Taxonomic assessment of the *Oxytropis* species from South-East of Kazakhstan

S. Almerekova[1, 2], S. Abugalieva[1, 2] ✉, N. Mukhitdinov[2]

[1] Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan
[2] Al-Farabi Kazakh National University, Almaty, Kazakhstan

The genus *Oxytropis* DC. is one of the largest genera in the Fabaceae family. The most plant species belonging to the *Oxytropis* genus have an important medicinal value. Currently the botanical taxonomy of the genus is complicated due to existence of many subgenera and sections that developed based on morphological traits. Also, in the literature there is luck of knowledge on phylogeny of *Oxytropis* species from Central Asian region. Therefore, the purpose of the present study was the clarification of taxonomic relationship of two *Oxytropis* species from South-East of Kazakhstan (*O. almaatensis* Bajt. and *O. glabra* DC.). The study was based on using phylogenetic analysis and haplotype network assessment based on sequences ITS (internal transcribed spacers), which is DNA marker of nuclear genome. Plant materials of *O. almaatensis* were collected from 2 populations in two neighboring Gorges in Trans Ili Alatau Mountains, *O. glabra* plant material was obtained from Herbarium of the Department of Biodiversity and Bioresources, al-Farabi Kazakh National University. Based on DNA sequences of ITS the phylogenetic and network relationships were investigated by using Neighbor Joining and Median Joining methods, respectively. The nucleotide sequences of ITS of *O. almaatensis* and *O. glabra* were aligned with sequences of 29 *Oxytropis* references found in the NCBI database. Out of the 601 aligned positions of ITS 33 (5.6 %) sites were found to be polymorphic nucleotides and used in evaluation of the genetic relationship of species. Constructed MJ haplotype network showed a very high congruence with the NJ phylogenetic tree. MJ network provided valuable additional hints in clarification of the taxonomic relationship among species involved in the analysis. In this study phylogenetic NJ tree and MJ network based on the variation of ITS sequences confirmed the monophyletic origin of the genus. The ITS haplotype network suggested that *O. glabra* is very diverse species and possibly played important role in the evolutionary processes of the genus in Central Asian region. The study is additional contribution in the molecular taxonomy of complex *Oxytropis* genus.

Key words: *Oxytropis*; *Oxytropis almaatensis*; *Oxytropis glabra*; DNA barcoding; haplotype network.

HOW TO CITE THIS ARTICLE:
Almerekova S., Abugalieva S., Mukhitdinov N. Taxonomic assessment of the *Oxytropis* species from South-East of Kazakhstan. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(2):285-290. DOI 10.18699/VJ18.362

УДК 582.738(574.4/5)
Поступила в редакцию 19.02.2018
Принята к публикации 07.03.2018

✉ e-mail: absaule@yahoo.com

Таксономическая оценка видов рода *Oxytropis* из Юго-Восточного Казахстана

Ш.С. Альмерекова[1, 2], С.И. Абугалиева[1, 2] ✉,
Н.М. Мухитдинов[2]

[1] Институт биологии и биотехнологии растений, Алматы, Казахстан
[2] Казахский национальный университет имени аль-Фараби, Алматы, Казахстан

Род *Oxytropis* DC. является одним из крупнейших родов семейства Fabaceae. Большинство видов растений, принадлежащих к данному роду, имеют важное лекарственное значение. В настоящее время ботаническая систематика рода затруднена в связи с наличием множества подродов и секций. Также в литературе отсутствуют данные о филогенетических взаимосвязях видов *Oxytropis* из Центральной Азии. В связи с этим целью настоящего исследования было уточнение таксономических взаимоотношений двух видов *Oxytropis* из Юго-Восточного Казахстана – *O. almaatensis* Bajt. и *O. glabra* DC. Осуществлены филогенетический анализ и оценка сети гаплотипов, базирующиеся на полиморфных последовательностях ITS (internal transcribed spacers), ДНК-маркера ядерного генома. Растительный материал *O. almaatensis* состоял из двух популяций, собранных в двух соседних ущельях Заилийского Алатау, растительный материал *O. glabra* был получен из гербарного образца кафедры биоразнообразия и биоресурсов Казахского национального университета имени аль-Фараби. Полученные полиморфные нуклеотидные последовательности ITS были использованы для анализа филогенетических взаимоотношений и сети гаплотипов с помощью методов Neighbor Joining (NJ) и Median Joining (MJ) соответственно. Последовательности ITS *O. almaatensis* и *O. glabra* сравнивали с последовательностями 29 образцов *Oxytropis*, полученными из базы данных GenBank (NCBI). Длина ITS составила 601 п. о., из них 33 (или 5.6 %) нуклеотида оказались полиморфными, что позволило использовать их в изучении генетического родства видов *Oxytropis*. В целом построенная сеть гаплотипов MJ позволила выявить высокую степень совпадения с филогенетическим древом NJ. Кроме того, применение MJ сети гаплотипов дало возможность получить ценные дополнительные данные для уточнения таксономических отношений между видами, вовлеченными в анализ. В этом исследовании филогенетическое древо и сеть гаплотипов, построенные на основе вариабельности последовательностей ITS, подтвердили монофилетическое происхождение рода. Построенная сеть гаплотипов позволила предположить, что *O. glabra* является высоковариабельным видом, который, возможно, играл важную роль в эволюционном процессе рода в Центральной Азии. Исследование внесло дополнительный вклад в изучение молекулярной таксономии рода *Oxytropis*.

