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PHYLOGENETIC TAXONOMY OF *ARTEMISIA* L. SPECIES FROM KAZAKHSTAN BASED ON *MAT*K ANALYSES

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The genus Artemisia *is one of the largest of the Asteraceae family. It is abundant and diverse, with complex taxonomic relations. In order to expand the knowledge about the classification of Kazakhstan species and compare it with classical studies,* mat*K genes of nine local species including endemic were sequenced. The infrageneric rank of one of them (*A. kotuchovii*) had remained unknown. In this study, we analysed results of sequences using two methods — NJ and MP and compared them with a median-joining haplotype network. As a result, monophyletic origin of the genus and subgenus Dracunculus was confirmed. Closeness of* A. kotuchovii *to other species of* Dracunculus *suggests its belonging to this subgenus. Generally,* mat*K was shown as a useful barcode marker for the identification and investigation of* Artemisia *genus.*

Key words: Artemisia, Artemisia kotuchovii*, DNA barcoding, haplotype network.*

INTRODUCTION

Artemisia of the family Asteraceae is a genus with great economic potential and importance. Species of this genus have been used in many aspects of life throughout the course of history (Lachenmeier, 2010; Petrovska, 2012). They have been used as medicinal, food, and ornamental plants (Weathers *et al*., 2011). A number of species have a very important medicinal significance, especially *Artemisia annua* L. (Klayman, 1985) and *Artemisia absinthium* L. (Lachenmeier, 2010). One of the most common examples is tarragon — *A. dracunculus* L. It is widespread in the wild across much of Eurasia and North America, and is cultivated for culinary and medicinal purposes. Its essential oils are used as antibacterial agents; dry leaves are added as flavourings for meat and fish (Aglarova *et al*., 2008; Obolskiy *et al.,* 2011).

Of the family Asteraceae, the tribe Anthemideae, genus *Artemisia* is one of the largest genera (Bremer and Humphries, 1993; Oberpreiler *et al*., 1995). It includes about 500 species, which are distributed in five subgenera (Vallčs and McArthur, 2001). Species of this genus are widespread in the Northern Hemisphere, particularly in Eurasian temperate zone and North America, and in South and North Africa (Bremer, 1994; Torrel *et al*., 1999). Due to the large amount of species in the genus, their classification is still complex and not fully completed. In earlier studies, the genus was subdivided into three subgenera (Poljakov, 1961; Kornkven *et al*., 1998). *Absinthium* and *Tridentatae* subg. were considered as the sections of *Artemisia* and *Seriphidum* subg., respectively. In more recent work already four subgenera were declared (Persson *et al*., 1974). *Absinthium* was proposed as a separate subgenus originated from the *Artemisia* subg. Based only on the capitula type and florets fertility, five major groups described as subgeneric or sectional rank (*Absinthium*, *Artemisia*, *Dracunculus*, *Seriphidium*, and *Tridentatae*) are more or less constantly found in classic studies confirmed by molecular data (Torrel *et al*., 1999). Previous phylogenetic studies on *Artemisia* showed monophyly of the genus and monophyly of the three main infrageneric groups (*Dracunculus*, *Seriphidium*, *Tridentatae*), whereas subgenera *Absinthium* and *Artemisia* were described as polyphyletic (Watson *et al*., 2002). Classical subgeneric separation based only on morphological traits was rearranged, because in some cases it was not supported by the traditional classifications (Sanz *et al.,* 2008; Tkach *et al*., 2008). Moreover, processes such as hybridisation, introgression, and polyploidisation are very common for these plants

and this makes understanding of their relations at the molecular level even more difficult (Winward and McArthur, 1995).

