Turczaninowia 25, 4: 5–11 (2022)
DOI: 10.14258/turczaninowia.25.4.1
http://turczaninowia.asu.ru

ISSN 1560–7259 (print edition)
TURCZANINOWIA
ISSN 1560–7267 (online edition)

УДК 581.9+575.1+57.087.3(235.222/.226)

DNA barcodes of the vascular flora of the Altai Mountain Country: type material of the Herbarium ALTB

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Keywords: Altai, biodiversity, endemics, flora, herbarium, plant DNA barcoding, sequencing.

Summary. The article presents first data of the work on DNA barcoding of type specimens of ALTB Herbarium (Barnaul, Russia). Obtained sequences of ITS and *trn*L-*trn*F, *trn*H-*psb*A markers of DNA were deposited in NCBI GenBank, and corresponding dataset was published in the GBIF (Global Biodiversity Information Facility).

ДНК-штрихкоды сосудистых растений флоры Алтайской горной страны: типовой материал Гербария ALTB

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Ключевые слова: Алтай, биоразнообразие, гербарий, ДНК-штрихкодирование растений, секвенирование, флора, эндемик.

Аннотация. В статье представлены первые результаты работы по ДНК-штрихкодированию типовых образцов, хранящихся в гербарии Алтайского государственного университета (ALTB, г. Барнаул, Россия). Полученные нуклеотидные последовательности маркерных фрагментов ДНК (ITS, *trn*L-*trn*F, *trn*H-*psb*A) внесены в базу данных NCBI GenBank, и соответствующий датасет опубликован в GBIF.

Поступило в редакцию 25.10.2022
Принято к публикации 28.11.2022

Submitted 25.10.2022
Accepted 28.11.2022

Introduction

Identification, naming, and classification of living organisms at the species level are the foundation of all biology and has become one of the indispensable criteria in biodiversity analysis and management, conservation, and breeding (Vu, Le, 2019). Genetic analysis is exclusively a DNA-based technology recognized as "DNA barcoding". In the global infrastructure of biodata, DNA barcoding plays a main role, first, to solve fundamental problems of biodiversity. This is a diagnostic technique that uses short DNA sequence(s) for effective and accurate identification of different group of organisms, as well as unknown species (Ankola et al., 2021). Using united protocols of DNA isolation and analysis allows to significantly increase the efficiency of research and, therefore, the relevance of the results obtained, as well as using of public data repositories (NCBI, EMBL-EBI, GBIF, DDBJ) makes in demand the results in other natural sciences, not only in biodiversity, ecology, and genetics.

Developing of methods of the DNA analysis led to the creation of "The Consortium for the Barcode of Life" (CBOL) and "The Barcode of Life Data System" (BOLD). These depositaries with keeping of separate markers (barcodes) are also in demand for taxa identification.

DNA barcoding has also a wide and expanding range of practical applications, including the protection of biodiversity and rare species and the prevention of their collection and illegal sale; the control of plant raw materials, herbal teas, honey, and other commercial products; the control of weeds, invasive species, and allergy-causing plants, etc. (Koltunova et al., 2019; Shneyer, Rodionov, 2019); the genotyping both cultivated (Chinnappareddy et al., 2013; Mitrova et al., 2015) and wild plants (Herden et al., 2016; Sinitsyna et al., 2016; Smirnov et al., 2017).

In global DNA barcoding, there is unresolved question regarding the approved set of markers specifically for plants (Shneyer, Rodionov, 2019). So far, the nuclear-encoded ribosomal internal transcribed spacer (ITS) region and the chloroplast intergenic spacer *trn*H-*psb*A have emerged as candidates for barcoding plants, followed by others including coding sequences from plastid genes *rbc*L and *mat*K, two loci now the most commonly used for plants (Kress, Erickson, 2007; Yao et al., 2010; Loera-Sánchez et al., 2020; Guo et al., 2022). These markers can be used separately or in combination with other markers or spacers. Since a standard plant barcode has been complicated by the trade-off that arises between the high variability of sequences and high conservation of primers, it is then recommended to simultaneously utilize more than one marker as a compromise that best matches the barcoding criteria (Lahaye et al., 2008; Shneyer, Rodionov, 2019; Guo et al., 2022).