Ключевые слова: *Oxytropis*; *Oxytropis almaatensis*; *Oxytropis glabra*; ДНК-баркодирование; сеть гаплотипов.

Oxytropis DC. with approximately 450 species, most of which are hairy perennial plants, is one of the largest genera in the family Fabaceae (Malyshev, 2008a). *Oxytropis* species are well distributed in Central Asia and rich in endemics, especially in mountain systems of Mongolian Altay, Tien Shan, Nanshan and Himalayas (Grubov, 2003). Grubov (2003) reported that Central Asia, along with West Asia, is the most important center of the speciation of genus *Oxytropis*. In Central Asia the genus consists of all the six subgenera and sixteen sections (Grubov, 2003). In northern Tien Shan the species composition of the genus *Oxytropis* has been studied by Abdulina (1978). Morphological studies of the species found in the northern Tien Shan region were carried out, the most convenient traits for diagnostics of taxa were identified, areas of endemic species and maps of their distribution have been specified (Abdulina, 1978). According to Malyshev (2008b) the genus is represented by 6 subgenera and 25 sections. Author clustered 25 sections according to the 50 quantitative alternative morphological characters (Malyshev, 2008b). Due to a large number of *Oxytropis* species, the taxonomy of this genus is still uncompleted.

In Kazakhstan *Oxytropis* is represented by 119 species, 36 of which are endemic (Baitenov, 1961). One of those endemic plant species is *Oxytropis almaatensis* Bajt. listed in the Red Book of Kazakhstan (2014). *O. almaatensis* is a narrow endemic species of Trans Ili Alatau range which belongs to the Tien Shan Mountains (Baitenov, 1961). According to the literature *O. almaatensis* has potential medicinal benefits. It contains phenol carboxylic acid which is helpful for coronary dilatation and flavonoid ramnazine which has antihypertensive properties (Grudzinskaya et al., 2014).

The DNA barcoding significantly contributed not only in plant species identification but also in the taxonomic relationship of poorly studied species (Techen et al., 2014; Li et al., 2015). Currently, this approach considered as an additional effective tool used in taxonomic studies of the genus *Oxytropis* (Archambault, Strömvik, 2012; Artyukova, Kozyrenko, 2012; Gao et al., 2013; Lu et al., 2014; Kholina et al., 2016; Tekpinar et al., 2016). For instance, first attempt to clarify taxonomy and biogeography of the genus in Alaska was carried out by Jorgensen (Jorgensen et al., 2003). The use of ITS (internal transcribed spacers) and RAPD (random amplified polymorphic DNA) markers has shown that north-eastern arctic populations in *O. arctica* and *O. campestris* were different from all other studied populations. The genetic subdivision probably reflects a Pleistocene barrier formed by the northern coastal ice shield (Jorgensen et al., 2003). To identify the phylogenetic relationship of Turkish *Oxytropis* species the *trn*L intron, *trn*L-F intergenic spacer, and *trn*V intron of chloroplast (cp) DNA were used (Tekpinar et al., 2016). According to Tekpinar (2016) *trn*L intron was the most variable region. Kholina et al. (2016) assessed phylogenetic relationships of Russian species of *Oxytropis* from subgenera *Oxytropis* and *Phacoxytropis* using *trn*H–*psb*A, *trn*L–*trn*F, and *trn*S–*trn*G intergenic spacer regions of chloroplast DNA (cpDNA) and genealogical haplotype network. This helped authors to clarify the phylogenetic relationships of the analysed species and sections within the subgenera.

