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

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

Summary. The article presents the second stage results of the work on DNA barcoding of type specimens of ALTB Herbarium (Barnaul, Russia). Obtained sequences of ITS and *trnL-trnF*, *trnH-psbA*, *rbcL* 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. Часть 2

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

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Аннотация. В статье представлены результаты второго этапа работы по ДНК-штрихкодированию типовых образцов, хранящихся в Гербарии Алтайского государственного университета (ALTB, г. Барнаул, Россия). Полученные нуклеотидные последовательности маркерных фрагментов ДНК (ITS, *trnL-trnF*, *trnH-psbA*, *rbcL*) внесены в базу данных NCBI GenBank, и соответствующий датасет опубликован в GBIF.

In 2023, we continued the DNA barcoding of type specimens of ALTB Herbarium (Barnaul, Russia, http://altb.asu.ru), as well as digitizing the collection (Vaganov et al., 2022a, b). The general fund of the ALTB Herbarium (South Siberian Botanical Garden, Barnaul, Russia) has more than 450 000 sheets. Of these, there are 338 items of typical material (as of date). The publication of data on DNA sequences and distribution of AMC plants in gene banks and GBIF is of modern standards.

DNA barcoding is considered as a strong and promising tool in molecular taxonomy 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 purpose of our work was to continue sequencing the main DNA markers as DNA barcode for type specimens of ALTB Herbarium.

At the second stage, we sequenced ITS region of nrDNA, *trnH–psbA* intergenic spacer, *trnL–trnF* intergenic spacer, and *trnL* intron and added to the study *rbcL* gene of plastid DNA.

Materials and methods

Material for the sequencing was dried parts (leaves, flowers) of type herbarium specimens from ALTB Herbarium. In total, the material from 110 samples (105 taxa of different taxonomic rank: species, subspecies, nothospecies, variations of 18 families) was selected.

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 fragments of nuclear (ITS1-5.8S-ITS – further ITS) and plastid (*trn*L-intron, *trn*L-*trn*F spacer – further *trn*L-F, *psb*A-*trn*H spacer – further *psb*A-*trn*H, *rbc*L gene – further *rbc*L) DNA was conducted in thermocyclers TECHNE TC-PLUS (Bibby Scientific, UK) and Bioer GeneExplorer (BIOER, China). PCR mix in volume 25 µl included 9,5 µl H_2O, 12,5 µl HS-qPCR color 2x mastermix (BioLabMix), 1 µl DNA, and 1 µl (10 mM) each primer.

We used the following primers: ITSfor–ITSrew (Kutsev et al., 2014), trnLF-f and trnLF-r (correspond Tab-c and Tab-f in Taberlet et al., 1991), psbA and trnH (Sang et al., 1997), rbcL1F and rbcL 819R, rbcL 595F and rbcL1379R (Hasebe et al., 1995) and programs: 5 °C – 4 min, (95 °C – 20 s, 55 °C – 45 s, 72 °C – 30 s) × 35 cycles, 72 °C – 7 min for nuclear DNA fragments; 95 °C – 4 min, (95 °C – 20 s, 65 °C – 45 s, 72 °C – 30 s) × 30 cycles, 72 °C – 7 min for plastid fragments (*trn*L-F, *psb*A-*trn*H:); 95 °C – 2 min, (95 °C – 30 s, 52 °C – 60 s, 72 °C – 90 s) × 35 cycles, 72 °C – 7 min for plastid fragments (*rbcL*).

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 1).