So, DNA barcoding is considered as a strong and promising tool in the field of molecular taxonomy for the taxonomists and conservation biologists worldwide to discover new species by performing unknown DNA sequence analysis on the DNA barcode database coupled with key morphological evidence (Ankola et al., 2021).

The Altai Mountain Country (AMC, Flora Altaica. http://altaiflora.asu.ru) is the highest modern uplift amongst the continental mountain countries in Siberia, as well as in Northern and Central Asia in general (Kamelin, 1998). This area occupies about 550 000 km^2 including the Chinese, Kazakh, Mongolian, and Russian Altai, as delimited by R. V. Kamelin (Kamelin, 2005; Vaganov et al., 2019). In 2002, David Olson and Eric Dinerstein singled Altai-Sayan territory as one of the 200 priority ecoregions of the world for global conservation of biodiversity in their work "The global 200 Priority ecoregions for global conservation" (Olson, Dinerstein, 2002). More than 2700 plant species, 300 of which are endemic, grow within the territory of the AMC (Vaganov et al., 2021). A list of 42 world scientific depositories containing the information on animals, plants and fungi findings of AMC placed in the Global Biodiversity Information Facility (GBIF) was obtained (Vaganov et al., 2019).

Plant biodiversity remains a potential source of novel human benefits, and the discovery of new taxa, as well as greater study of known taxa (Erst et al., 2022). Endemic species, those restricted in their distribution to a relatively small geographic area, are the most vulnerable to extinction (Chichorro et al., 2019; Erst et al., 2022). The type material of herbarium collections can play the key role in DNA barcoding in the study of new plant species, which are endemic in most cases.

The general fund of the ALTB Herbarium (South Siberian Botanical Garden, Barnaul, Russia) has more than 450 000 sheets. Of these, there are 334 items of typical material (as of date 20.11.2022). The publication of data on DNA sequences and distribution of AMC plants in gene banks and GBIF is one of the indicators of active work in the field of genetics and biodiversity informatics at the level of modern standards. In 2022, within the framework of

the RSF project "Study of Phytodiversity and Genetic Resources of the Altai Mountainous Country Based on Big Data", the process of DNA barcoding of the type material of the ALTB Foundation was started and work was carried out to digitize the collection (http://altb.asu.ru).

So, the purpose of our work was to sequence the main DNA markers as DNA barcode for type specimens of ALTB Herbarium. At the first stage, we chose 3 popular markers – ITS region of nrDNA, *trn*H–*psb*A intergenic spacer, and *trn*L–*trn*F intergenic spacer and *trn*L intron of plastid DNA.

Materials and methods

For molecular genetic study, we took material (little part of the dried plant) from 110 specimens of 72 type taxa of different taxonomic rank (species, subspecies, nothospecies, variations, etc.) of 16 families of the ALTB Herbarium, mainly from the territory of AMC. After revision of the type material for the analysis, the most numerous genera by number of representatives were *Alchemilla* L., *Veronica* L., *Potentilla* L. and *Gagea* Salisb.

DNA isolation and amplification were conducted in Laboratory of Bioengineering of the South Siberian Botanical Garden of Altai State University according to standard techniques (Kutsev, 2009). DNA was isolated using DiamondDNA kit (LLC "ABT", Russia) according to the manufacturer's instructions.

Amplification of the marker fragments of nuclear DNA (ITS1-5.8S-ITS) and chloroplast DNA (*trn*L-intron, *trnL-trn*F spacer, *psb*A–*trn*H spacer) was carried out on the thermocycler TC-Plus (Techne Workbench, United Kingdom) in 30 μl reaction mix included 12 μl H_2O, 15 μl HS-Taq PCR-Color (2x) mastermix (BioLabMix), 1 μl DNA, and 1 μl (10 mM) each primer. We used the following primers: ITSfor and ITSrew (Kutsev et al., 2014), trnLF-f and trnLF-r (Taberlet et al., 2007), trnH and psbA (Shaw et al., 2007) and amplification programs:

95 °C – 4 min., (95 °C – 20 sec., 56 °C – 30 sec., 72 °C – 1 min.) × 35 cycles, 72 °C – 7 min. for nuclear DNA fragments; 94 °C – 4 min., (95 °C – 30 sec., 60 °C – 30 sec., 72 °C – 1 min.) × 35 cycles, 72 °C – 5 min. for plastid fragments (*trn*L-intron, *trn*L-*trn*F spacer); 94 °C – 4 min., (95 °C – 30 sec., 64 °C – 30 sec., 72 °C – 45 sec.) × 35 cycles, 72 °C – 7 min. for plastid fragments (*trn*H–*psb*A spacer).