In *Oxytropis* taxonomy studies, along with estimated phylogenetic trees, several successful analyses were included haplotype network approach (Artyukova, Kozyrenko, 2012; Kholina et al., 2016, 2017). For instance, the three intergenic spacers *psb*A–*trn*H, *trn*L–*trn*F, and *trn*S–*trn*G of cpDNA of rare and endemic plant species of Buryatia in four populations from Barguzin and Yeravna depressions were studied (Kholina et al., 2017). Therefore, the assessment of combinations of haplotype network and phylogenetic trees might provide valuable insights into understanding the microevolutionary process for closely related species.

As in the literature there is luck of knowledge on phylogeny of *Oxytropis* species from Central Asian region, the purpose of the present study was the clarification of taxonomic relationship of two *Oxytropis* species from South-East of Kazakhstan (*O. almaatensis* Bajt. and *O. glabra* DC.). The taxonomic analysis of *Oxytropis* taxa was relied on using phylogenetic analysis and haplotype network assessment by using the variability of the ITS nucleotide sequences. The study was conducted in the frame of the nation-wide research project DNA barcoding of wild flora of Kazakhstan (Turuspekov, Abugalieva, 2015) that combined efforts of local botanists and geneticists from Biotechnology Research Organizations, Botanical Gardens, National Nature Parks and Reserves as well as project "Informational system for molecular genetic and botanical documentation of wild flora in Kazakhstan". It is another contribution to the description of the genetic variation of wild flora in Kazakhstan (Adams, Turuspekov, 1998; Turuspekov et al., 2002, 2014; Genievskaya et al., 2017).

Materials and methods

Sample collections and DNA extraction. Samples of leaves from *O. almaatensis* were collected from 2 populations in two different Gorges in Trans Ili Alatau Mountains (Big Almaty gorge and Small Almaty gorge) in 2015 and 2016, five plant samples from each population were chosen for the genetic analysis. *O. glabra* plant material was obtained from Herbarium of the Department of Biodiversity and Bioresources, al-Farabi Kazakh National University. For the construction haplotype network and phylogenetic tree ITS sequences were taken from NCBI (https//www.ncbi.nlm.nih.gov/genbank/). DNA was extracted using CTAB protocol (Doyle J.J., Doyle J.L., 1987) and stored at –20 °C until use.

DNA amplification and sequencing. PCR fragments were amplified from nuclear ribosomal complex including ITS1 and ITS2 (White et al., 1990). PCR was performed by using Veriti Thermo cycler (Applied Biosystems, Foster City, CA, USA). PCR reaction (total volume 16 μl) contained 4 mM of each dNTP, 6.4 mM of primer mix, 1.6 U of Taq DNA polymerase and 80 ng of total genomic DNA. The entire ITS-1, 5.8S, and ITS-2 region was polymerase chain reaction (PCR)-amplified using primers ITS1nF (5′-AGAAGTCGTAACAAGGTTTC CGTAGG-3′) and ITS4nR (5′-TCCTCCGCTTATTGATAT GC-3′) with annealing temperature 58 °C (White et al., 1990). PCR products were run in 1.5 % agarose gel electrophoresis at 80 V voltage for 40 min. Single bands with expected sizes around 650 bp were cut out from gels and purified using ULTRAPrep® Agarose Gel Extraction Mini Prep Kit (AHN Biotechnologie GmbH, Nordhausen, Germany) according to the protocol provided by the company. Purified DNA amplicons were used for the sequence reactions with forward and reverse primers separately. All reactions were performed

Haplotypes formed from the analysis of ITS sequences *Oxytropis* species and the outgroup