Central Asia is a centre of the genus *Artemisia* origin and one of the most important centres of its diversification (McArthur and Plummer, 1978; Wang, 2004). According to previous studies, most of the Asian species belong to the *Seriphidium* subg. (Poljakov, 1961; Tkach *et al*., 2008). In Kazakhstan, there are around 80 species mostly growing in steppes and deserts (Pavlov *et al.*, 1966). In general, endemic and rare species of the genus *Artemisia* in Kazakhstan are poorly studied and their place in subgenera classification is not well described. Only a few works were dedicated to their biochemistry and economical importance (Goryaev *et al*., 1962; Nikitina *et al*., 1964). The main aim of our study was to reveal the complex relations of subgenera in the genus *Artemisia* growing in Kazakhstan and compare results with traditional classifications based on both morphological traits (Poljakov, 1961) and molecular data (Watson *et al.,* 2002; Sanz *et al.*, 2008; Garcia *et al*., 2011). Another important objective was to determine the place of one unranked local species — *A. kotuchovii* — in the subgenera classification. Additionally, we tested two different methods for phylogenetic taxonomy (joining and maximum parsimony) and compared them to haplotype networking analysis.

This work represents a new direction in the study of Kazakhstan native flora. In the past, only a few reports were related to the description of the genetic variation of local flora (Adams *et al*., 1998; Turuspekov *et al.*, 2002). Therefore, the study is an expansion of a research oriented towards description of endemic, rare, and economically important species of the country and part of cooperative nation-wide project (Turuspekov and Abugalieva, 2015) for genotyping of plant accessions using DNA barcoding. The project combined efforts of local botanists and geneticists from biotechnology research organisations, botanical gardens, state nature parks, and reserves. In the last 20 years, DNA barcoding has shown itself as a powerful and efficient tool for sample identification and phylogeny of new and poorly studied species (Hebert *et al*., 2003; Hebert *et al*., 2005; Kress *et al*., 2017).

MATERIALS AND METHODS

Materials sampling. Nine populations of *Artemisia* species were collected from different places of central, southeastern, eastern, and western regions of Kazakhstan (Table 1, Fig. 1). For the reconstruction of intragenus topology sequences of twenty one *Artemisia* taxa were taken from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

DNA extraction, amplification and sequencing. Three plants from each population were chosen for the genetic analysis. Total genomic DNA was extracted from dry leaf material according to the modified Dellaporta DNA extraction protocol (Dellaporta *et al.,* 1983). Individual DNA samples were analysed separately. PCR fragments were amplified for the *maturase* K gene of the chloroplast genome (*mat*K) (Naeem *et al*., 2014).

All PCR reactions were carried out in 16 μl volumes in a Veriti Thermo cycler (Applied Biosystems, Foster City, CA, USA). One PCR reaction 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. Protocols for PCR reactions were taken from Jun *et al.* (2012). Primers chosen for PCR included *mat*K-F (5'-CCTATCCATCTGGAAATCTTAG-3') and *mat*K-R (5'-GTTCTAGCACAAGAAAGTCG-3') with annealing

Table 1

LIST OF *ARTEMISIA* SPECIES COLLECTED IN KAZAKHSTAN AND THEIR GENBANK ACCESSIONS NUMBERS

Region	Species	No. of collected plants	GenBank accession number *mat*K
Central KZ (Karkaraly, Bol'shoe lake)	*A. radicans* Kupr.	20 plants	MG282056
Eastern KZ (Valley of Kurchum River)	*A. gmelinii* Web. ex Stechm.	27 plants	MG282059
Eastern KZ (Southern Altai-Tarbagatai spine)	*A. kotuchovii* Kupr.	13 plants	MG282057
Eastern KZ (Kurchum River)	*A. sublessingiana* (Kell.) Krasch. ex Poljak.	20 plants	MG282053
Southeastern KZ (Almaty State Nature Reserve)	*A. santolinifolia* Turcz. ex Besser.	20 plants	MG282055
Southeastern KZ (Karatau State Nature Reserve)	*A. scopaeformis** Ledeb.	20 plants	MG282054
Southeastern KZ (Altyn Emel National Park)	*A. terrae-albae* Krasch.	20 plants	MG282052
Southeastern KZ (Karatau State Nature Reserve)	*A. transiliensis** Poljak.	21 plants	MG282051
Western KZ (West Karatau)	*A. gurganica* Willd.	20 plants	MG282058

* indicates endemic species for the Kazakhstan territory (Pavlov *et al*., 1966).

