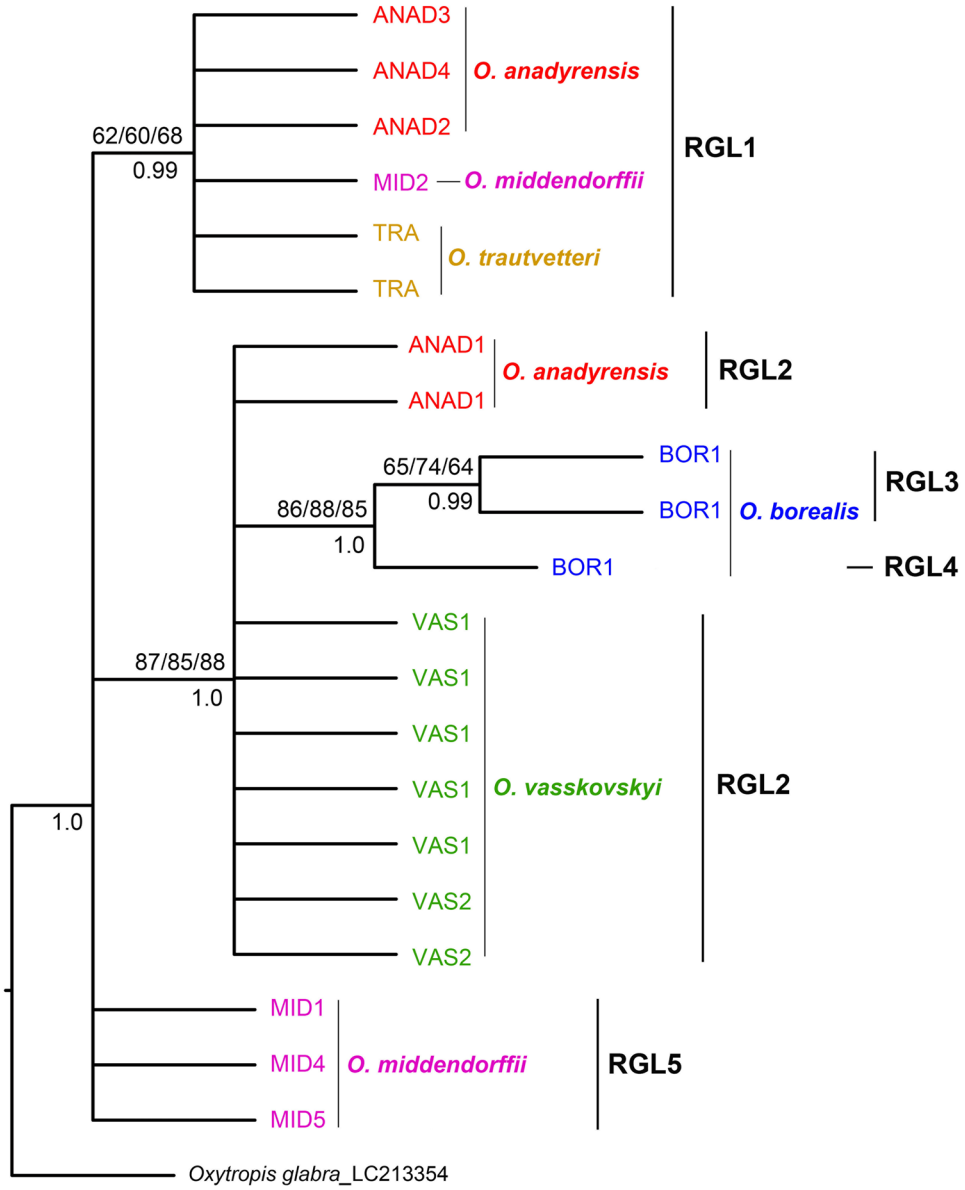

Fig. 3 (*Grayscale*) Phylogenetic MP-tree of the ITS nrDNA ribotypes (RGL1–RGL5). The numbers above and below branches indicate bootstrap values (BI > 50%) for MP/NJ/ML analyses and Bayesian posterior probabilities (PP > 0.95) for BI analysis, respectively. Population codes correspond to those in Table 1. The ITS sequence of *O. glabra* (GenBank LC213354) was used as an outgroup. (*RGB Color*) Phylogenetic MP-tree of the ITS nrDNA ribotypes (RGL1–RGL5). The numbers above and below branches indicate bootstrap values (BI > 50%) for MP/NJ/ML analyses and Bayesian posterior probabilities (PP > 0.95) for BI analysis, respectively. Population codes correspond to those in Table 1. The ITS sequence of *O. glabra* (GenBank LC213354) was used as an outgroup. Each species is written in a specific coloured font

Discussion

The analysis of nucleotide polymorphism of the *psbA–trnH* + *trnL–trnF* + *trnS–trnG* of cpDNA for *O. anadyrensis*, *O. borealis*, *O. middendorffii*, *O. trautvetteri*, and *O. vasskovskyi* sect. *Gloeocephala* from Northeast Asia revealed low levels of haplotype diversity and nucleotide diversity (Table 1). Comparisons of the current results with the previously obtained data for *Oxytropis* sect. *Orobia*, *Arctobia*, *Verticillares*, *Polyadena*, and *Xerobia* from Asian Russia (Table 5) showed that the populations of *Oxytropis* sect. *Orobia*, *Arctobia*, and *Gloeocephala* inhabited more high latitudes, or arctic latitudes, were characterised by a lower level of genetic diversity. This is likely largely due to the history of the range formation of almost entirely arctic sections, such as *Gloeocephala* and *Arctobia*. During repeated Pleistocene glaciation–interglacial