Haplotype	Cluster and haplogroup	Species	GenBank Accession number	Sections
Hap_1	IV	*O. revoluta*	LM653251	*Atctobia*
Hap_2	IV	*O. retusa*	LM653264	*Orobia*
Hap_3	III	*O. racemosa*	HQ199320	*Verticillares*
		O. ochrantha	GQ422820	
Hap_4	II	*O. pilosa*	AF121759	*Chrysantha*
Hap_5	II	*O. pallasii*	KM053395	*Chrysantha*
Hap_6	III	*O. oxyphylla*	FR839000	*Verticillares*
		O. intermedia	LM653257	*Xerobia*
		O. inschanica	HQ199322	
Hap_7	III	*O. microphylla*	KP338205	*Polyadena*
Hap_8	III	*O. maydelliana*	HQ176486	*Orobia*
Hap_9	III	*O. mandshurica*	LM653236	*Janthina*
Hap_10	III	*O. lanata*	LM653259	*Verticillares*
Hap_11	II	*O. kansuensis*	KJ143718	*Mesogaea*
Hap_12	IV	*O. kamtschatica*	LM653247	*Atctobia*
Hap_13	IV	*O. hidakamontana*	LM653263	*Atctobia*
Hap_14	III	*O. filiformis*	HQ199321	*Janthina*
Hap_15	III	*O. evenorum*	LM653239	*Orobia*
Hap_16	II	*O. deflexa*	HQ176481	*Mesogaea*
Hap_17	III	*O. chankaensis*	FR839001	*Verticillares*
Hap_18	III	*O. campestris*	HQ176475	*Orobia*
Hap_19	III	*O. caerulea*	GU217599	*Janthina*
Hap_20	III	*O. borealis*	AF121758	*Gloeocephala*
Hap_21	II	*O. aciphylla*	GQ422806	*Lycotriche*
Hap_22	I	*O. glabra*	LC213354	*Mesogaea*
Hap_23	I	*O. glabra*	KJ143729	*Mesogaea*
Hap_24	I	*O. glabra*, *O. glabra*	KJ143719, GQ265958	*Mesogaea*
Hap_25	I	*O. glabra*	GQ265961	*Mesogaea*
Hap_26	I	*O. glabra**	In this study	*Mesogaea*
Hap_27	I	*O. almaatensis**	MG 282028, in this study	*Eumorpha*
Hap_28	Outgroup	*A. polaris*	AF121714	–
Hap_29	Outgroup	*A. mollissimus*	AF121719	–

with the BigDye Terminator Cycle Sequencing technology (Applied Biosystems, Foster City, CA, USA). Sequencing was carried out using an ABI 3130 DNA analyzer (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA).

Sequence alignment. The ITS sequences were aligned in MEGA 6 (Tamura et al., 2013) by using Neighbor Joining method (NJ) (Saitou, Nei, 1987), the 1000 replication bootstrap test was applied. The sequences of *O. almaatensis* and *O. glabra* were aligned with other *Oxytropis* species sequences obtained from NCBI reference database (https//www.ncbi.nlm.nih.gov/genbank/). The ITS sequences of five samples of *O. almaatensis* were identical, consequently one sample was selected for the next analysis and deposited to the NCBI database (MG 282028) (see Table).

Haplotype Network was reconstructed using the Median Joining method (Bandelt et al., 1999) in PopART v.1.7 (Leigh, Bryant, 2015). The aligned sequences were converted into Nexus file format in DNASP v5.10 (Librado, Rozas, 2009) for the operations in the PopART software (version 1.7).

Results

Phylogenetic tree analyses based on ITS sequences

The DNA sequences of ITS of *O. almaatensis* and *O. glabra* were aligned with sequences of 29 *Oxytropis* references extracted from NCBI, and *Astragalus polaris* and *Astragalus mollissimus* were chosen as the outgroup taxa. The length of ITS (including ITS1, 5.8S, and ITS2) region for *Oxytropis* was

