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PLANT GENETICS

The Evolutionary Pathways of *Oxytropis* Species of the Section *Verticillares* at the Center of the Section Origin

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Abstract—Genetic diversity and phylogenetic relationships of *Oxytropis* species from the section *Verticillares* were studied on the basis of nucleotide polymorphism of cpDNA intergenic spacers *psbA-trnH*, *trnL-trnF*, and *trnS-trnG* at the center of the section origin (Baikal Siberia and adjacent territories of Southern Siberia, Mongolia, and China). Moreover, for the first time, the reconstruction of phylogenetic relationships of species from the section *Verticillares* based on the analysis of ITS nrDNA has been performed. The article summarizes new material on the topic under study and data for a number of previously unstudied species and populations; 84.4% of populations of 11 species are characterized by a high level of chloroplast haplotype diversity (*h* varies from 0.700 to 1.000). The majority of populations (71.9%) have high haplotype diversity with low nucleotide diversity. Three haplogroups revealed in the genealogical network of chlorotypes indicate that there are different evolutionary pathways of the species included in these groups: divergence of genetically isolated taxa in the zone of sympatry presumably on the basis of ecological specialization; incomplete sorting of lineages with conservation of ancestral polymorphism in combination with hybridization of weakly diversified taxa; allopatric divergence and polyploidization. The analyses of chloroplast and nuclear genome markers indicate the rapid adaptive radiation of *Oxytropis* species from the section *Verticillares*.

Keywords: genetic diversity, phylogenetic relationships, chloroplast DNA, ITS nrDNA, Fabaceae, *Oxytropis*, *Verticillares*

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INTRODUCTION

The genus *Oxytropis* DC. of the family Fabaceae includes from 310 [1] to 450 species [2], which mostly grow in Asia. It is assumed that the genus evolved from ancient species of the genus *Astragalus* L. at the Miocene—Pliocene boundary about 5.6 million years ago in the mountains of Southern Siberia [3, 4]. Later, the secondary centers of speciation arose, the largest of which was Baikal Siberia, where a complex of new species was formed on the basis of migrants from Western Siberia [3].

Baikal Siberia is located in the south of Eastern Siberia, adjacent to Lake Baikal and covers the Baikal region, Western Transbaikalia, the eastern part of the Eastern Sayan, and the Vitim Plateau [5]. The Baikal speciation center is unique and rich in the species composition of flora, which is due to orography and climate features of this region, as well as its buffer position between North and Central Asia [6, 7]. It is Baikal Siberia with the adjacent territories of Northern Mongolia and China that is considered the homeland of *Oxytropis* species of the section *Verticillares* DC. (=*Baicalia* Bunge) [8] and the main arena of the evolution of the section [9]. The two names of the section are due to the fact that at first botanists did not use the term "section" and, thus, in 1825 Decandol classified the species with verticillate disposition of leaflets into a separate group (*Verticillares*) [10]. The monographer of the *Oxytropis* genus Alexander Bunge [11] divided the group *Verticillares* DC. in two natural sections, *Baicalia* Bunge, which included nonfragrant plants that do not have glands, and *Polyadena* Bunge, i.e., fragrant plants with many glands. M.G. Popov [12] conferred the rank of section to the *Verticillares* group and gave the name *Verticillares* DC. instead of *Baicalia* for the sake of historical justice [10]. Taking into account the ecology and modern distribution of the section species, it was suggested that the section separated at the beginning of the Pleistocene, i.e., about 2.5 million years ago [8].

The *Verticillares* section contains about 50 species, including 25 in Asian Russia [13], 23 in Siberia [14], 22 in Mongolia [15], and 15 in China [1]. The species of this section have two main distribution centers: (1) Southern Siberia, Mongolia, and Northeastern China; (2) the Asian Arctic, with the ranges of Arctic species being more fragmented [16]. The section consists of 17 subsections, ten of which are monotypic [9, 10, 17]. The large number of subsections for the group of 50 species indicates a high phylogenetic differentiation [10]. A phenetic analysis of species based on 54 diagnostic morphological features revealed a partial discrepancy with the arrangement of species by subsections, as well as a number of other contradictions [10].

A total of 13 species grow in the center of origin of the section in Baikal Siberia: *Oxytropis bargusinensis* Peschkova, *O. heterotricha* Turcz., *O. interposita* Sipl., *O. lanata* (Pall.) DC., *O. lasiopoda* Bunge, *O. myriophylla* (Pall.) DC., *O. oxyphylla* (Pall.) DC., *O. oxyphylloides* M. Pop., *O. prostrata* (Pall.) DC., *O. selengensis* Bunge, *O. stukovii* Palibin, *O. tompudae* M. Pop., and *O. turczaninovii* Jurtzev [7]. Of these representatives, *O. heterotricha* and *O. oxyphylloides* are mountain species, *O. tompudae* is a forest species, *O. interposita* is azonal, and the rest are steppe species [7]. The chromosome numbers are known for nine out of the 13 species [13, 18, 19]. Most of the species in the place of origin of the section have a diploid chromosome number ($2n = 16$), similar to the related species of the section *Verticillares* from adjacent areas of Southern Siberia, Mongolia, and China, while Arctic species from the subsection *Inaequiseptatae* Jurtz. are polyploids ($2n = 32, 48, 64$) (Table 1), indicating indirectly that the species of Baikal Siberia are more ancient [13]. *O. turczaninovii* exhibits karyological polymorphism, and diploid and polyploid races are found in natural populations ($2n = 16, 32$) [13, 18].

The species *O. stukovii* and *O. tompudae* are rare species with a narrow ecological niche and are listed in *The Red Book of Zabaykalsky Krai* (2017); *O. tompudae*, *O. bargusinensis*, and *O. oxyphylloides* are endemic to Baikal Siberia and are listed in *The Red Book of the Irkutsk Oblast* (2020). *O. lanata* has a rather extensive but disjunctive range; the species is ecologically confined to sandy habitats, the destruction of which threatens the existence of populations; and hence it was included in *The Red Book of Zabaykalsky Krai* (2017).

Molecular markers of the nuclear and chloroplast genomes are popular in molecular biology to assess genetic variation, population structure, divergence of species, and phylogenetic relationships. The reconstruction of phylogenetic relationships of the *Oxytropis* species, including species of the section *Verticillares* by means of sequencing the ITS region (ITS1–5.8S rDNA–ITS2) of the ribosomal operon of nuclear DNA (rDNA) [20, 21] and ITS rDNA + *trnL–trnF* of chloroplast DNA (cpDNA) [4], showed that relationships between species even at the level of sections remained unresolved. Our earlier studies of species of the genus *Oxytropis* using intergenic spacers (IGS) *psbA–trnH* + *trnL–trnF* + *trnS–trnG* of cpDNA showed that the Southern Siberian species of the section *Verticillares* form a separate monophyletic group in the haplotype network [22]. For a range of species of the section *Verticillares* in the steppe flora of Baikal Siberia [23], data on the divergence degree of their chloroplast genomes were obtained. Next, the genetic diversity and population structure of medicinal species *O. lanata*, *O. myriophylla*, and *O. oxyphylla* of Baikal Siberia have been studied quite well [24].