Table 1

Studied type herbarium specimens for DNA barcoding from the ALTB Herbarium
(Taxa in the 3rd column are presented in the alphabet order. If the taxon has the second name in brackets, this name is in priority and accepted in international databases, i. p. NCBI)

No.	ALTB barcode (specimen voucher)	Taxon	ITS NCBI number	*trnL-F* NCBI number	*ps*bA-trnH NCBI number	*rbcL* NCBI number
1.	1100036151	*Acantholimon karabajeviorum* Lazkov	–	–	OP672191*	–

Table 1 (continued)

No.	ALTB barcode (specimen voucher)	Taxon	ITS NCBI number	*trnL-F* NCBI number	*ps*bA-trnH NCBI number	*rbcL* NCBI number
2.	1100000034	*Achillea schmakovii* Kupr.	OP558098	OP644542	OP672197	–
3.	1100000120	*Aconitum khanminthunii* A. A. Solovjev et Shmakov	OR722521*	OP644535*	OP672189*	–
4.	1100052034	*Alchemilla laxescens* Czkalov	–	–	OP672201*	–
5.	1100054125	*Alchemilla mininzonii* Czkalov	–	–	OP672204*	–
6.	1100052026	*Alchemilla oirotica* Czkalov	–	–	OP672200*	–
7.	1100051985	*Alchemilla pseudobungeana* Czkalov	–	–	OP672199*	–
8.	1100053450	*Alchemilla pustynensis* Czkalov	–	–	OP672205*	–
9.	1100052042	*Alchemilla vorotnikovii* Czkalov	–	–	OP672202*	–
10.	1100054109	*Alchemilla zimoenkensis* Czkalov	–	–	OP672203*	–
11.	1100036152	*Anoplocaryum tenellum* A. L. Ebel et Rudaya	OR722536*	–	OP672196*	–
12.	1100044812	*Aquilegia aradanica* Shaulo et Erst	OP558088	–	–	–
13.	1100045130	*Aquilegia synakensis* Shaulo et Erst	OP558087	OP644532*	–	–
14.	1100000089	*Artemisia elenae* Kupr.	OP558095*	OP644538*	OP672192*	–
15.	1100036105	*Astragalus lenensis* Shemetova, Shaulo et Lomon.	OP558084*	OR763017*	–	–
16.	1100000045	*Cancrinia krasnoborovii* Khanm.	OR722522*	–	OR818577*	–
17.	1100036087	*Corydalis subverticillata* Lazkov	–	OP644537*	–	–
18.	1100036080	*Dontostemon senilis* subsp. *gubanovii* D. A. German (***Dontostemon gubanovii* (D. A. German) D. A. German**)	OP558071	OR763014	OP672171*	–
19.	1100037147	*Draba czuensis* Revuschkin et A.L. Ebel	OR722529*	–	OP672170*	–
20.	1100037155	*Draba sibirica* var. *eurycarpa* A. L. Ebel	OR722531	OR763015	OR818570*	–
21.	1100000110	*Elymus tzvelevii* Kotukhov (***Campeiostachys schrenkiana* (Fisch. et C. A. Mey. ex Schrenk) Drobow**)	OR722534	OR763020	OP672193	–
22.	1100053752	*Eremurus czatkalicus* Lazkov	–	–	OP672208*	–
23.	1100000112	*Eritrichium alpinum* Ovczinnikova	OR722535*	–	OP672194*	–
24.	1100036469	*Eritrichium kamelinii* Ovczinnikova	OR722523*	–	OP672195*	–
25.	1100036088	*Erysimum kotuchovii* D. A. German	OP558070*	OP644519*	OP672169*	–

Table 1 (continued)