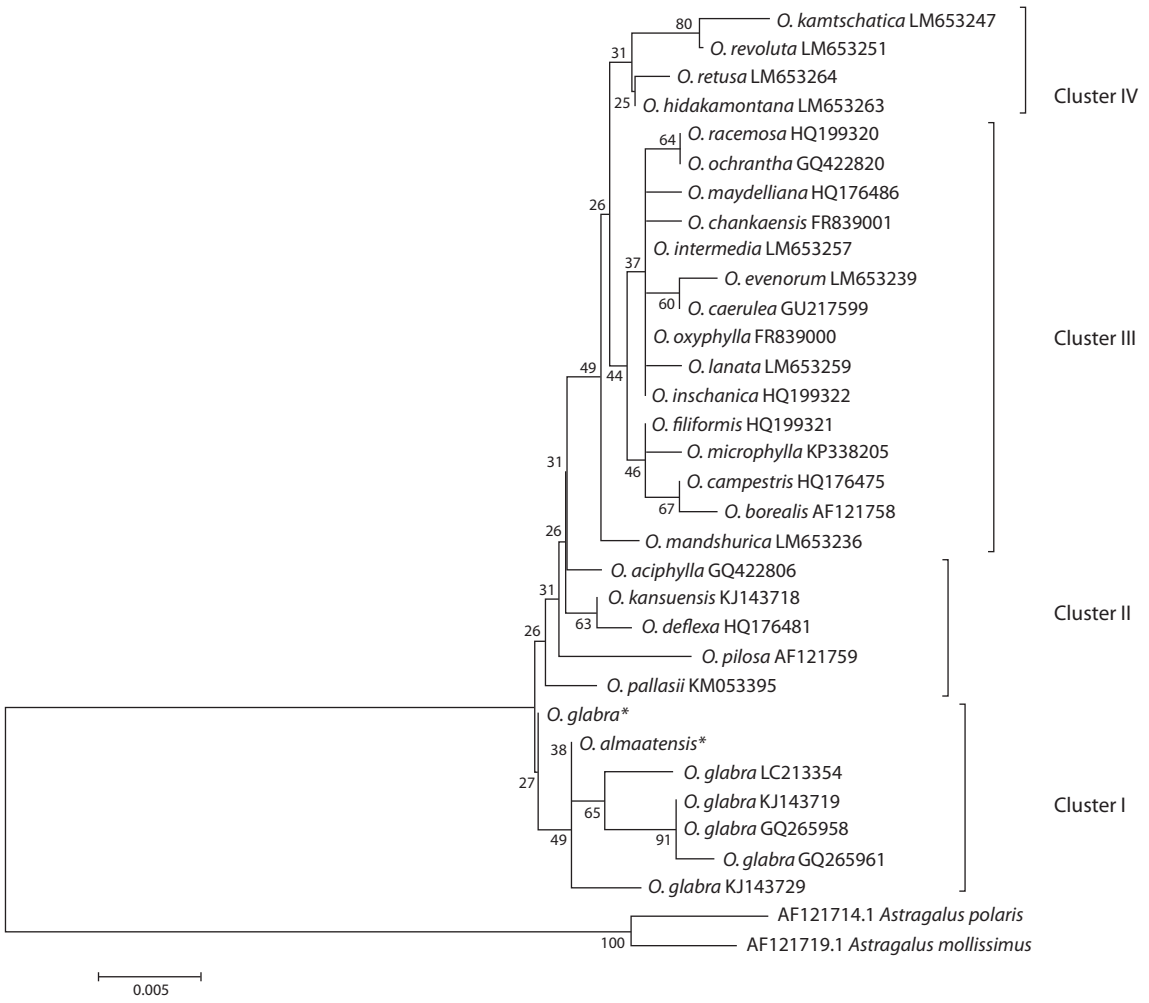

Fig. 1. Neighbor Joining phylogenetic tree reconstructed from the analysis of ITS sequences of *Oxytropis* and outgroup species.

601 bp. 33 (5.6 %) sites out of the 601 aligned positions of ITS were polymorphic without outgroup. Singleton variable sites was 16, parsimony informative sites was 17.

The ITS nucleotide dataset consisted from *O. almaatensis*, *O. glabra* sequenced in this study, as well as 29 *Oxytropis* species and two outgroup species (*A. polaris*, *A. mollissimus*) collected from the NCBI database. The NJ tree clustered all *Oxytropis* accessions into four clusters and separated from the outgroup (Fig. 1). Sequences of *O. almaatensis* and *O. glabra* grouped with five references of *O. glabra* from NCBI in Cluster I. The Cluster II grouped together *O. pallasii*, *O. pilosa*, *O. kansuensis*, *O. deflexa*, and *O. aciphylla.* The Cluster III was represented by *O. oxyphylla*, *O. intermedia*, *O. inschanica*, *O. microphylla*, *O. maydelliana*, *O. filiformis*, *O. evenorum*, *O. lanata*, *O. racemosa*, *O. ochrantha*, *O. chankaensis*, *O. campestris*, *O. caerulea*, *O. borealis*, *O. mandshurica.* The Cluster IV was represented by following species from NCBI: *O. kamtschatica*, *O. hidakamontana*, *O. revoluta*, *O. retusa*.

Haplotype network analyses based on ITS sequences

Twenty-nine haplotypes were identified for the ITS region in 33 accessions of *Oxytropis* genus and outgroup species in the network association analysis (Fig. 2). The results suggested that Hd = 0.991 (haplotype diversity), $\pi = 0.01498$ (nucleotide diversity), and $k = 8.86553$ (average number of nucleotide differences). The 29 haplotypes generated four haplogroups that corresponded to the NJ phylogenetic tree.

The largest haplotype H6 included *O. oxyphylla*, *O. intermedia*, *O. inschanica* (from NCBI) in haplogroup III. The next largest haplotype H3 contained *O. racemosa*, *O. ochrantha* from NCBI from the same haplogroup III. Haplotype 24 included two references of one species *O. glabra* in haplogroup I. Local species *O. glabra* and *O. almaatensis* generated two different haplotypes, H26 and H27, respectively, in haplogroup I (see Fig. 2, Table).