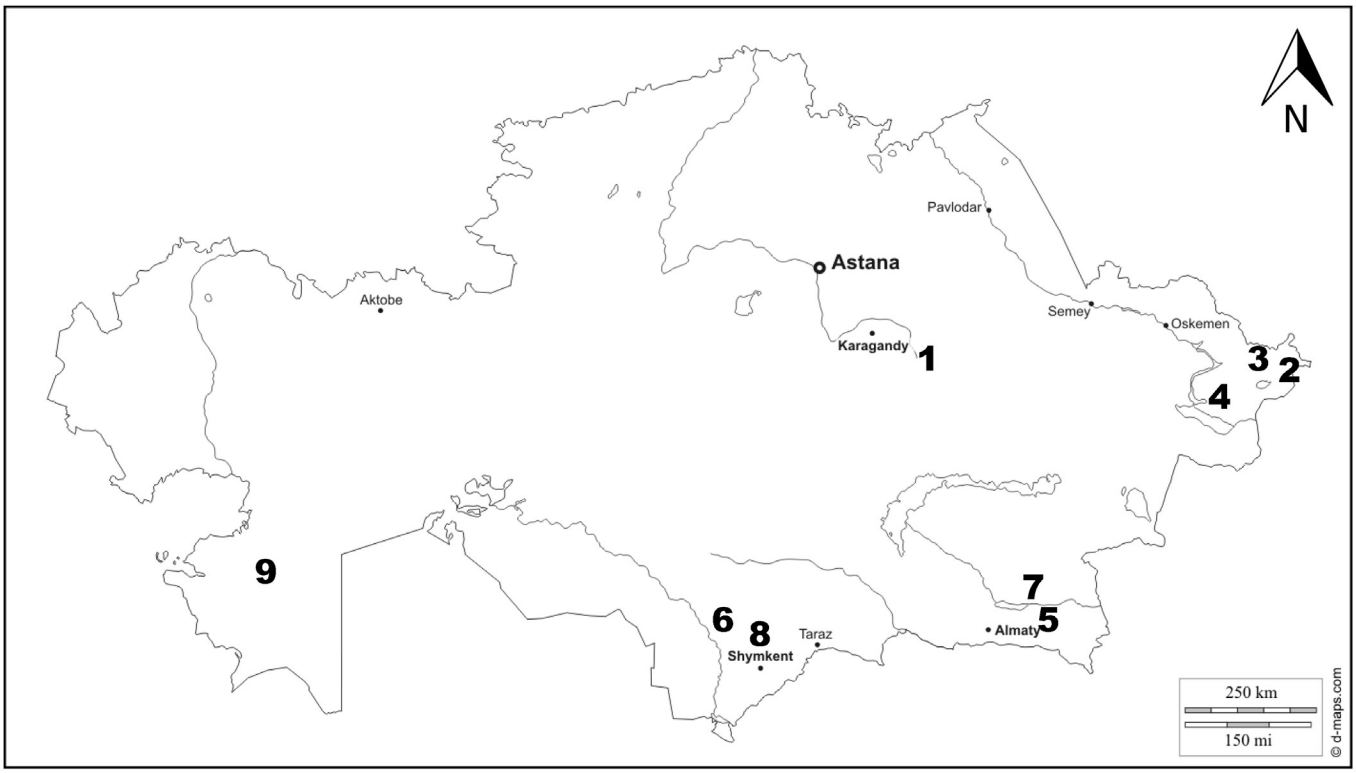

Fig. 1. Collected sites of *Artemisia* species.