cycles, northern populations had sharply reduced areas and effective number of population (bottleneck effect), while during recolonisation of postglacial territories, the populations were restored from preserved refugia (founder effect). Similar processes also influenced the structure of variability of other plant species inhabiting Northeast Asia. For example, the study of the phylogeography of *Alnus alnobetula* (Ehrh.) K. Koch. (Betulaceae) based on sequencing of IGS cpDNA (Hantemirova and Marchuk 2021) showed that eleven populations from Magadan Oblast, Kamchatskiy Krai, and Chukchi Autonomous District were monomorphic; of these, five populations from Kamchatskiy Krai had the same haplotype. According to the authors, this can be explained by the bottleneck effect during glaciations of the Kamchatka Peninsula. The presence of individual haplotypes in the populations of Chukotka and Magadan Oblast, and a high interpopulation differentiation indicated that, during climatic fluctuations in the Pleistocene, there were numerous refugia, and the geographic barriers between them prevented gene flow between populations, which resulted in the formation of morphologically distinct subspecies (Hantemirova and Marchuk 2021). Therefore, the same factors could have influenced the substantial intraspecific divergence of *O. anadyrensis*, *O. borealis*, and *O. middendorffii* that inhabited this territory.

It is also known that narrow endemic species show low level of genetic diversity. The values of haplotype diversity in the endemic *O. anadyrensis* are much lower (Table 1) than even in the populations of the narrow endemic *O. neimonggolica* C. W. Chang & Y. Z. Zhao (*h* varies from 0.250 to 0.679) inhabiting the north of China (Wang et al. 2021).