Discussion

The traditional taxonomy of the genus *Oxytropis* is still unresolved and has many difficulties. Therefore the application of haplotype network and phylogenetic tree methods using polymorphic molecular markers is essential additional asset molecular taxonomy analyses of complicated genera. In this study phylogenetic NJ tree and MJ network based on the variation of ITS sequences confirmed the monophyletic origin of the genus (see Figs. 1, 2). This result is well in line with previously published results (Archambault, Strömvik, 2012;

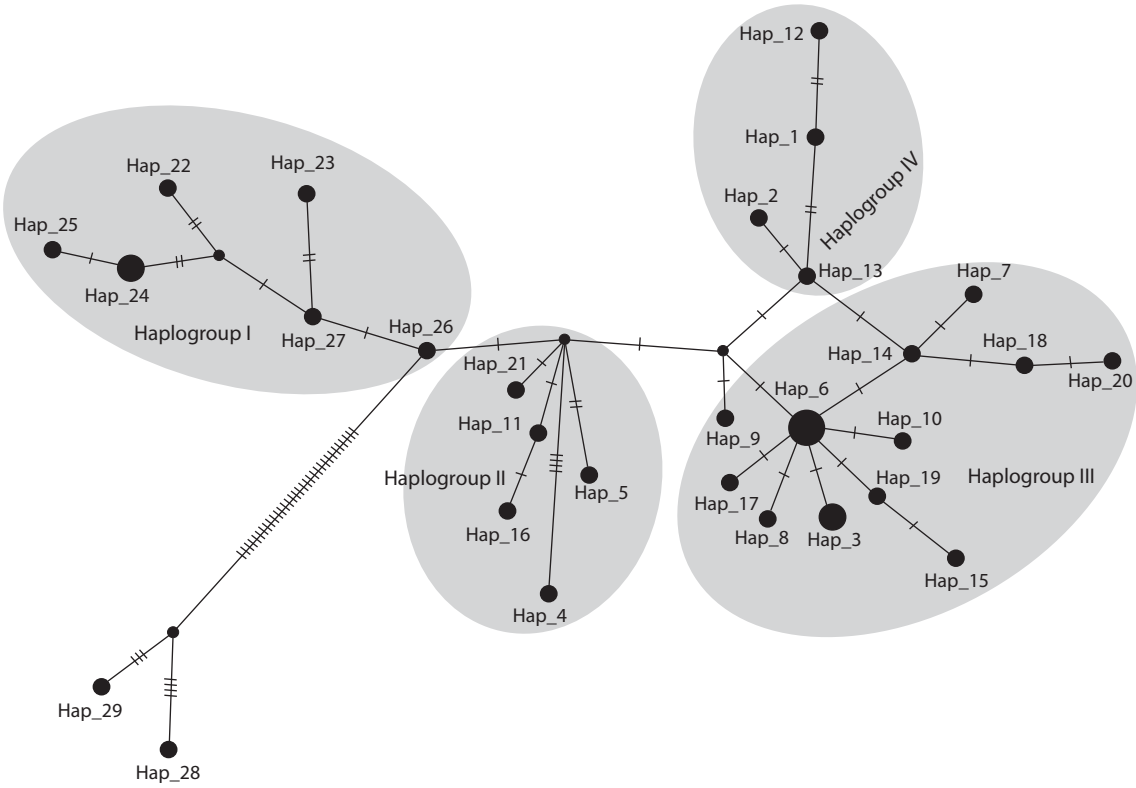

Fig. 2. The Median-joining haplotype network of ITS of *Oxytropis* and outgroup species.

Gao et al., 2013; Lu et al., 2014). The other important outcome is that the majority of species in this study formed four distinct clusters. The first cluster in the tree and first haplogroup in the network consisted only of two species, *O. glabra* and *O. almaatensis*. In general, as shown in previous studies (Archambault, Strömvik, 2012; Kholina et al., 2016) and in present work, the botanical classification is rarely coincided with produced phylogenetic trees, which is further complicate the analyses of the evolutionary processes in the genus *Oxytropis*.

The phylogenetic tree showed that *O. glabra* (section *Mesogaea*) and *O. almaatensis* (section *Eumorpha*) are genetically close to each other within the genus *Oxytropis*. This result is suggesting that there is a possibility of existance of extinct or extant group of relative species that can be evolutionary closely associated with both *O. glabra* and *O. almaatensis*. Therefore, additional studies should be done to clarify this hypothesis.