No.	ALTB barcode (specimen voucher)	Taxon	ITS NCBI number	*trnL-F* NCBI number	*ps*bA-trnH NCBI number	*rbcL* NCBI number
26.	1100036413	*Erysimum mongolicum* D. A. German	OP558076	OP644523*	OP672174*	–
27.	1100044814	*Fritillaria sonnikovae* Shaulo et Erst	OP558092	OP644534*	OP672186	–
28.	1100000091	*Gagea azutavica* Kotukhov	OP558091*	–	OP672185*	–
29.	1100000029	*Gagea goljakovii* Levichev	OP558090*	OR763018*	OR818576*	–
30.	1100000106	*Gagea kuraiensis* Levichev	–	–	OP672184*	–
31.	1100000057	*Gagea shmakoviana* Levichev	OR722528	–	OP672183	–
32.	1100000025	*Gagea xiphoidea* Levichev	OP558089	OP644533	OP672182	–
33.	1100000033	*Hieracium nasimovae* Stepanov	OP558096*	OP644539*	OR818578*	–
34.	1100036112	*Leiospora exscapa* var. *pilosa* A. L. Ebel	OP558073*	OP644521*	OP672173*	–
35.	1100000065	*Neogaillonia botschantzevii* Lincz. (***Plocama botschantzevii* (Lincz.) M. Backlund et Thulin**)	OP558094	OR763019	–	–
36.	1100000095	*Oxytropis kaspensis* Krasnob. et Pshenich.	OP558097*	OP644541*	OR818580*	–
37.	1100053760	*Phlomoides hypoviridis* Lazkov	–	OP644544*	OP672209*	–
38.	1010000112	*Polypodium kamelinii* Shmakov	–	–	–	OR805770*
39.	1010000083	*Polypodium* × *vianei* Shmakov	–	–	OP672188*	OR805769*
40.	1100053142	*Potentilla* × *chemalensis* Kechaykin	–	OP644546*	OR818584*	–
41.	1100053442	*Potentilla* × *habievii* Kechaykin	OR722527*	OR763022*	OP672211*	–
42.	1100054132	*Potentilla* × *jakovlevii* Kechaykin et Shmakov	–	–	OP672213*	–
43.	1100053645	*Potentilla approximata* Bunge	–	–	OR818582	–
44.	1100053507		OR722526*	–	OR818583	–
45.	1100042998	*Potentilla friesenii* Kechaykin et Shmakov	–	OR763023*	OP672212*	–
46.	1100000004	*Potentilla junatovii* Rudaya et A. L. Ebel	OR722525*	OP644545*	OP672210*	–
47.	1100035874	*Potentilla khanminczunii* Keczaykin et Shmakov	–	–	OP672207*	–
48.	1100053474	*Potentilla rudolfii* Keczaykin et Shmakov	OR722524*	OR763021*	OP672206*	–
49.	1100051995	*Potentilla tuvinica* Artemov	–	–	OR818581*	–
50.	1100036421	*Ptilotrichum canescens* var. *elongatiforme* A. L. Ebel	–	OP644522*	OR818571*	–
51.	1100000011	*Ranunculus schmakovii* Erst	OP558085*	OP644531*	OR818575*	–
52.	1100000001	*Ranunculus tuvinicus* Erst	OP558086*	–	–	–

Table 1 (continued)