This paper studies the genetic diversity and clarifies phylogenetic relationships between species of the section *Verticillares*. A large amount of material was additionally collected for different populations and species growing in Baikal Siberia, and also the Arctic species of the section from the subsections *Inaequiseptatae* and *Uniflorae* Jurtz. were also included in the analysis. On the basis of the previous data [23, 25], *O. interposita* was excluded from the analysis because of the significant isolation of the chloroplast genome of this species from the species of the section *Verticillares* and the genetic proximity of *O. interposita* to the species of the section *Polyadena* [25]. The purpose of this study is to assess the genetic diversity of *Oxytropis* species of the section *Verticillares* in the center of the section origin and to reconstruct phylogenetic relationships on the basis of the analysis of the nucleotide polymorphism of IGS *psbA–trnH* + *trnL–trnF* + *trnS–trnG* cpDNA and ITS rDNA.

MATERIALS AND METHODS

The study material included 327 plants from 45 natural habitats of 19 species from seven subsections of the section *Verticillares* (Table 1, Fig. 1). The species of Southern Siberia and adjacent territories included subsection *Oxyphylliformes* Jurtz.—*O. bargusinensis* (30 specimens), *O. ochrantha* (5), *O. oxyphylla* (75), *O. pumila* (1), *O. reverdattoi* (2), *O. selengensis* (18), *O. tompudae* (8), *O. turczaninovii* (14); subsection *Salinae* Jurtz.—*O. mongolica* (1), *O. prostrata* (30), *O. stukovii* (14); subsection *Lanatae* Jurtz.—*O. lanata* (61); subsection *Myriophyllae* Jurtz.—*O. myriophylla* (54); subsection *Gobicola* (Bunge) Malysch.—*O. gracillima* (5); Arctic species included subsection *Inaequiseptatae* Jurtz.—*O. scheludjakovae* (3), *O. schmorgunoviae* (1), *O. sverdrupii* (1), *O. wrangelii* (4); subsection *Uniflorae* Jurtz.—*O. putoranica* (1). We studied previously several populations of some species [22, 23]. The names of the species and subsections are given according to L.I. Malyshev [10].

The methods of DNA isolation, amplification, and sequencing of IGS *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* are described in our previous works [26, 27]. The ITS rDNA was amplified with the ITS1 and ITS4 primers under the reaction conditions and temperature regime given in [28]. The nucleotide sequences of forward and reverse chains were determined using an ABI 3500 genetic analyzer (Applied Biosystems, United States) at the Joint-Use Center "Biotechnology and Genetic Engineering" (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far

Table 1. The studied populations of 19 *Oxytropis* species from the section *Verticillares*, place of growth, sample size, and codes

Species, number of chromosomes (reference), place of growth (number of specimens)	Coordinates lat. N; long. E	Population code
Species of Southern Siberia		
Subsection *Oxyphylliformes* Jurtz.		
O. bargusinensis, 2*n*—no data		
1. Republic of Buryatia, Valley of Alla River (15)	54°43′12″; 110°40′48″	BARKUR
2. Republic of Buryatia, near the village Urzhil (15)	54°3′36″; 110°21′36″	BARBAR
O. ochrantha, 2*n* = 16 [65]		
1. China, Inner Mongolia, south of Shilin-Hot (5)	43°43′50; 116°6′36″	**OCHR**
O. oxyphylla, 2*n* = 16 [13]		
1. Zabaikalsky krai, near Lake Nozhii (5)	50°47′24″; 114°47′24″	OXY1
2. Republic of Buryatia, near the village Novoselenginsk (16)	51°4′12″; 106°36′0″	OXY2
3. Republic of Buryatia, near the village Komsomol’skoye (8)	52°28′48″; 111°5′24″	OXY3
4. Mongolia, Bulgan Aimag, near the Rashaant Sum (5)	47°24′36″; 103°39′36″	OXY4
5. Republic of Buryatia, near the village Maly Kunaley (10)	50°36′36″; 107°49′48″	**OXY5**
6. Republic of Buryatia, near the village Mondy (10)	51°41′24″; 100°54′0″	**OXY6**
7. Zabaykalsky krai, near the village Domna (11)	51°49′48″; 113°5′24″	**OXY7**
8. Irkutsk oblast, near the village Usury (10)	53°49′48″; 107°5′24″	**OXY8**
O. pumila, 2*n* = 16 [13]		
1. Altai Republic, Chuiskaya Steppe (1)	49°30′0″; 88°19′48″	PUM
O. reverdattoi, 2*n* = 16 [13]		
1. Republic of Khakassia, near the village Vesennee (1)	54°34′48″; 90°40′48″	**REVER1**
2. Republic of Khakassia, near the village Borets (1)	54°28′12″; 90°21′36″	**REVER2**
O. selengensis, 2*n* = 32 [13]		
1. Republic of Buryatia, near the village Unegetei (18)	52°10′12″; 108°36′0″	SEL
O. tompudae, 2*n*—no data		
1. Republic of Buryatia, near the village Maisk (8)	54°36′0″; 110°46′48″	TOMP
O. turczaninovii, 2*n* = 16, 32 [13, 18]		
1. Republic of Buryatia, near the village Dyrestuy (5)	50°38′24″; 106°0′36″	**TURCZ1**
2. Republic of Buryatia, near the village Udinsk (9)	52°7′12″; 109°9′0″	**TURCZ2**
Subsection *Salinae* Jurtz.		
O. mongolica, 2*n*—no data		
1. Republic of Tuva, the western shore of Lake Khadan (1)	51°19′12″; 94°28′48″	MONG
O. prostrata, 2*n*—no data		
1. Zabaykalsky krai, near Lake Zun-Torey (12)	50°7′48″; 115°42′36″	**PROST1**
2. Zabaykalsky krai, near Lake Barun-Torey (10)	50°0′0″; 115°43′12″	**PROST2**
3. Zabaykalsky krai, near Lake Kunkur (8)	50°42′36″; 114°53′24″	**PROST3**
O. stukovii, 2*n* = 16 [19]		
1. Zabaykalsky krai, near Lake Nozhii (4)	50°48′36″; 114°50′24″	STUK1
2. Zabaykalsky krai, near Lake Kunkur (10)	50°42′36″; 114°53′24″	**STUK2**
Subsection *Lanatae* Jurtz.		
O. lanata, 2*n* = 16 [13]		
1. Republic of Buryatia, near the village Unegetei (20)	52°10′12″; 108°36′0″	LAN1
2. Republic of Buryatia, near the village Urzhil (15)	54°3′36″; 110°21′36″	LAN2
3. Mongolia, Central Aimag, near the Argalant sum (5)	47°49′48″; 105°52′48″	LAN3
4. Irkutsk oblast, Olkhon Island, Nyurgonskaya Bay (10)	53°16′48″; 107°34′48″	**LAN4**

Table 1. (Contd.)