The ITS network suggested that *O. glabra* is highly polymorphic species and one of their haplotype (Hap_26) is the closest point to two outgroup species of *Astragalus* (Hap_26 and Hap_29) (see Fig. 2). As both haplotypes, Hap_26 and Hap_27, represented two genetically close species sampled in southeast Kazakhstan, it can be speculated that these regions might associate with one of the centers of diversification for this genus.

The second group of species consisted of five following species – *O. pilosa*, *O. pallasii*, *O. kansuensis*, *O. deflexa*, and *O. aciphylla*. In previously published articles the majority of these species was often clustered together with *O. glabra* (Archambault, Strömvik, 2012; Artyukova, Kozyrenko, 2012; Kholina et al., 2016). In this study, the haplotype network separated these two groups as all five species of the second cluster were bound to the same median vector (*mv*) (see Fig. 2). Thus, it is a possibility that species may have the same extinct or extant predecessor, which is genetically close to *O. kansuensis*, *O. deflexa*, and *O. aciphylla*. Most populated groups of *Oxytropis* species formed the third cluster (haplogroup III) that has a connection to the *O. mandshurica* via a common *mv* in the network (Fig. 2). Similarly, *O. mandshurica* (Hap_9) using the same *mv* was also connected to haplogroup IV, represented by four Far East species (Kholina et al., 2016). It is interesting that the network is suggesting a close genetic relationship between *O. filiformis* (haplogroup III) and *O. hidakamontana* (haplogroup IV) despite their clasterization in different sub clades (see Fig. 2).

In general, the constructed haplotype network showed a very high congruence with the NJ phylogenetic tree. As generated NJ dendrogram showed a relatively low bootstrap value indices; the network provided valuable additional hints in clarification of the taxonomic relationship among species involved in the analysis. The study is another contribution in the molecular taxonomy of complex *Oxytropis* genus.

Acknowledgments
The authors would like to acknowledge the funding of the grant AP05131621 from the Ministry of Education and Sciences of the Republic of Kazakhstan.

Conflict of interest
The authors declare no conflict of interest.

References

Abdulina S.A. Endemic species of the genus *Oxytropis* DC. in Northern Tien Shan. Bull. Acad. Sci. Kazakh SSR. 1978:66-71.

Adams R.P., Turuspekov Y. Taxonomic reassessment of some Central Asian and Himalayan scale-leaved taxa of *Juniperus* (Cupressaceae) supported by random amplification of polymorphic DNA. Taxon. 1998;47:75-84.

Archambault A., Strömvik M.V. Evolutionary relationships in *Oxytropis* species, as estimated from the nuclear ribosomal internal transcribed spacer (ITS) sequences point to multiple expansions into the Arctic. Botany. 2012;90:770-779. DOI 10.1139/b2012-023.

Artyukova E.V., Kozyrenko M.M. Phylogenetic relationships of *Oxytropis chankaensis* Jurtz. and *Oxytropis oxyphylla* (Pall.) DC. (Fabaceae) inferred from the data of sequencing of the ITS region of the nuclear ribosomal DNA operon and intergenic spacers of the chloroplast genome. Genetika. 2012;48:186-193. DOI 10.1134/S1022795411110032.

Baitenov M.S. *Oxytropis almaatensis* Bajt. sp. nova. Flora Kazahstana. T. V. [Flora of Kazakhstan. Vol. V]. Ed. N.V. Pavlov. Alma-Ata: Akademija Nauk Kazakhskoy SSR, 1961;330-410. (in Russian)

Bandelt H.J., Forster P., Röhl A. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 1999;16(1):37-48.

Doyle J.J., Doyle J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 1987;19:11-15.

Gao L., Lu P., Jin F., Enhebayaer E., Gao J. *Trn*L-F sequences analysis and molecular phylogeny of 10 species of *Oxytropis*. Acta Botanica Boreali-Occidentalia Sinica. 2013;2:266-271.

Genievskaya Y., Abugalieva S., Zhubanysheva A., Turuspekov Y. Morphological description and DNA barcoding study of sand rice (*Agriophyllum squarrosum*, Chenopodiaceae) collected in Kazakhstan. BMC Plant Biology. 2017;17(Suppl.1):177. DOI 10.1186/s12870-017-1132-1

Grubov V.I. Plants of Central Asia – Plant Collection from China and Mongolia. Vol. 8b. Legumes, Genus *Oxytropis*. Enfield Taylor & Francis, 2003.