Concentration of the DNA probe was determined fluorometrically by NanoPhotometer P360 Implen (Hamburg, Germany), as well as with electrophoresis in 1.5 % agarose gel using DNA ladder Step50plus (BioLabMix). PCR products were purified using magnetic buds CleanMag DNA (Evrogen, Russia) according to the manufacturer's instructions. Purified products were sequenced by Sanger-method in SB RAS Genomics Core Facility (Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia).

Obtained sequences were analyzed in Chromas 2.6.4, and then, in BLAST – for the sample confirmation. The resulted sequences were submitted in the international NCBI GenBank (see Table).

Table

Studied type herbarium specimens for DNA barcoding from the ALTB Herbarium

№	ALTB barcode (specimen voucher)	Taxon	ITS region NCBI accession number	*trn*L-*trn*F NCBI accession number	*trn*H-*psb*A NCBI accession number
1	1100036088	*Erysimum kotuchovii* D. A. German (***Erysimum quadrangulum* Desf.**)	OP558070	OP644519	OP672169
2	1100036413	*Erysimum mongolicum* D. A. German	OP558076	OP644523	OP672174
3	1100037147	*Draba czuensis* Revuschkin et A. L. Ebel			OP672170
4	1100036080	*Dontostemon senilis* subsp. *gubanovii* D. A. German (***Dontostemon gubanovii* (D. A. German) D. A. German**)	OP558071		OP672171
5	1100036137	*Smelowskia calycina* (Stephan ex Willd.) C. A. Mey. var. *brachycarpa* A. L. Ebel (***Smelowskia calycina* (Stephan ex Willd.) C. A. Mey.**)	OP558072		
6	1100036112	*Leiospora exscapa* (C. A. Mey.) F. Dvořák var. *pilosa* A. L. Ebel (***Leiospora exscapa* (C. A. Mey.) F. Dvořák**)	OP558073	OP644521	OP672173

Table (continued)

№	ALTB barcode (specimen voucher)	Taxon	ITS region NCBI accession number	*trn*L-*trn*F NCBI accession number	*trn*H-*psb*A NCBI accession number
7	1100036421	*Ptilotrichum canescens* var. *elongatiforme* A. L. Ebel		OP644522	
8	1100036145, 1100036146	*Sterigmostemum schmakovii* Kamelin et D. A. German	OP558074, OP558075		
9	1100036405	*Thellungiella botschantzevii* D. A. German (***Eutrema botschantzevii* (D. A. German) Al-Shehbaz et Warwick**)	OP558077		
10	1100036104	*Veronica reverdattoi* Krasnob.		OP644520	OP672172
11	1100037634	*Veronica spicata* subsp. *kamelinii* Kosachev	OP558082	OP644528	OP672179
12	1100037430	*Veronica* × *altaica* Kosachev	OP558079	OP644525	OP672176
13	1100036422	*Veronica* × *austrosibirica* Kosachev (***Veronica* × *altaica***)	OP558080	OP644526	OP672177
14	1100035080	*Veronica* × *sapozhnikovii* Kosachev	OP558081	OP644527	OP672178
15	1100036586, 1100035772	*Veronica* × *schmakovii* Kosachev	OP558083	OP644529, OP644530	OP672180, OP672181
16	1100036446	*Veronica* × *smirnovii* Kosachev et D. A. German	OP558078	OP644524	OP672175
17	1100036105	*Astragalus lenensis* Shemetova, Shaulo et Lomon.	OP558084		
18	1100000011	*Ranunculus schmakovii* Erst	OP558085	OP644531	
19	1100000001	*Ranunculus tuvinicus* Erst	OP558086		
20	1100000120	*Aconitum khanminthunii* A. A. Solovjev et Shmakov		OP644535	OP672189
21	1100045130	*Aquilegia synakensis* Shaulo et Erst	OP558087	OP644532	
22	1100044812	*Aquilegia aradanica* Shaulo et Erst	OP558088		
23	1100000091	*Gagea azutavica* Kotukhov	OP558091		OP672185
24	1100000029	*Gagea goljakovii* Levichev	OP558090		
25	1100000106	*Gagea kuraiensis* Levichev			OP672184
26	1100000057	*Gagea shmakoviana* Levichev			OP672183
27	1100000025	*Gagea xiphoidea* Levichev	OP558089	OP644533	OP672182
28	1100044814	*Fritillaria sonnikovae* Shaulo et Erst	OP558092	OP644534	OP672186
29	1100000098	*Waldsteinia tanzybeica* Stepanov			OP672187
30	1010000083	*Polypodium* × *vianei* Shmakov			OP672188
31	1100000022	*Scorzonera veresczaginii* Kamelin et S. V. Smirn. (***Takhtajaniantha veresczaginii* (Kamelin et S. V. Smirn.) Kamelin et S. V. Smirn.**)	OP558093	OP644536	OP672190
32	1100036087	*Corydalis subverticillata* Lazkov		OP644537	
33	1100036151	*Acantholimon karabajeviorum* Lazkov			OP672191
34	1100000065	*Neogaillonia botschantzevii* Lincz. (***Plocama botschantzevii* (Lincz.) M. Backlund et Thulin**)	OP558094		
35	1100000089	*Artemisia elenae* Kupr.	OP558095	OP644538	OP672192
36	1100000033	*Hieracium nasimovae* Stepanov	OP558096	OP644539	
37	1100036071	*Viola* × *talmensis* Vl. V. Nikitin (***Viola* × *vilnaensis* W. Becker**)		OP644540	
38	1100000110	*Elymus tzvelevii* Kotukhov (***Campeiostachys schrenkiana* (Fisch. et C. A. Mey. ex Schrenk) Drobow**)			OP672193