Species, number of chromosomes (reference), place of growth (number of specimens)	Coordinates lat. N; long. E	Population code
5. Republic of Buryatia, near the village Tunka (11)	51°44′24″; 102°27′36″	**LAN5**
Subsection *Myriophyllae* Jurtz.		
O. myriophylla, $2n = 16$ [13]		
1. Zabaykalsky krai, near the village Tsugol (5)	51°1′12″; 115°36′0″	MYR1
2. Republic of Buryatia, near the village Udinsk (21)	52°7′12″; 109°9′0″	MYR2
3. Republic of Buryatia, near the village Komsomol'skoye (8)	52°28′48″; 11°5′24″	MYR3
4. Republic of Buryatia, near the village Mukhor-Konduy (6)	52°29′24″; 113°17′24″	**MYR4**
5. Republic of Buryatia, near the village Bichura (8)	50°37′48″; 107°40′12″	**MYR5**
6. Zabaykalsky krai, near the village Soktuy-Milozan (6)	50°37′48″; 117°40′12″	**MYR6**
Subsection *Gobicola* (Bunge) Malysch.		
O. gracillima, $2n = 16$ [65]		
1. Mongolia, Central Aimag, near the Argalant sum (5)	47°49′48″; 105°52′48″	GRAC
Arctic species		
Subsection *Inaequiseptatae* Jurtz.		
O. scheludjakovae, $2n = 16, 32, 48$ [13]		
1. Magadan oblast, road to Merenga village. MHA* (1)	62°3′36″; 156°4′48″	SCHEL1
2. Magadan oblast, Zamkovaya Mountain. MAG* (1)	63°21′0″; 152°37′48″	**SCHEL2**
3. Yakutia, Yakutsk, Botanical Garden of the North-Eastern Federal University (1)	62°0′36″; 129°42′0″	**SCHEL3**
O. schmorgunoviae, $2n = 48$ [13]		
1. Magadan oblast, 70 km below the Seymchan, left bank of the Kolyma River. MAG* (1)	62°37′48″; 151°7′48″	**SCHMOR**
O. sverdrupii, $2n = 48$ [13]		
1. Western Chukotka, Ayon Island, the Utatgyr River. LE* (1)	69°53′24″; 168°1′12″	SVERD
O. wrangelii, $2n = 64$ [13]		
1. Magadan oblast, near the Gulf of Cragin. MAG* (1)	69°47′24″; 174°46′12″	**WRANG1**
2. Chukotka Autonomous Okrug, Wrangel Island, Somnitel'naya Bay. MAG* (2)	71°6′0″; –179°37′12″	**WRANG2**
3. Chukotka Autonomous Okrug, Wrangel Island, Gusinaya River. MAG* (1)	70°56′24″; –179°37′12″	**WRANG3**
Subsection *Uniflorae* Jurtz.		
O. putoranica, $2n = 16$ [13]		
1. Southwest Taymyr, Putorana Plateau, near Lake Ayan. MW* (1)	69°20′24″; 93°33′36″	**PUTOR**

The populations studied in this work are in bold. * Herbarium acronym: LE—Vascular Plants Herbarium of the Komarov Botanical Institute, St. Petersburg; MAG—Herbarium of the Institute of Biological Problems of the North, Magadan; MHA—Herbarium of the Main Botanical Garden, Moscow; MW—Herbarium of the Moscow State University, Moscow.

Eastern Branch, Russian Academy of Sciences). The DNA sequences of each region were assembled and edited using the Staden Package v1.5 software [29], then aligned in SeaView v4.7 [30], and combined by DnaSP v. 5.0 [31]. A matrix of combined sequences of three cpDNA regions was used to estimate the number of haplotypes and haplotype (h) and nucleotide (π) diversity (for populations with five or more specimens) in the Arlequin v. 3.5 software [32]. The genealogical haplotype networks were constructed in the Network v5.0 software [33] using the Median Joining (MJ) algorithm, by encoding each deletion/insertion, regardless of its size, as a single mutational event. The *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* nucleotide sequences of cpDNA (GenBank accession numbers LT856572, LT856585, and LT856598, respectively) and ITS rDNA (LR898464) that we obtained earlier for *O. glabra* (Lam.) DC. from the section *Mesogaea* Bunge of the subgenus *Phacoxytropis* Bunge [26, 34] were used as the outgroup.

RESULTS

The nucleotide sequences of IGS *psbA–trnH*, *trnL–trnF*, and *trnS–trnG* cpDNA were obtained for 151 specimens from populations of species *O. ochran-*

Fig. 1. The map with sampling points of the *Oxytropis* section *Verticillares* from 45 natural habitats. For population codes, see Table 1. The ranges of the studied species are given in [66].

tha (OCHR), *O. oxyphylla* (OXY5–OXY8), *O. reverdattoi* (REVER1, REVER2), *O. turczaninovii* (TURCZ1, TURCZ2), *O. prostrata* (PROST1–PROST3), *O. stukovii* (STUK2), *O. lanata* (LAN4, LAN5), *O. myriophylla* (MYR4–MYR6), *O. scheludjakovae* (SCHEL2, SCHEL3), *O. wrangelii* (WRANG1–WRANG3), and *O. putoranica* (PUTOR). The sequences of previously identified haplotypes (chlorotypes) of the section *Verticillares* species [22, 23] were added to the combined matrix of three regions; the length of the combined matrix after alignment was 2546 sites. A total of 46 polymorphic sites were found, of which 18 were parsimony informative and 28 were singleton sites. A total of 137 chlorotypes were identified, 54 of them were new, and their sequences were deposited in DDBJ/ENA/Genbank-INSDC (Table 2). Of the 19 studied species, only three had the species-specific nucleotide substitutions and inserts: *O. lanata* had A at position 1223 of the combined matrix (IGS *trnL–trnF*) and C at position 2228 (*trnS–trnG*); *O. ochrantha* carried insertion of five nucleotides (GTATT) at position 418–422 (*psbA–trnH*); *O. wrangelii* had insertion of nine nucleotides (GAAGAAAAT) at position 349–357 (*psbA–trnH*).

Table 2 presents data on the genetic diversity of 32 populations of 11 species from the section *Verticillares*.