In addition to glaciers, other factors affect the level and distribution of variability, such as the lack of suitable habitats, and topographic and climatic barriers. As a result, disunited populations that existed in isolation for a long time show unusually high interpopulation differences. Thus, for *O. anadyrensis*, *O. borealis*, and *O. middendorffii*, the interpopulation differences accounted for 99–100% of the total variation (Table 3). Even closely spaced populations of *O. vasskovskyi* were rather highly differentiated, and the interpopulation differences accounted for more than 50% of the total variation (Table 3). A similarly high population differentiation (from 70 to 98%) was identified between isolated populations of *O. czukotica*, *O. exserta*, *O. kamtschatica*, *O. revoluta*, and *O. pumilio* sect. *Arctobia* (Kholina et al. 2020), between distant populations of *O. ochotensis* (87.03%), and between insular and continental populations of *O. ruthenica* (88.98%) sect. *Orobia* (Kozyrenko et al. 2020). At the same time, for most *Oxytropis* sect. *Verticillares*, *Polyadena*, and *Xerobia* from South Siberia, the interpopulation variability accounted for no more than 25% (Kholina et al. 2019, 2021a, 2021b), excluding populations of *O. caespitosa* sect. *Xerobia* (about 88%) (Kholina et al. 2021b) and *O. glandulosa* sect. *Polyadena* (about 75%) (Kholina et al. 2018b).

A similar level of interpopulation differentiation was also found in species of other genera. For example, analysis of cpDNA variability in populations of *Sophora linearifolia* Griseb. (Fabaceae) from Central Argentina (Alercia et al. 2017) showed that the differences between identified

Table 5 Genetic diversity of *Oxytropis* species of six sections from Asian Russia according to cpDNA data

Section (Number of studied species/populations/of which are monomorphic)	Diversity		References
	Haplotype diversity	Nucleotide diversity	
Northeast Asia			
Gloeocephala (5/7/4)	0.143–0.692	0.0001–0.0005	Present data
Orobia (5/12/2)	0.154–0.872	0.0002–0.0016	Kozyrenko et al. (2020)
Arctobia (9/15/2)	0.154–0.583	0.0002–0.0050	Kholina et al. (2020)
South Siberia			
Verticillares (6/13/0)	0.634–1.000	0.0003–0.0045	Kholina et al. (2019)
Polyadena (6/13/1)	0.133–0.911	0.0001–0.0059	Kholina et al. (2021a)
Xerobia (7/17/1)	0.327–1.000	0.0001–0.0090	Kholina et al. (2021b)

phylogroups accounted for 77.77% of the total variation, which was presumably due to geological and climatic processes in the studied region. For *Tugarinovia mongolica* Iljin (Asteraceae) growing in Inner Mongolia (Northwest China) (Zhao et al. 2019), according to IGS cpDNA sequencing data, in the southern group of populations, 100% of the variability belonged to the interpopulation component and could be due to habitat fragmentation resulting from climate fluctuations, lack of long-distance dispersal, and geographic isolation.

A high level of interpopulation differences in *O. anadyrensis*, *O. borealis*, and *O. middendorffii*, as well as a low and statistically insignificant level of genetic differences between all studied *Oxytropis* sect. *Gloeocephala* (Table 3) indicates that intensive microevolutionary processes are currently taking place in this section. This is supported by the fact that *O. anadyrensis*, *O. borealis*, and *O. middendorffii* each have two haplogroups, that have significantly diverged from each other (Table 3; Fig. 2), and each of these haplogroups is a separate lineage. The formation of cpDNA lineages in *O. anadyrensis* (haplogroup V, ANAD1 population from Kamchatskiy Krai and haplogroup VI, ANAD2–ANAD4 populations from Chukchi Autonomous District), separated by a distance of about 2000 km, and in *O. middendorffii* (haplogroup VIII, MID1 population from Kamchatskiy Krai and haplogroup VII, MID2–MID5 populations from Taimyr Peninsula), separated by a distance of about 4000 km (Figs. 1 and 2), is caused by the isolation by distance and the absence of gene exchange between them. In *O. borealis*, the distance between two cpDNA lineages (haplogroup III, BOR2 population from Chukchi Autonomous District and haplogroup IV, BOR1 and BOR3 populations from Chukchi Autonomous District) was about 200 km (Figs. 1 and 2); however, natural geographic barriers could also restrict gene flow. The presence of several cpDNA lineages within a species was also revealed earlier in *Oxytropis* species from other sections. For example, in *O. glandulosa*, the derivation of two main independent lineages was due to topographic barriers between populations and the difference in the ploidy level (Kholina et al. 2018b); in *O. ruthenica*, the formation of lineages was caused by the historical isolation of insular populations (Kozyrenko et al. 2020). Thus, the formation of cpDNA lineages in *Oxytropis*, including the species of sect. *Gloeocephala* in Northeast Asia indicates that diversification processes are currently active.