Table (continued)

№	ALTB barcode (specimen voucher)	Taxon	ITS region NCBI accession number	*trn*L-*trn*F NCBI accession number	*trn*H-*psb*A NCBI accession number
39	1100000112	*Eritrichium alpinum* Ovczinnikova			OP672194
40	1100036469	*Eritrichium kamelinii* Ovczinnikova			OP672195
41	1100036152	*Anoplocaryum tenellum* A. L. Ebel et Rudaya			OP672196
42	1100000095	*Oxytropis kaspensi*s Krasnob. et Pshenich.	OP558097	OP644541	
43	1100053760	*Phlomoides hypoviridis* Lazkov		OP644544	OP672209
44	1100053752	*Eremurus czatkalicus* Lazkov			OP672208
45	1100000034	*Achillea schmakovii* Kupr.	OP558098	OP644542	OP672197
46	1100037062	*Saussurea revjakinae* S. V. Smirn.		OP644543	OP672198
47	1100052034	*Alchemilla laxescens* Czkalov			OP672201
48	1100054125	*Alchemilla mininzonii* Czkalov			OP672204
49	1100052026	*Alchemilla oirotica* Czkalov			OP672200
50	1100051985	*Alchemilla pseudobungeana* Czkalov			OP672199
51	1100053450	*Alchemilla pustynensis* Czkalov			OP672205
52	1100052042	*Alchemilla vorotnikovii* Czkalov			OP672202
53	1100054109	*Alchemilla zimoenkensis* Czkalov			OP672203
54	1100042998	*Potentilla friesenii* Kechaykin et Shmakov			OP672212
55	1100000004	*Potentilla junatovii* Rudaya et A. L. Ebel		OP644545	OP672210
56	1100035874	*Potentilla khanminczunii* Keczaykin et Shmakov			OP672207
57	1100053474	*Potentilla rudolfii* Keczaykin et Shmakov			OP672206
58	1100053142	*Potentilla* × *chemalensi*s Kechaykin		OP644546	
59	1100053442	*Potentilla* × *habievii* Kechaykin			OP672211
60	1100054132	*Potentilla* × *jakovlevii* Kechaykin et Shmakov			OP672213

Results and discussion

The first stage result of DNA barcoding of the ALTB type material was the publication of molecular data on plant species relatively recently described in science, mainly from the AMC territory, of which a significant proportion belongs to rare and endemic ones.