Table 2. The genetic diversity of *Oxytropis* section *Verticillares* and the accession numbers for the nucleotide sequences *psbA*–*trnH*, *trnL*–*trnF*, and *trnS*–*trnG* cpDNA in DDBJ/ENA/Genbank-INSDC

Population code (reference)	Genetic diversity		Accession number in DDBJ/ENA/Genbank-INSDC		
	haplotype (SD)	nucleotide (SD)	*psbA*–*trnH*	*trnL*–*trnF*	*trnS*–*trnG*
BARKUR [34]	0.762 (0.096)	0.0011 (0.0007)	LT856473–LT856488	LT856506–LT856521	LT856539–LT856554
BARBAR [34]	0.895 (0.070)	0.0016 (0.0009)			
OCHR	**0.700 (0.218)**	**0.0007 (0.0005)**	**OQ622432–OQ622434**	**OQ604977–OQ604979**	**OQ605014–OQ605016**
OXY1 [23]	0.900 (0.161)	0.0007 (0.0005)	LT994867–LT994891; **OQ622410–OQ622431**	LT994921–LT994945; **OQ604955–OQ604976**	LT996010–LT996034; **OQ604992–OQ605013**
OXY2 [23]	0.958 (0.036)	0.0023 (0.0013)			
OXY3 [23]	0.964 (0.077)	0.0045 (0.0026)			
OXY4 [23]	1.000 (0.177)	0.0042 (0.0029)			
OXY5	**0.956 (0.059)**	**0.0020 (0.0012)**			
OXY6	**0.533 (0.180)**	**0.0005 (0.0004)**			
OXY7	**0.800 (0.114)**	**0.0006 (0.0005)**			
OXY8	**0.978 (0.054)**	**0.0035 (0.0020)**			
PUM [23]	–	–	MH174939	LT996251	LT996252
REVER1	–	–	**OQ622459; OQ622460**	**OQ604909; OQ604910**	**OQ604938; OQ604939**
REVER2	–	–			
SEL [23]	0.634 (0.127)	0.0009 (0.0006)	MH174937; LT996052–LT996057	LT996045–LT996051	LT996038–LT996044
TOMP [34]	0.893 (0.111)	0.0026 (0.0016)	LT856566–LT856571	LT856579–LT856584	LT856592–LT856597
TURCZ1	**0.700 (0.218)**	**0.0003 (0.0003)**	**OQ622437–OQ622441**	**OQ604887–OQ604891**	**OQ604916–OQ604920**
TURCZ2	**0.583 (0.183)**	**0.0006 (0.0005)**			
MONG [22]	–	–	LN898501	LN898613	LN898625
PROST1	**0.894 (0.063)**	**0.0014 (0.0009)**	**OQ622442–OQ622458**	**OQ604892–OQ604908**	**OQ604921–OQ604937**
PROST2	**0.956 (0.059)**	**0.0020 (0.0012)**			
PROST3	**0.964 (0.077)**	**0.0010 (0.0007)**			
STUK1 [23]	0.833 (0.222)	0.0005 (0.0005)	LT994892–LT994894	LT994946–LT994948	LT996035–LT996037
STUK2	**0.711 (0.117)**	**0.0004 (0.0003)**			

Table 2. (Contd.)

Population code (reference)	Genetic diversity		Accession number in DDBJ/ENA/Genbank-INSDC		
	haplotype (SD)	nucleotide (SD)	*psbA–trnH*	*trnL–trnF*	*trnS–trnG*
LAN1 [23]	0.895 (0.051)	0.0010 (0.0006)	LT994841–LT994858; **OQ622400–OQ622402**	LT994895–LT994912; **OQ604945–OQ604947**	LT994949–LT994966; **OQ604982–OQ604984**
LAN2 [23]	0.895 (0.052)	0.0010 (0.0006)			
LAN3 [23]	0.900 (0.161)	0.0017 (0.0012)			
LAN4	**0.378 (0.181)**	**0.0003 (0.0002)**			
LAN5	**0.545 (0.072)**	**0.0029 (0.0017)**			
MYR1 [23]	0.700 (0.218)	0.0003 (0.0003)	LT994859–LT994866; **OQ622403–OQ622409**	LT994913–LT994920; **OQ604948–OQ604954**	LT996002–LT996009; **OQ604985–OQ604991**
MYR2 [23]	0.767 (0.057)	0.0007 (0.0005)			
MYR3 [23]	0.821 (0.101)	0.0008 (0.0005)			
MYR4	**0.867 (0.129)**	**0.0005 (0.0004)**			
MYR5	**0.857 (0.108)**	**0.0014 (0.0009)**			
MYR6	**0.733 (0.155)**	**0.0006 (0.0005)**			
GRAC [23]	1.000 (0.126)	0.0042 (0.0027)	MH174938, LT996058–LT996061	LT996062–LT996066	LT996067–LT996071
SCHEL1 [22]	–	–	LN898574; **OQ622437; OQ622438**	LN898536; **OQ604980; OQ604981**	LN898648; **OQ605017; OQ605018**
SCHEL2	–	–			
SCHEL3	–	–			
SCHMOR	–	–	**OQ622461**	**OQ604911**	**OQ604940**
SVERD [22]	–	–	LN898508	LN898620	LN898632
WRANG1	–	–	**OQ622463–OQ622465**	**OQ604913–OQ604915**	**OQ604942–OQ604944**
WRANG2	–	–			
WRANG3	–	–			
PUTOR	–	–	**OQ622462**	**OQ604912**	**OQ604941**

SD—standard deviation. Bold type indicates the populations whose data on the genetic diversity and accession numbers were obtained in this work. (–) The genetic parameters were not estimated owing to the small sample size (less than five specimens).

High values of haplotype diversity (0.700 to 1.000) were revealed for 27 populations (84.4%), and low values of nucleotide diversity (from 0.0003 to 0.0029) were found in 28 populations (87.5%). A combination of the high haplotype diversity and medium nucleotide diversity was found in OXY3, OXY4, OXY8, and GRAC populations, and medium haplotype and low nucleotide diversity was observed in OXY6, SEL, TURCZ2, and LAN5 populations (12.5%, respectively). Only the LAN4 *O. lanata* population from the Olkhon Island of Irkutsk oblast is characterized by low haplotype diversity ($h = 0.378$). Thus, the majority of the studied populations (23 populations, 71.9%) of the *Verticillares* section species exhibit a high level of haplotype and low level of nucleotide diversity.