No.	ALTB barcode (specimen voucher)	Taxon	ITS NCBI number	*trnL-F* NCBI number	*p*sbA-trnH NCBI number	*rbcL* NCBI number
53.	1100037062	*Saussurea revjakinae* S. V. Smirn.	OR722537*	OP644543*	OP672198*	–
54.	1100000022	*Scorzonera veresczaginii* Kamelin et S. V. Smirn. (***Takhtajaniantha veresczaginii* (Kamelin et S. V. Smirn.) Zaika, Sukhor. et N. Kilian)**	OP558093*	OP644536*	OP672190*	–
55.	1100036137	*Smelowskia calycina* var. *brachycarpa* A. L. Ebel	OP558072*	–	OR818569*	–
56.	1100036145	*Sterigmostemum schmakovii* Kamelin et D. A. German	OP558074	–	OR818572*	–
57.	1100036146		OP558075	OR763016*	OR818573*	–
58.	1100036405	*Thellungiella botschantzevii* D. A. German (***Eutrema botschantzevii* (D. A. German) Al-Shehbaz et Warwick**)	OP558077	–	OR818574	–
59.	1100037430	*Veronica* × *altaica* Kosachev	OP558079	OP644525*	OP672176	–
60.	1100036422	*Veronica* × *austrosibirica* Kosachev	OP558080*	OP644526*	OP672177*	–
61.	1100035080	*Veronica* × *sapozhnikovii* Kosachev	OP558081*	OP644527*	OP672178	–
62.	1100036586	*Veronica* × *schmakovii* Kosachev	OP558083*	OP644529*	OP672180	–
63.	1100035772		–	OP644530*	OP672181	–
64.	1100036446	*Veronica* × *smirnovii* Kosachev et D. A. German	OP558078*	OP644524*	OP672175	–
65.	1100036104	*Veronica reverdattoi* Krasnob.	OR722530*	OP644520*	OP672172	–
66.	1100037634	*Veronica spicata* subsp. *kamelinii* Kosachev	OP558082	OP644528*	OP672179	–
67.	1100036071	*Viola* × *talmensis* Vl. V. Nikitin	OR722533*	OP644540*	OR818579*	–
68.	1100000098	*Waldsteinia tanzybeica* Stepanov **(syn. *Geum tanzybeicum* (Stepanov) Smedmark)**	OR722532	–	OP672187	–
69.	1010000078	*Woodsia gorovoii* Krestsch. et Shmakov	–	–	–	OR805771*

Results and discussion

Totally, DNA was isolated from 103 samples (7 samples – duplicates). The most numerous genera by number of representatives were *Alchemilla* L., *Veronica* L., *Potentilla* L., and *Gagea* Salisb. mainly from the territory of Altai Mountain Country.

During the work, primers and optimal conditions for PCR were selected. As a result of amplification, sequencing and computer processing, the following nucleotide sequences were deciphered:

ITS – 46 samples (45 taxa), *trnL-trnF* – 38 samples (38 taxa), *psbA-trnH* – 61 samples (59 taxa), *rbcL*1 – 3 samples (3 taxa), *rbcL*2 – 3 samples (3 taxa).

We obtained the following lengths of DNA fragments:

ITS – from 583 bp in *Veronica reverdattoi* (OR722530) to 735 bp in *Saussurea revjakinae*

(OR722537), *trnL-F* – from 649 bp in *Draba sibirica* var. *eurycarpa* (OR763015) to 989 bp in *Elymus tzvelevii* (OR763020), *psbA-trnH* – from 204 bp in *Acantholimon karabajeviorum* (OP672191) to 623 bp in *Elymus tzvelevii* (OP672193).

For 9 type taxa of Pteridophytes stored in ALTB, the *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) gene was amplified. This fragment is routinely used in phylogenetic studies of ferns (Hasebe et al., 1995; Vaganov et al., 2020). *RbcL*1 and *rbcl*2 fragments were successfully obtained and deciphered for 3 taxa, and then combined into a single *rbcL* gene sequence and uploaded to the gene bank. Obtained fragment lengths: *Polypodium* × *vianei* has 1339 bp (OR805769), *Polypodium kamelinii* – 1096 bp (OR805770), *Woodsia gorovoii* – 1309 bp (OR805771).

151 sequences of nuclear and chloroplast DNA fragments were obtained for 66 taxa. 148 sequences were deciphered and uploaded to the NCBI GenBank (see Table 1). Of them, 109 were not previously published in NCBI (marked by asterisk (*) in the Table 1). Large amount of newly published markers can be explained by endemism and rarity of the taxa studied.

The taxa with nuclear and plastid markers deciphered were united in the dataset "DNA barcodes of the vascular flora of the Altai Mountain Country: type material of the Herbarium ALTB" (Vaganov et al., 2022b).

As a result of the work, the database "DNA barcodes of vascular plants of Altai, mountains of South Siberia and the Far East" (Vaganov et al., 2023) was published.

The research results unite molecular genetics and digital technologies and can help to solve problems in biodiversity, evolution, ecology, and systematics of rare and endemic plants.

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