One number denotes sampling point for the population of one species: 1 – *A. radicans* Kupr.; 2 – *A. gmelinii* Web. ex Stechm.; 3 – *A. kotuchovii* Kupr.; 4 – *A. sublessingiana* (Kell.) Krasch. ex Poljak.; 5 – *A. santolinifolia* Turcz. ex Besser.; 6 – *A. scopaeformis** Ledeb.; 7 – *A. terrae-albae* Krasch.; 8 – *A. transiliensis** Poljak.; 9 – *A. gurganica* Willd. * Indicates endemic plants for Kazakhstan territory (Pavlov *et al*., 1966).

temperature 50 °C and expected sizes of amplicons 784 bp (obtained from Asterales according to GenBank data).

Whole volume of each PCR product was checked by electrophoresis in 1.5% agarose gel at 80 V voltage for 40 min. Single bands with expected sizes for *mat*K were visualised, cut out from gel and purified using the 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 with the BigDye Terminator Cycle Sequencing technology (Applied Biosystems, Foster City, CA, USA) according to protocols of the company.

Alignment and phylogenetic analyses. Generated *mat*K sequences of the samples were imported in MEGA 6 (Tamura *et al*., 2013) software. Results were evaluated by different methods used for phylogenetic reconstructions — neighbour-joining (Bhattacharyya, Mukherjee, 2017) and maximum parsimony (Bryant *et al*., 2017).

The final alignment was imported into DNASP v5.10 (Librado and Rozas, 2009) and converted into Roehlfile format for the operations in the Network software (version 4.6; http://fluxus-engineering.com). In addition, the nucleotide sequences for *mat*K of local species were aligned with sequences of *Artemisia* species from the NCBI reference database. The genetic structure was assessed through median-joining haplotype networks (Bandelt *et al*., 1999) using the Network software. Post-processing calculation was done without the MP criterion ($\varepsilon = 0$).

RESULTS

DNA sequencing. DNA sequences of 784 bp of the *maturase* K gene (*mat*K) were obtained from nine local *Artemisia* species and aligned in MEGA 6.06 together with available *Artemisia* references from the GenBank. *Tanacetum parthenium* L., *Achillea ptarmica* L., and *Anthemis cotula* L. from the same tribe Anthemideae (Asteraceae) were chosen as the outgroups. In total, 9 sites with gaps and 17 polymorphic sites were detected for the studied *Artemisia* species. Nine of those sites were singleton variable sites and other eight were parsimony informative sites (Fig. 2). In this study we used two sets of data: 1) Kazakhstan species with specimens from Genbank; 2) Kazakhstan species only. There were no differences among DNA sequences in analysed three individual plants within nine studied species from Kazakhstan. The sequences of *mat*K of the nine species were deposited to the NCBI database (Table 1).

Phylogenetic and haplotype network analyses of local species and GenBank specimens. The first tree was reconstructed using the NJ method (bootstrap 1000) for 33 species, including outgroups (Fig. 3). All species of the genus

№	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Nucleotide position	147	170	216	233	258	259	260	261	262	263	264	265	266	278	280	350	404	421	445	473	507	511	537	602	611	632
KJ372399.1 *Artemisia roxburghiana**	G	C	A	A	C	T	T	G	C	A	G	A	A	G	C	T	C	T	G	T	G	G	C	T	G	G
KF648716.1 *Artemisia vulgaris**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
JQ412200.1 *Artemisia afra**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Artemisia radicans	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Artemisia sublessingiana	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Artemisia scopaeformis	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
Artemisia transiliensis	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
GQ434109.1 *Artemisia gmelinii**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
FN668458.1 *Artemisia arctisibirica**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
JQ173391.1 *Artemisia sieversiana**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
KF530805.1 *Artemisia japonica**	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	C	.
KC474124.1 *Artemisia borealis* subsp. *richardsoniana**	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	C	.
JQ173388.1 *Artemisia capillaris**	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	C	.
HM989797.1 *Artemisia scoparia**	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	C	.
JN894047.1 *Artemisia campestris**	T	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	.	.	.	.	.	C	.
KC474133.1 *Artemisia tilesii**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A
JQ173387.1 *Artemisia annua**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A
JQ173390.1 *Artemisia sacrorum**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A
KC474129.1 *Artemisia hyperborea (A. furcata)**	T	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
HM989729.1 *Artemisia lactiflora**	T	.	G	.	-	-	-	-	-	-	-	-	-	.	.	.	.	A	.	.	.	.	.	.	.	.
HM989726.1 *Artemisia argyi**	T	.	G	.	-	-	-	-	-	-	-	-	-	.	.	.	.	A	.	.	.	.	.	.	.	.
JQ173389.1 *Artemisia igniaria**	T	.	G	.	-	-	-	-	-	-	-	-	-	.	.	.	.	A	.	.	.	.	.	.	.	.
JN894044.1 *Artemisia absinthium**	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	A	.	G	.	.
HQ593182.1 *Artemisia dracunculus**	T	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	G	.	.	.	.	C	.
AF456776.1 *Artemisia tridentata**	T	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	.	.	.
Artemisia gurganica	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.	.	.
Artemisia terrae-albae	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	.	.	.	.
Artemisia santolinifolia	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	A	.	.	.	.	.	.	.	.
Artemisia kotuchovii	T	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	A	G	.	.	.	.	C	.
Artemisia gmelinii	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.