To reconstruct the genealogical relationships between chlorotypes, the *O. glabra* sequences (LT856572, LT856585, LT856598) were added to the combined matrix as the outgroup and the length polymorphism of mono- and dinucleotide repeats was removed. A total of 55 chlorotypes (H1–H55) were identified, and only two (H14 and H36) were shared by several species: the H14 chlorotype was found in seven species (*O. oxyphylla*, *O. selengensis*, *O. stukovii*, *O. bargusinensis*, *O. reverdattoi*, *O. turczaninovii*, and *O. prostrata*); and H36 was found in two (*O. bargusinensis* and *O. tompudae*). The relationship between the chlorotypes is shown in Fig. 2a. All chlorotypes of the 19 species of the *Verticillares* section are connected within a single network, which includes three haplogroups (I, II, III), each of which contains a star-like structure with one of the chlorotypes at the center. Haplogroup I is central in the network, and haplogroups II and III are terminal. Haplogroup I is formed by the chlorotypes of clearly separated species *O. ochrantha*, *O. mongolica*, *O. lanata*, *O. gracillima*, and *O. myriophylla* with the chlorotype H11 of *O. myriophylla* at the center. Furthermore, the H12 chlorotype of *O. myriophylla* is connected via many mutational steps with the chlorotype of *O. glabra*. Haplogroup II includes chlorotypes of closely related species *O. bargusinensis*, *O. oxyphylla*, *O. pumila*, *O. reverdattoi*, *O. selengensis*, *O. tompudae*, *O. turczaninovii*, *O. prostrata*, and *O. stukovii* with the H14 chlorotype shared by seven species at the center. Haplogroup III combines the H29 chlorotype of *O. oxyphylla* and all the chlorotypes of Arctic species: *O. scheludjakovae*, *O. schmorgunoviae*, *O. sverdrupii*, *O. putoranica,* and *O. wrangelii*, which originated from the central hypothetical (extinct, or not identified in this sample) chlorotype (Fig. 2a). In haplogroups I and II, alternative connections (loop structures) between chlorotypes were identified, and hence the relationships between populations of each species cannot be established unambiguously. In addition, in haplogroups I and II, except for *O. mongolica*, neighboring chlorotypes are mainly connected by 1–2 mutational transitions, while in haplogroup III some chlorotypes are 4–5 mutational steps apart (Fig. 2a). Chlorotypes of *O. oxyphylla* were found in two haplogroups: H14–H28 as part of haplogroup II and chlorotype H29 of the OXY8 population (9 specimens out of 10 studied) in haplogroup III, indicating the existence of two phyletic lineages. There is no clear geographical pattern in the distribution of chlorotypes in haplogroups, with the exception of a separate group of chlorotypes in Arctic species.

The ITS rDNA nucleotide sequences from 137 specimens that include all the chlorotypes of cpDNA identified in this study of all the studied *Oxytropis* species, except for *O. pumila* owing to the lack of material, are characterized by the same length (603 bp) and low/medium nucleotide variation. The sequence sizes of ITS1, 5.8S rDNA gene, and ITS2 were 227, 164, and 212 bp, respectively. Out of 603 sites, 20 were variable, of which 17 were parsimony informative: eight substitutions (positions 28, 73, 119, 122, 166, 175, 178, and 223) in ITS1 and nine (positions 405, 415, 427, 458, 485, 531, 538, 548, and 549) in ITS2. In 137 sequences, 17 ribotypes (RH1–RH17) were identified, the sequences of which are deposited in DDBJ/ENA/Genbank-INSDC (Table 3). The network of genealogical relationships of ribotypes (Fig. 2b) reveals a well-defined star-like structure with the RH3 ribotype at the center, which is shared by six species of Southern Siberia: *O. lanata*, *O. oxyphylla*, *O. selengensis*, *O. turczaninovii*, *O. tompudae*, and *O. stukovii*. In addition, the following species have shared ribotypes: *O. lanata* and *O. bargusinensis* (RH1); *O. ochrantha*, *O. stukovii*, and *O. oxyphylla* (RH2); *O. myriophylla* and *O. oxyphylla* (RH4). All Arctic species of the section *Verticillares* have individual ribotypes (Fig. 2b).

DISCUSSION

The section *Verticillares* is a quite well isolated group of species from the genus *Oxytropis*. The section presumably emerged from the macromutant that turned out to acquire advantageous mutations and gave rise to new taxa [3]. The formation of new species took place in a region with a numerous variation of relief, including submontane depressions, flat uplands, chain of mountains, and intermountain basins, the flora of which forms “steppe islands.” The heterogeneous relief and various climatic conditions triggered evolutionary transformations of the species in this group, their diversification, and emergence of complex relationships, which was shown earlier when studying the morphological diversity of the species of the section *Verticillares* [9, 10].

Our analysis of the species from the section *Verticillares* using cpDNA data reflects a high level of species diversity in the center of formation of the section and a complex picture of their phylogenetic relationships. Thus, out of 32 studied populations, no monomorphic population was found. The populations of both quite widespread species (*O. oxyphylla* and *O. lanata*) and narrow endemic species (*O. bargusinensis* and *O. tom-*