The identified species-specific marker in the *trnS–trnG* IGS cpDNA in *O. trautvetteri* supports the opinion of a number of botanists (Vassilchenko and Fedchenko 1948; Voroshilov 1982; Pavlova 1989) that this taxon is an independent species. Regarding the relationship between *O. anadyrensis* and *O. middendorffii*, our data (the level of nucleotide divergence, which turned out to be higher than that between the "good" species *O. middendorffii–O. vasskovskyi*, *O. borealis–O. trautvetteri*, and *O. borealis–O. vasskovskyi* (Table 4), the absence of shared chlorotypes, and the presence of specific molecular markers in cpDNA lineages) indicate the status of *O. anadyrensis* as an independent species, which is consistent with the existing opinion (Vassilchenko and Fedchenko 1948; Pavlova 1989; Yakubov 2014).

The intraspecific ITS nrDNA polymorphism in *O. anadyrensis*, *O. borealis*, and *O. middendorffi* apparently reflects the allopolyploid origin of these hexaploids (2n = 48) because of hybridisation of polymorphic diploid ancestors. In addition, the shared ribotype RGL1 in *O. anadyrensis* and *O. middendorffii*, as well as the shared ribotype RGL2 in *O. anadyrensis* and *O. vasskovskyi*, may be partly due to the putative origin of *O. anadyrensis*: Yurtsev (1986) suggested that this species originated through ancient hybridisation between *O. middendorffii* and *O. vasskovskyi*.

A combination of a few nrDNA ribotypes and a wide variety of cpDNA chlorotypes, as revealed in *Oxytropis* sect. *Gloeocephala* (five species, five ribotypes and 20 chlorotypes), was also found in species of other sections. For example, for five species of the sect. *Orobia*, six ribotypes and 39 chlorotypes were identified (Kozyrenko et al. 2020), for nine species of the sect. *Arctobia*, ten ribotypes and 55 chlorotypes were identified (Kholina et al. 2020), and for seven species of the sect. *Xerobia*, two ribotypes and 67 chlorotypes were identified (Kholina et al., 2021b). It is likely that the reasons for this are the common origin, relatively recent divergence, and rapid adaptive radiation of the species. Based on the analysis of nuclear and chloroplast markers, it was found that *Oxytropis* species are characterised by rapid radiation (Shavvon et al. 2017), similar to other genera of the family Fabaceae, for example, *Lupinus* (Drummond et al. 2012) and *Astragalus* (Bagheri et al. 2017). This rapid radiation can be accompanied by rapid isolation, as has been shown for the species of *Indigofera bungeana* complex (Fabaceae) (Zhao et al. 2017), and is also highly probable for *Oxytropis* sect. *Gloeocephala*.

Thus, the analysis of nucleotide polymorphism of the *psbA–trnH* + *trnL–trnF* + *trnS–trnG* IGS cpDNA for *Oxytropis* sect. *Gloeocephala* from Northeast Asia confirmed the status of *O. anadyrensis* and *O. trautvetteri* taxa as separate species. Our data suggest that, at present, in *O. anadyrensis*, *O. borealis*, and *O. middendorffii*, intensive microevolutionary processes are occurring, resulting in the formation of separate cpDNA lineages. Northeast Asia characterized by high species richness of *Oxytropis* (Sandanov et al. 2022). These results confirm Yurtsev's hypothesis (Yurtsev 1999) that the Asian sector of Megaberingia is the main centre for the diversity and diversification of Arctic legumes.

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Declarations

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

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