Fig. 2. Polymorphic sites of *Artemisia* species detected in *mat*K region. * Indicates specimens take from GenBank with their accession numbers. Endemic species for Kazakhstan are indicated in bold.

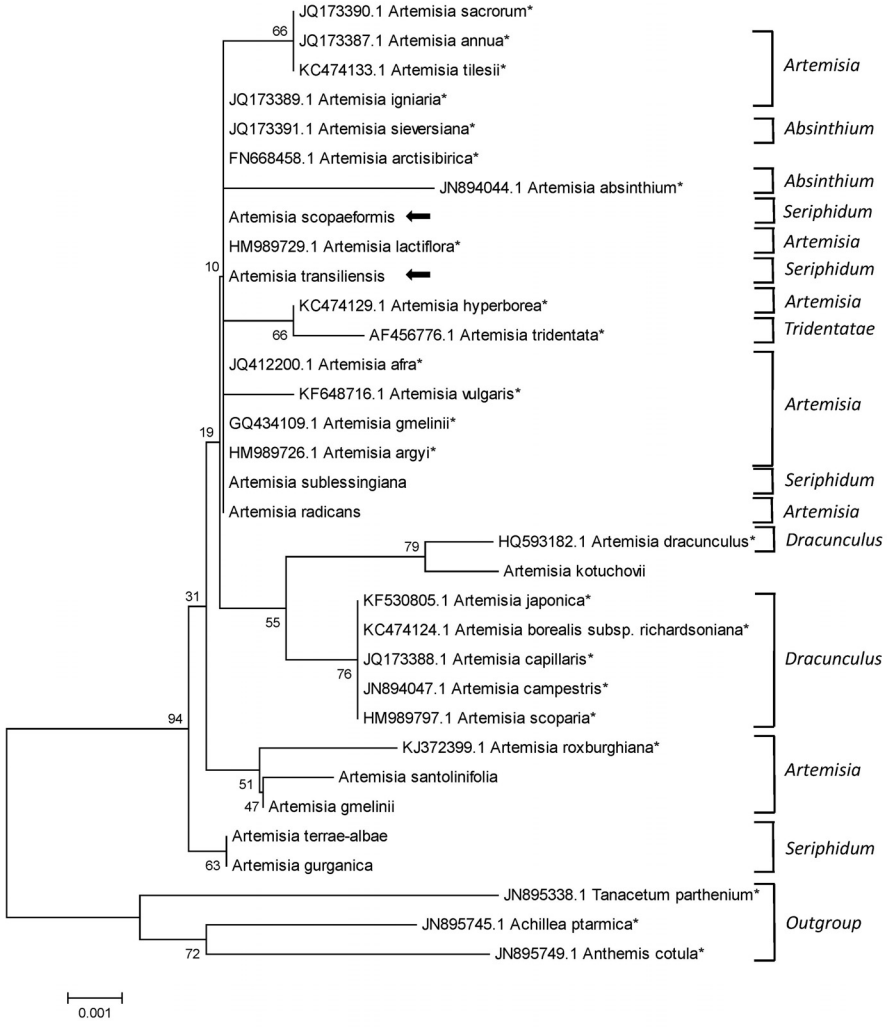

Fig. 3. Neighbour-joining phylogenetic tree results from the analysis of *mat*K sequences of nine local, twenty-one GenBank *Artemisia* species and three outgroup taxa. The subgenera classifications are given according to Poljakov, 1961. The lengths