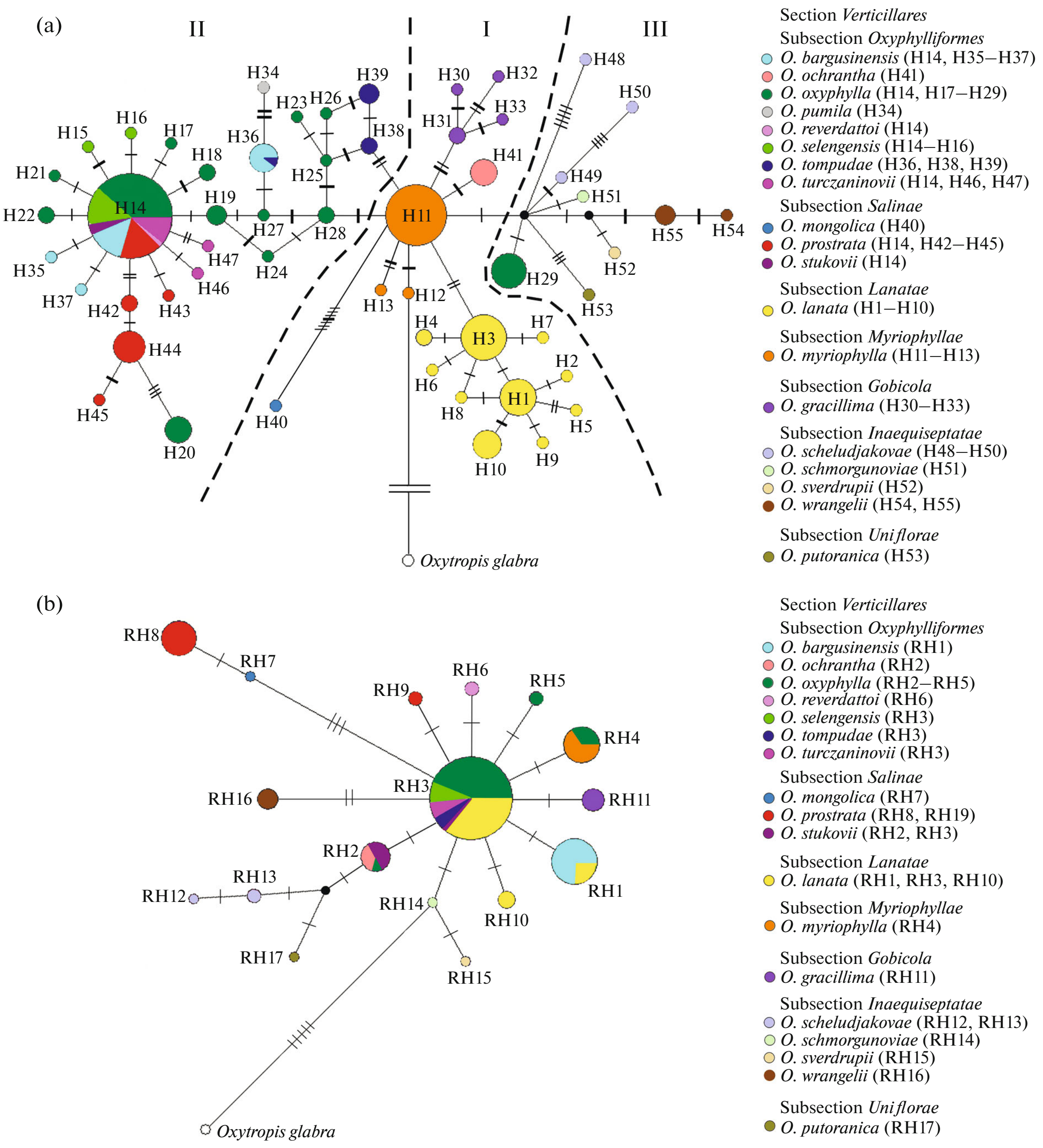

Fig. 2. The genealogical relationships between haplotypes of the *Oxytropis* section *Verticillares* reconstructed using the MJ algorithm: (a) genealogical network of cpDNA chlorotypes (H1–H55); (b) genealogical network of ITS rDNA ribotypes (RH1–RH17). The size of circles indicates the occurrence frequency of chlorotypes and ribotypes, small black circles are hypothetical chlorotypes and ribotypes, thin intersecting lines on the branches are mutational events, and thick black intersecting lines are indels. Dashed line separates the chlorotype network into haplogroups I, II, and III. Mutations for *O. glabra* used as outgroup are not shown and are not considered.

pudae) are highly polymorphic. The majority of the studied populations (84.4%), except for the isolated island population LAN4 of *O. lanata*, exhibit a high level of haplotype diversity (Table. 2), similar to populations of *Oxytropis* species from the sections *Polyadena* [25] and *Xerobia* [35] in Baikal Siberia (maximum values $h = 0.911$ and $h = 1.000$, respectively). The nucleotide diversity in the populations of *O. ochrantha* and *O. myriophylla* species located closer to the center of origin of the section (Southern Siberia) was 0.0007 and 0.0003–0.0014, respectively (Table 2), while in the populations of the same species located in

Table 3. Ribotypes of ITS rDNA for the *Oxytropis* section *Verticillares* and accession numbers for the nucleotide sequences in DDBJ/ENA/Genbank-INSDC

Species	Ribotype	Accession number in DDBJ/ENA/Genbank-INSDC
O. bargusinensis	RH1	OQ625437
O. ochrantha	RH2	OQ625441
O. oxyphylla	RH2, RH3, RH4, RH5	OQ625442–OQ625445
O. reverdattoi	RH6	OQ625452
O. selengensis	RH3	OQ625448
O. tompudae	RH3	OQ625449
O. turczaninovii	RH3	OQ625450
O. mongolica	RH7	OQ625453
O. prostrata	RH8, RH9	OQ625454, OQ625455
O. stukovii	RH2, RH3	OQ625446, OQ625447
O. lanata	RH1, RH3, RH10	OQ625438–OQ625440
O. myriophylla	RH4	OQ625451
O. gracillima	RH11	OQ625456
O. scheludjakovae	RH12, RH13	OQ625457, OQ625458
O. schmorgunoviae	RH14	OQ625459
O. sverdrupii	RH15	OQ625460
O. wrangelii	RH16	OQ625461
O. putoranica	RH17	OQ625462

Northern China (≈40°–41° N), but to the south of the territories that we studied, the nucleotide diversity turned out to be significantly lower (0.000093 and 0.000095, respectively) [36]. In general, the low level of nucleotide diversity is consistent with the low mutation rate in the chloroplast genome in the genus *Oxytropis*, which is 8.9×10^{-10} substitutions per site per year [37].

The combination of the high level of haplotype diversity and the low level of nucleotide diversity for most of the studied populations of *Oxytropis* section *Verticillares* is also characteristic of other previously studied *Oxytropis* species in Southern Siberia [38] and of *O. diversifolia* ($h = 0.880 \pm 0.074$; $\pi = 0.00061 \pm 0.00020$) from the Inner Mongolia (China) [39]. This combination suggests the rapid population growth from a small number of founders, while the time interval should be sufficient to restore haplotype variation by means of mutations, but insufficient to accumulate significant nucleotide differences in DNA sequences [40, 41].

The complex nature of the phylogenetic relationships between the studied species of the section *Verticillares* is indicated by the distribution of chlorotypes in the genealogical network (Fig. 2a). Chlorotypes of five species from five subsections, *O. ochrantha* of subsection *Oxyphylliformes*, *O. mongolica* of subsection *Salinae*, *O. gracillima* of subsection *Gobicola*, and *O. myriophylla* and *O. lanata* of monotypic subsections *Myriophyllae* and *Lanatae*, respectively, are grouped in haplogroup I according to the species affiliation, even in the case of joint growth of the *O. myriophylla* and *O. lanata* populations in the same habitats with plants species of haplogroup II (Figs. 1, 2a). A previous paper [23] showed a high degree of genetic differentiation of *O. lanata*, *O. myriophylla*, *O. gracillima*, and *O. mongolica* from other species of the section *Verticillares.* The high genetic distances between each of the above-mentioned species and other species of the section, as well as the species-specific nucleotide substitutions and inserts identified in this work in *O. lanata* and *O. ochrantha*, indicate a significant divergence of their chloroplast genomes. There may be various reasons for such divergence of species growing in the sympatric zone, but the ecological specialization of species appears the most important.

B.A. Yurtsev [9] discussed the ways of morphological evolution of the section *Verticillares* species and assumed that the evolutionary transformations occur owing to adaptation to various habitat conditions, i.e., growth in high mountains, on saline, rocky, and sandy substrates. The fact that ecological differentiation promotes genetic divergence was repeatedly shown for several plant species inhabiting complex heterogeneous landscapes [42–47]. In a range of cases, genetic divergence is associated specifically with the habitat substrate [42, 45], as was found, including for representatives of rare species of the *Astragalus* genus, a sister group to the genus *Oxytropis*, growing in the intermountain region of western North America. The diversification of these species was due to the interaction of demographic features and edaphic factors [44].

