Dushenkov et al. "DNA barcoding." CUNY Academic Works 2021: 1-2

Published online 9 June 2021

DNA BARCODING OF THE HIGH-ALTITUDE ARTEMISIA AND NEPETA SPECIES

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ABSTRACT

DNA barcoding was performed for four medicinal plant species from the mountain region of Tajikistan. The nucleotide sequences for *Artemisia sieberi*, *Artemisia scoparia, Artemisia vulgaris*, and *Nepeta glutinosa* were deposited into the GenBank at the National Center for Biotechnology Information.

INTRODUCTION

Since when in 2003 Paul Hebert, University of Guelph, [1] used the term "DNA barcode," this method of employing specific regions of DNA for species identification became a fast-growing area of research and implementation. Projects around the world target establishing DNA barcodes for all groups of living organisms and make these data publicly available in order to help understand, conserve, and utilize the world's biodiversity [2]. DNA barcoding has a wide 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*.[3]

In 2009 the CBOL (Consortium for the Barcodes of Life) Executive Committee proposed a plant DNA barcode consisting of a combination of two chloroplast DNA regions representing fragments of the *rbcL* and *matK* genes. In addition, it was reported that the addition of either an *ITS* (Internal transcribed spacer) region or *trnH–psbA* spacer increased intrageneric resolution.[3] The *ITS* and *trnH-psbA* combination is being successfully used for plant species identification and differentiation. [4]

Artemisia L. is a complex genus of medicinal importance. Publicly available chloroplast genomes of a few *Artemisia* species are insufficient to resolve taxonomic discrepancies at the species level. Also, there is a need to understand the geographical variation at the genetic level. DNA-based techniques have been widely used for assessing diversity and authentication of plant species of medicinal importance.

MATERIAL AND METHODS

Plant material: Plant material was collected during the summer of 2018 by Dr. Shifo Kurbonbekova. Plant species identification confirmed by Dr. Dovutsho Navruzshoev. Arial parts of the plant were dried and sent to Rutgers, The State University of New Jersey for processing.

Artemisia sieberi Besser, Bull. Soc. Imp. Naturalistes Moscou 9: 80 (1836): Gorno-Badakhshan Autonomous Region, Tajikistan, elevation 2166 m, GPS - 37.4918667°, 071.5957167°.

Artemisia scoparia Waldst. & Kit., Descr. Icon. Pl. Hung. 1: 66, t. 65: Rushon, Gorno-Badakhshan Autonomous Region, Tajikistan, left bank of the Gund river, Eastern slope of the Shugnan range, elevation 2766 m, GPS - 37.9019444°, 072.2116667°.

Artemisia vulgaris L., Sp. Pl. 2: 848 (1753): Gorno-Badakhshan Autonomous Region, Tajikistan, left bank of the Gund river, Eastern slope of the Shugnan range, elevation 3150 m, GPS - 37.8808333°, 072.2630556°.

Nepeta glutinosa Benth., Labiat. Gen. Spec. 735 (1835): Gorno-Badakhshan Autonomous Region, Tajikistan, Eastern slope of the Shugnan range, elevation 2400 m, GPS - 37.9036111°, 072.2088889°.

DNA barcoding: DNA isolation was done on dry plant material, using a modified CTAB method [5]. DNA concentration was measured in a NanoDrop (Thermo Scientific, USA) spectrophotometer, then DNA was diluted to 100 ng/μl for PCR reactions. PCR was done using primers designed to amplify the *ITS*

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Table 1. Primers used to amplify barcodes.

Primer name	Primer sequence	Reference	Primer pair
ITSF	CCTTATCATTTAGAGGAAGGAG	[6]	*ITS*
ITSR	TCCTCCGCTTATTGATATGC	[6]	
trnH_modF	CGCATGGTGGATTCACAATCC	Modified [7]	*trnH-psbA*
psbA_R	GTTATGCATGAACGTAATGCTC	[7]	

nuclear barcode, and the *trnH-psbA* plastid barcode in one of the following programs: 94 °C 5 min, 34 cycles of 94 °C 30 sec, 59 °C 30 sec, 72 °C 1 min, then 72 °C 10 min, or 94°C 5 min, 34 cycles of 94 °C 30 sec, 56 °C 30 sec, 72 °C 1 min, then 72 °C 10 min. PCR products were enzymatically purified using ExoSap-IT (Affymetrix) and sequenced at a commercial facility. Primers used to amplify barcodes are listed in Table 1.

The sequence data were directly submitted to GenBank at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov). GenBank® is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (Nucleic Acids Research, 2013 Jan;41(D1):D36-42) Table 2. GenBank is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI.

Table 2. GenBank accession numbers for nucleotide sequences

Artemisia_vulgaris_submit.sqn	Artemisia_vulgaris	MN504999
Artemisia_ITS_submit.sqn	Artemisia_sieberi	MN505000
Artemisia_ITS_submit.sqn	Artemisia_scoparia	MN505001
Artemisia_trnH-psbA_submit.sqn	Artemisia_scoparia	MN505003
Artemisia_trnH-psbA_submit.sqn	Artemisia_sieberi	MN505004
Nepeta_glutinosa_submit.sqn	Nepeta_glutinosa	MN505005

We provided DNA barcodes for four important species, however, there is a great need to produce DNA barcodes for medicinal plants from a variety of regions in Tajikistan.

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