In total, it was deciphered 102 nuclear and chloroplast DNA sequences of 60 taxa of vascular plants from the ALTB type material: 29 fragments (28 taxa) of nuclear-encoded ribosomal internal transcribed spacer (ITS) region, 28 fragments (27 taxa) of the chloroplast intergenic spacer *trn*L-*trn*F and *trn*L-intron, and 45 fragments (44 taxa) coding sequences from *trn*H–*psb*A spacer. The above data on DNA sequences were not equally successfully obtained for all taxa. In some samples, concentration of the PCR product was not enough to sequence. As a rule, the success of DNA extraction and further amplification was depended on the quality of the herbarium material.

The length of the ITS region in the data set was from 617 bp in *Neogaillonia botschantzevii* Lincz. (*Plocama botschantzevii* (Lincz.) M. Backlund et Thulin) to 701 bp in *Hieracium nasimovae* Stepanov., length of the *trn*L-*trn*F fragment – from 724 bp in *Oxytropis kaspensis* Krasnob. et Pshenich. to 960 bp in *Potentilla* × *chemalensis* Kechaykin, length of the trnH–psbA spacer – from 204 bp in *Acantholimon karabajeviorum* Lazkov to 623 bp in *Elymus tzvelevii* Kotukhov (*Campeiostachys schrenkiana* (Fisch. et C. A. Mey. ex Schrenk) Drobow).

Each obtained nucleotide sequence was downloaded in Genbank and identified by BLAST. In the most cases, the percent identity was 90–100 %. If it was less, it meant this taxon was absent in the database. The results are common for barcode

evaluations of endemic species, libraries of reference sequences in GenBank are poorly covered (Hebert et al., 2004; Erst et al., 2022).

The sequences were prepared and placed in GenBank with a unique number assigned (Table).

First column of the Table is presented barcode of the type specimen in ALTB Herbarium (Virtual Herbarium ALTB. http://altb.asu.ru). The second column includes names of type specimens as they are called on the herbarium labels. If this name is obsolete and is a synonym now, then the current name under which the taxon is registered with the NCBI is given in brackets. All taxonomic nomenclature was verified by POWO service (https://powo.science.kew.org/).

The taxa, for which both nuclear and chloroplast DNA sequences were obtained, were combined into a dataset and published in the Global Biodiversity Information Facility (Vaganov et al., 2022) through the Integrated Publishing Toolkit (IPT) data publisher's operator node (http://altb.asu.ru/ipt). The dataset "DNA barcodes of the vascular flora of the Altai Mountain Country: type material of the Herbarium ALTB" has information on DNA sequences (the term "associatedSequences" of the Darwin Core specification), data on the places of collection of type material ("decimalLatitude", "decimalLongitude"), links to digitized images of the herbarium on the Internet and other information, including labels.

Conclusion

The results of the study combine molecular genetics and digital technologies, and the end-to-end number of the type collection of ALTB Herbarium is integrated into the biodata architecture of GenBank and GBIF. In the future, this approach will make it possible to obtain objective results for solving the tasks on biodiversity, evolution, and ecology of endemic and other promising plant species. General open access to the original data of the study will allow identification of taxa and trace the dynamics of their area more reasonably and accurately. In the absence of other evidence, DNA barcoding creates hypotheses regarding new species rather than outright discovering them (Taylor, Harris, 2012; Guo et al., 2022). But it should be noted that barcoding must supplement morphological data for species description (Guo et al., 2022). In the applied aspect, the identification of plant objects directly affects the solution of social problems of environmental safety, is included in the food and health agenda, and is no less significant for nature protection activities in the transboundary territory of Russia, Kazakhstan, China, and Mongolia.

Acknowledgements

The research was supported by RSF (project No. 22-24-20002, https://rscf.ru/project/22-24-20002/).