In *O. myriophylla*, the H11 and H12 chlorotypes are probably ancestral to other species of the section, since one of them occupies a central position in the network, and the other is closely related to the outgroup. This species has a wider ecological amplitude than the other species of haplogroup I; i.e., it grows in rocky steppes, on steppe slopes, in pine and birch forests, and on sandy areas [1, 13, 14], while the other species of haplogroup I, i.e., *O. ochrantha*, *O. mongolica*, *O. gracillima*, and *O. lanata*, occur in sandy areas of steppes and coastal sands of rivers and lakes, with *O. lanata* being the most highly specialized species, psammophyte [1, 9, 13, 14]. The narrow substrate specificity was noted earlier for *Oxytropis* species, in particular, for the species of section *Arctobia*: *O. czukotica* Jurtz. plants are acidophiles and inhabit acidic rocks; *O. gorodkovii* Jurtz. plants are calciphiles and are restricted to carbonate rocks [48]. Despite their morphological proximity (both species are part of the *O. nigrescens* s. l. complex [48]), the species reveal clear genetic isolation according to variation of chloroplast and nuclear markers [49]. Thus, the narrow specialization to depleted sandy soils could have made a certain contribution to the formation of separate phyletic lineages of species of the section *Verticillares*, whose chlorotypes form haplogroup I in the genealogical network.

The most common chlorotype H14 is the nucleus of haplogroup II (Fig. 2a), which indicates the common origin of the species forming this haplogroup. The highest frequency of H14 is observed in populations of *O. oxyphylla*. This species belongs to the ancient low specialized species of the section [8] and is type species of the subsection *Oxyphylliformes*, which is the largest and oldest within the section *Verticillares* [9, 17]. Next, H14 is shared by *O. stukovii*, *O. prostrata*, and a range of species that make up the cycle of *O. oxyphylla* s. ampliss. (*O. reverdattoi*, *O. selengensis*, *O. turczaninovii*, and *O. bargusinensis*; B.A. Yurtsev defined *O. bargusinensis* as "the not yet described Barguzin race" [9, 17]). The *O. oxyphylla* s. ampliss. cycle also includes *O. tompudae*, which carries the H36 chlorotype shared with *O. bargusinensis* and two chlorotypes forming a loop structure with *O. oxyphylla* chlorotypes (Fig. 2a). This star-like topology assumes a rapid gene flow in populations of small effective size that were not separated by ancient barriers and experienced rapid and recent expansion [40]. The demographic data that we obtained earlier for some populations of *O. oxyphylla* [24] indicate a possible recent demographic expansion of the species. The combination of the high haplotype diversity and the low nucleotide diversity characteristic of all populations of the section is also an indirect indication of the relatively recent and rapid demographic expansion (Table 2).

The occurrence of shared chlorotypes in different species found only in haplogroup II may be due to several causes, the main ones of which are as follows: (1) conservation of the ancestral form polymorphism; (2) incomplete divergence of genealogical lineages (incomplete lineage sorting, ILS) during and after speciation; (3) introgressive hybridization between close species [46, 50–55]. In some cases, there is a mutual influence of these factors. Introgression is most often detected in the sympatry zone, in jointly living populations of different species. The absence of a clear geographical regularity in chlorotype distribution in haplogroup II indicates incomplete lineage sorting [46, 54]. This is quite probable, since, taking into account the time of the section *Verticillares* emergence estimated to be about 2.5 million years ago [8], not enough time has passed for complete divergence of plastid genomes in the species of the *O. oxyphylla* s. ampliss cycle. In addition, the presence of the internal haplotype with multiple mutational connections in the network (H14 with 13 connections in this work), high frequency, and wide geographical distribution points to ancestral polymorphism ([55] and the references therein). A study of the distribution patterns in this group of species showed that all the species have narrow isolated ranges in certain areas of the mountainous territories of Southern Siberia [56] and are descendants of the common ancestral species that had a continuous zonal distribution in the cryophilic landscapes of the Miocene–Pliocene [7]. Such extensive expansion could have led to widespread establishment of ancestral polymorphism before these species underwent morphological diversification. Currently, they exist as small isolated populations [56]. Therefore, there is a high probability that presence of the shared chlorotype in several species of the section is associated more with conservation of ancestral polymorphism, rather than with the current gene exchange.

At the same time, the influence of hybridization on evolutionary processes in haplogroup II species cannot be excluded. Introgression and hybridization of species of the genus *Oxytropis*, including species of the section *Verticillares*, were described previously [9, 10, 12–14, 17]. Thus, the hybrid origin of *O. bargusinensis*, *O. prostrata*, and *O. tompudae*, as well as the ability of *O. oxyphylla* and *O. lanata* to hybridize, was noted. It is likely that the appearance of polyploids in the subsection *Oxyphylliformes* (*O. selengensis* and the karyological race *O. turczaninovii* with $2n = 32$) is the result of hybridization. Therefore, it is assumed that both hybridization and the general polymorphism of the ancestral form could have played an important role in the formation of evolutionary relationships between species of the *O. oxyphylla* s. ampliss. cycle, which is observed currently (Fig. 2a). Therefore, the picture of phylogenetic relationships between chlorotypes of species in haplogroup II indicates complex processes of speciation and subsequent diversification of species in the *O. oxyphylla* s. ampliss. cycle, as well as of the *O. stukovii* and *O. prostrata* species.

This picture of phylogenetic relationships arising from the mutual influence of ancestral polymorphism and hybridization and the high level of diversity observed even in narrow endemics (as in *O. bargusinensis* and *O. tompudae* in this paper) were repeatedly shown for groups of closely related species from various topographically heterogeneous regions, which represent, as a rule, hotspots of biodiversity. The examples include the *Centaurea* species (mountains of continental Greece) [50], three species of Alpine oak (the Eastern Himalayas region and the Handuan Mountains) [51], the *Spiraea* species (Qinghai–Tibet Plateau) [52], the *Acanthophyllum* species (the Irano-Turanian floristic region) [53], and the *Oxytropis* species of the section *Xerobia* that we studied previously (Southern Siberia and adjacent territories) [35] and that come from the same region as the species of the section *Verticillares*. This area (the territory of origin and the main area where species of the section *Verticillares* are distributed) also practically coincides with one of the most important floristic diversity nodes called the Selenginsky-Daursky node in Southern Siberia [57]. Evidently, *O. oxyphylla*, *O. reverdattoi*, *O. selengensis*, *O. turczaninovii*, *O. bargusinensis*, *O. tompudae*, *O. stukovii*, *O. prostrata*, and *O. pumila*, whose chlorotypes form haplogroup II, have an independent species status, but they are genetically very close to each other, which indicates the relative youth of this group and actively ongoing microevolutionary processes in the center of the section origin.