Grudzinskaya L.M., Gemedzhieva N.G., Nelina N.V., Karzhaubekova J.J. Annotirovannyj spisok lekarstvennyh rastenij Kazahstana: Spravochnoe izdanie [Annotated checklist of medicinal plants in Kazakhstan: a reference book]. Almaty, 2014. (in Russian)

Jorgensen J.L., Stehlik I., Brochmann C., Conti E. Implications of ITS sequences and RAPD markers for the taxonomy and biogeography of the *Oxytropis campestris* and *O. arctica* (Fabaceae) complexes in Alaska. Am. J. Bot. 2003;90(10):1470-1480. DOI 10.3732/ajb.90.10.1470.

Kholina A.B., Kozyrenko M.M., Artyukova E.V., Sandanov D.V., Andrianova E.A. Phylogenetic relationships of the species of *Oxytropis* DC. subg. *Oxytropis* and *Phacoxytropis* (Fabaceae) from Asian Russia inferred from the nucleotide sequence analysis of the intergenic spacers of the chloroplast genome. Russ. J. Genet. 2016;52(8):780-793. DOI 10.1134/S1022795416060065.

Kholina A., Kozyrenko M., Artyukova E., Sandanov D., Selyutina I., Chimitov D. Plastid DNA variation of the endemic species *Oxytropis glandulosa* Turcz. (Fabaceae). Turkish J. Bot. 2017;42(1):38-50. DOI 10.3906/bot-1706-11.

Leigh J.W., Bryant D. PopART full-feature software for haplotype network construction. Methods Ecol. Evol. 2015;6(9):1110-1116. DOI 10.1111/2041-210X.12410.

Li X., Yang Y., Henry R.J., Rossetto M., Wang Y., Chen S. Plant DNA barcoding from gene to genome. Biol. Rev. 2015;90:157-166. DOI 10.1111/brv.12104.

Librado P., Rozas J. DnaSP v5 a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009;25(11):1451-1452.

Lu P., Gao L., Jin F., Enhebayaer E. Molecular phylogeny of 10 species of *Oxytropis* based on psbA-trnH sequences. Acta Bot. Yunnanica. 2014;3:279-284. DOI 10.1093/bioinformatics/btp187. DOI 10.7677/ynzwyj201413135.

Malyshev L. Diversity of the genus *Oxytropis* in Asian Russia. Turczaninowia. 2008a;11(4):5-141.

Malyshev L.I. Phenetics of the subgenera and sections in the genus *Oxytropis* DC. (Fabaceae) bearing on ecology and phylogeny. Contemp. Probl. Ecol. 2008b;1(4):440-444. DOI 10.1134/S1995425508040073.

National Center for Biotechnology Information. U.S. National Library of Medicine, Rockville Pike, 1988. https //www.ncbi.nlm.nih.gov. Accessed 2 August 2017.

Saitou N., Nei M. The neighbor-joining method. A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 1987;4:406-425.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6 Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 2013;30(12):2725-2729. DOI 10.1093/molbev/mst197.

Techen N., Parveen I., Pan Z., Khan I. DNA barcoding of medicinal plant material for identification. Curr. Opin. Biotechnol. 2014;25:103-110. DOI 10.1016/j.copbio.2013.09.010.

Tekpinar A., Erkul S.K., Aytac Z., Kaya Z. Phylogenetic relationships among native *Oxytropis* species in Turkey using the *trn*L intron, *trn*L-F IGS, and *trn*V intron cpDNA regions. Turkish J. Bot. 2016;40(5):472-479. DOI 10.3906/bot-1506-45.

The Red Book of the Republic of Kazakhstan. Almaty: ArtPrint XXI, 2014.

Turuspekov Y., Abugalieva S. Plant DNA barcoding project in Kazakhstan. Genome. 2015;58(5):290.

Turuspekov Y., Abugalieva S., Ermekbayev K., Sato K. Genetic characterization of wild barley populations (*Hordeum vulgare* ssp. *spontaneum*) from Kazakhstan based on genome wide SNP analysis. Breed. Sci. 2014;64(4):399-403. DOI 10.1270/jsbbs.64.399.

Turuspekov Y., Adams R.P., Kearney C.M. Genetic diversity in three perennial grasses from the Semipalatinsk nuclear testing region of Kazakhstan after long-term radiation exposure. Biochem. Syst. Ecol. 2002;30(9):809-817. DOI 10.1016/S0305-1978(02)00021-2.

White T.J., Bruns T., Lee S., Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: a Guide to Methods and Applications. 1990;18(1):315-322.