REFERENCES / ЛИТЕРАТУРА

Ankola K., Mahadevegowda L. G., Melichar T., Boregowda M. H. 2021. Chapter 18. DNA barcoding: nucleotide signature for identification and authentication of livestock. In: Sukanta Mondal, Ram Lakhan Singh (eds.). *Advances in Animal Genomics.* Academic Press. Pp. 299–308. DOI: 10.1016/B978-0-12-820595-2.00018-7

Chichorro F., Juslen A., Cardoso P. 2019. A review of the relation between species traits and extinction risk. *Biological Conservation* 237: 220–229. DOI: 10. 1016/j.biocon.2019.07.001

Chinnappareddy L. R. D., Khandagale K., Chennareddy A., Ramappa V. G. 2013. Molecular markers in the improvement of Allium crops. *Czech J. Genet. Plant Breed.* 49: 131–139. DOI: 10.17221/111/2013-CJGPB

Erst A. S., Nikulin A. Yu., Nikulin V. Yu., Ebel A. L., Zibzeev E. V., Sharples. M. T., Baasanmunkh S., Choi H. JAE, Olonova M. V., Pyak A. I., Gureyeva I. I., Erst T. V., Kechaykin A. A., Luferov A., Maltseva S. Yu., Nobis M., Lian L., Wang W. 2022. Distribution analysis, updated checklist, and DNA barcodes of the endemic vascular flora of the Altai mountains, a Siberian biodiversity hotspot. *Systematics and Biodiversity* 20(1): 1–30. DOI: 10.1080/14772000.2022.2049391

Guo M., Yuan C., Tao L., Cai Ya., Zhang W. 2022. Life barcoded by DNA barcodes. *Conservation Genetics Resources* 14: 351–365. DOI: 10.1007/s12686-022-01291-2

Hebert P. D. N., Stoeckle M. Y., Zemlak T. S., Francis C. M. 2004. Identification of birds through DNA Barcodes. *PLoS Biology* 2:e312. DOI: 10.1371/journal.pbio.0020312

Herden T., Hanelt P., Friesen N. 2016. Phylogeny of *Allium* L. subgenus *Anguinum* (G. Don. ex W. D. J. Koch) N. Friesen (Amaryllidaceae). *Molecular Phylogenetics and Evolution* 95: 79–93. DOI: 10.1016/j.ympev.2015.11.004

Kamelin R. V. (ed.). 2005. *Flora Altaica. 1.* Barnaul: AzBuka. 340 pp. [In Russian] (***Камелин Р. В.*** (ред.). Флора Алтая. Т. 1. Барнаул: АзБука, 2005. 340 с.).

Koltunova A. M., Sinitsyna T. A., Skaptsov M. V., Uvarova O. V., Kutsev M. G. 2019. Molecular genetic markers of plants. In: *From bioproducts to bioeconomy: materials of the III interregional scientific and practical conference (with*

international participation) (Barnaul, November 7–8, 2019). Barnaul: Publishing house of Altai State University. Pp. 72–78. [In Russian] (***Колтунова А. М., Синицына Т. А., Скапцов М. В., Уварова О. В., Куцев М. Г.*** Молекулярно-генетические маркеры растений // От биопродуктов к биоэкономике: материалы III межрегион. науч.-практ. конф. (с междунар. участием) (г. Барнаул, 7–8 ноября 2019 г.). Барнаул: Изд-во Алт. ун-та, 2019. С. 72–78).

Kress W. J., Erickson D. L. 2007. A two-locus global DNA barcode for land plants: the coding *rbc*L gene complements the non-coding *trn*H–*psb*A spacer region. *PLoS ONE* 2:e508. DOI: 10.1371/journal.pone.0000508

Kutsev M. G. 2009. *Fragment Analysis of Plant DNA: RAPD, DAF, ISSR*. Barnaul: ARTIKA. 164 pp. [In Russian] (***Куцев М. Г.*** Фрагментный анализ ДНК растений: RAPD, DAF, ISSR. Барнаул: ARTIKA, 2009. 164 с.).

Kutsev M. G., Uvarova O. V., Sinitsyna T. A. 2014. A set of synthetic oligonucleotides for amplification and sequencing of ITS1-5.8S-ITS2 of vascular plants / Patent for invention No. 2528063, copyright holder FGBOU VPO "Altai State University", issued on 07/16/14, priority 02/18/2013. [In Russian] (***Куцев М. Г., Уварова О. В., Синицына Т. А.*** Набор синтетических олигонуклеотидов для амплификации и секвенирования ITS1-5,8S-ITS2 сосудистых растений. Патент на изобретение № 2528063, правообладатель ФГБОУ ВПО «Алтайский государственный университет», выдан 16.07.14, приоритет 18.02.2013).