The chlorotypes of five Arctic species, endemic with a narrow ecological amplitude, *O. scheludjakovae*, *O. schmorgunoviae*, *O. sverdrupii*, and *O. wrangelii* from the subsection *Inaequiseptatae* and *O. putoranica* from the subsection *Uniflorae*, form haplogroup III (Fig. 2a). *O. wrangelii* and *O. putoranica*, as rare species, are included in *The Red Book of the Chukotka Autonomous Okrug* (2008) and in *The Red Book of Krasnoyarsk Krai* (2022), respectively; *O. sverdrupii*, as a narrow endemic that lives only on the Ayon Island, is included in *The Red Book of the Russian Federation* (2008). These species are spatially isolated from the group of South Siberian species of the section, and their chlorotypes are genetically isolated both from each other and from the chlorotypes of other species, which indicates the independent evolution of Arctic taxa and the limited gene flow between them. The exception in this haplogroup is the H29 chlorotype of *O. oxyphylla*, which makes up the second phyletic lineage, the appearance of which may be caused by convergent evolution. Owing to the fact that the analysis of Arctic species was performed using a small number of herbarium specimens, it is difficult to draw any conclusions on this group, but some features need mentioning. Previously, B.A. Yurtsev [48], characterizing the morphoecological differentiation of species from the *Inaequiseptatae* subsection, distinguished the morphological and evolutionary series, starting from *O. schmorgunoviae* (endemic to the steppe communities of the Kolyma Basin and Western Chukotka), involving *O. sverdrupii* (endemic to the Ayon Island, inhabitant of dry tundra), and finishing with *O. wrangelii* (endemic to the Wrangel Island and the east of the Chukchi Peninsula, inhabitant of dry and medium-moist tundra). The evolution in this series is driven by the expansion to wetter habitats and adaptation to a colder climate; herein, *O. schmorgunoviae* and *O. sverdrupii* are hexaploids ($2n = 48$), and in *O. wrangelii*, the number of chromosomes increases to $2n = 64$. In the genealogical network (Fig. 2a), the chlorotypes of these species (H51 of *O. schmorgunoviae*, H52 of *O. sverdrupii*, H54 and H55 in *O. wrangelii*) are arranged in the same sequence starting from a hypothetical central chlorotype of this haplogroup. This arrangement of chlorotypes may be partly explained by the ecological specialization of these species, which also may explain isolated position of the H53 chlorotype of *O. putoranica*, an endemic calciphile in the Putorana Plateau and the Byrranga Mountains of the Taymyr that inhabits gravelly tundra on mountain slopes in wind-blown areas and limestones [48]. The arrangement of chlorotypes involving three *O. scheludjakovae* populations appears intriguing since they are so genetically isolated from each other that the number of mutational steps between their chlorotypes exceeds that between the chlorotypes of some species in the section (Fig. 2a). This may probably be due to allopatric divergence in remote isolated populations. In *O. scheludjakovae*, the presence of karyological races with the number of chromosomes 16, 32, and 48 was noted [13]; however, these data were obtained for populations quite remote from those studied in this paper, but it is assumed that polyploidization processes also had an impact on the deep intraspecific divergence of *O. scheludjakovae*. An analysis of genealogical relationships between chlorotypes of *Oxytropis* species [22] revealed proximity of *O. scheludjakovae* and *O. sverdrupii* species to the section *Orobia*, which is consistent with B.A. Yurtsev's opinion [48] that species of the section *Orobia* can hybridize with species of the subsection *Inaequiseptatae* of the section *Verticillares*. It is possible that polyploid races *O. scheludjakovae* appeared as a result of hybridization. Therefore, it is assumed that the evolutionary processes in the group of Arctic species, whose chlorotypes are combined in haplogroup III, are driven by ecological specialization, allopatric divergence, and hybridization to form polyploid races and species, but expanded samples of populations and species of this group need to be studied in order to obtain justified conclusions.

The genealogical network of ribotypes (Fig. 2b), similar to the chlorotype network, includes a star-like structure that is evidence of the expansion; the RH3 ribotype is located at the center of this structure and reveals 11 mutational connections. This is a dominant and potential ancestral ribotype shared by six southern Siberian taxa, including *O. lanata*, whose chlorotypes are well divergent from the chlorotypes of other haplogroup II species (Fig. 2a). This star-like arrangement of ribotypes, as mentioned earlier, is explained by the common origin of species of the section *Verticillares*, their relatively recent divergence, conservation of ancestral polymorphism, and incomplete lineage sorting, as well as hybridization between taxa with weak reproductive barriers in the sympatry zone. In addition, the star-like structure may indicate the rapid adaptive radiation of its taxa. It was found previously using nuclear and chloroplast markers that the rapid radiation is characteristic of the genus *Oxytropis* [4], as well as other genera of the family Fabaceae: *Lupinus* [58], *Astragalus* [59], *Sophora* [60], *Indigofera* [61], and others. We also showed the shared ribotype for six species of *Oxytropis* belonging to three different subgenera *Phacoxytropis*, *Tragacanthoxytropis*, and *Oxytropis* of the genus *Oxytropis* [26]. The ribotypes of Arctic species are isolated and have no shared ribotypes with southern Siberian species, which indicates their relatively early divergence from this group.

It is noted that the number of identified chlorotypes (54) in 18 studied species of the section *Verticillares* (*O. pumila* is not taken into account owing to the lack of ribotype) exceeds the number of ribotypes (17) by more than three times. This is typical of the *Oxytropis* species of other sections, for example, the section *Orobia*—five studied species, 39 chlorotypes, and six ribotypes [62]; *Arctobia*—nine species, 55 chlorotypes, and ten ribotypes [49]; and *Xerobia*—seven species, 69 chlorotypes, and two ribotypes [35]. Similar differences were also noted in other plant species [61, 63, 64]. This discordance may occur owing to the rapid radiation and incomplete lineage sorting, as well as because of different modes of inheritance (uniparental or biparental inheritance) and distribution of chloroplast and nuclear genomes (dispersal by seeds only or by pollen and seeds). The chloroplast genome inherited from one parent carries only half of the effective population size, compared to the ITS nuclear gene, which spreads both with pollen and seeds, and therefore, the divergence process of phyletic lineages in the nuclear DNA takes a longer time than in the chloroplast DNA.

The study of the *Verticillares* section species in the focus of origin of the section based on chloroplast genome markers showed that most of the studied populations are characterized by combination of the high haplotype and low nucleotide diversity, which suggests rapid population expansion from a small number of founders. By the nature of the revealed genealogical relationships between chlorotypes, different evolutionary pathways for the species of the section may be assumed: (1) divergence of well genetically isolated taxa in the sympatry zone probably driven by the ecological specialization; (2) incomplete lineage sorting with conservation of ancestral polymorphism plus hybridization between weakly divergent taxa; (3) allopatric divergence and polyploidization. The data from the analysis of chloroplast and nuclear genome markers indicate rapid adaptive radiation of species in the section *Verticillares*. Evidently, the center of origin of the section (Baikal Siberia and adjacent areas of southern Siberia, Mongolia, and China) is currently the center of active speciation and high genetic diversity. Taking into account the fact that the section *Verticillares* includes rare, endemic, and valuable medicinal species, this work may be used as the basis for identification of centers of genetic diversity, their conservation, and monitoring of the state of species.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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