Lahaye R., van der Bank M., Bogarin D., Warner J., Pupulin F., Gigot G., Maurin O., Duthoit S., Barraclough T. G. 2008. DNA barcoding the floras of biodiversity hotspots. *Proc. Natl. Acad. Sci. U.S.A.* 105(8): 2923–2928. DOI: 10.1073/pnas.0709936105

Loera-Sánchez M., Studer B., Kölliker R. 2020. DNA barcode trnH-psbA is a promising candidate for efficient identification of for-age legumes and grasses. *BMC Res Notes* 13: 35. DOI: 10.1186/s13104-020-4897-5

Mitrova K., Svoboda P., Ovesna J. 2015. The selection and validation of a marker set for the differentiation of onion cultivars from the Czech Republic. *Czech J. Genet. Plant Breed.* 51(2): 62–67. DOI: 10.17221/16/2015-CJGPB

Olson D., Dinerstein E. 2002. The global 200: Priority ecoregions for global conservation. *Annals of the Missouri Botanical Garden* 89(2): 199–224. DOI: 10.2307/3298564

Shaw J., Lickey E. B., Schilling E. E., Small R. L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *Am. J. Bot.* 94: 275–288. DOI: 10.3732/ajb.94.3.275

Shneyer V. S., Rodionov A. V. 2019. Plant DNA Barcodes. Biology Bulletin Reviews, 9, 4: 295–300. DOI: 10.1134/S207908641904008X

Sinitsyna T. A., Herden T., Friesen N. 2016. Dated phylogeny and biogeography of the Eurasian *Allium* section *Rhizirideum* (Amaryllidaceae). *Plant Syst. Evol.* 302(9): 1311–1328. DOI: 10.1007/s00606-016-1333-3

Smirnov S., Skaptsov M., Shmakov A., Fritsch R., Friesen N. 2017. Spontaneous hybridization among *Allium tulipifolium* and *A. robustum* (*Allium* subg. *Melanocrommyum*, Amaryllidaceae) under cultivation. *Phytotaxa* 303(2): 155–164. DOI: 10.11646/phytotaxa.303.2.5

Taberlet P., Coissac E., Pompanon F., Gielly L., Miquel C., Valentini A. 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.* 35, e14. DOI: 10.1093/nar/gkl938

Taylor H. R., Harris W. E. 2012. An emergent science on the brink of irrelevance: A review of the past 8 years of DNA barcoding. *Molecular Ecology Resources* 12: 377–388. DOI: 10.1111/j.1755-0998.2012.03119.x

Vaganov A. V., Shmakov A. I., Gudkova P. D. 2019. Global data on biodiversity of the Altai mountain country, presented in the world's scientific depositories. *Acta Biologica Sibirica* 5(2): 95–101. DOI: 0.14258/abs.v5.i2.5937

Vaganov A. V., Shmakov A. I., Smirnov S. V., Usik N. A., Shibanova A. A., Kechaykin A. A., Kosachev P. A., Kopytina T. M., Zholnerova E. A., Medvedeva K. E., Zaikov V. F., Sinitsyna T. A., Shalimov A. P., Antonyuk E. V., Gudkova P. D., Dmitriev D. A., Batkin A. A., Kasatkin D. E., Belkin D. L. 2021. Virtual Herbarium ALTB: collection of vascular plants of the Altai Mountain Country. *Biodiversity Data Journal* 9: e67616.

Vaganov A. V., Sinitsyna T. A., Kutsev M. G., Zholnerova E. A., Kechaykin A. A., Kosachev P. A., Smirnov S. V., Shmakov A. I. 2022. *DNA barcodes of the vascular flora of the Altai Mountain Country: type material of the Herbarium ALTB.* Version 1.2. Altai State University. Occurrence dataset (accessed via GBIF.org on 2022-11-22). DOI: 10.15468/gtxnbz

Vu H., Le L. 2019. Bioinformatics analysis on DNA barcode sequences for species identification: a review. *Annu. Res. Rev. Biol.* 34(1): 1–12.

Yao H., Song J., Liu C., Luo K., Han J., Li Y., Pang X., Xu H., Zhu Y., Xiao P., Chen S. 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS ONE* 5(10): e13102. DOI: 10.1371/journal.pone.0013102