of branches are based on maximum composite likelihood and numbers at nodes shows a probability bootstrap. * denotes GenBank species with reference numbers from the NCBI database. Black arrows indicate endemic species.

Artemisia formed one large clade separately from outgroups. This *Artemisia* clade was further subdivided into four subclades. The first subclade included two local species (*A. terrae-albae* and *A. gurganica*) belonging to the

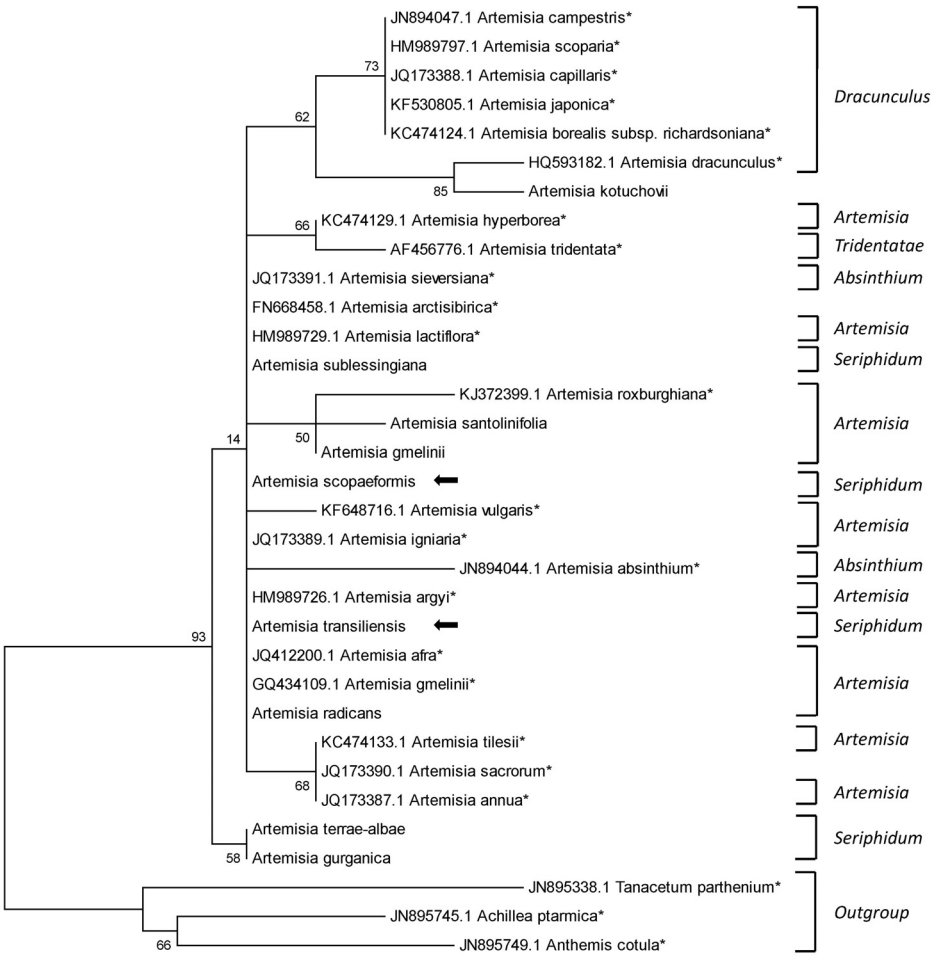

Fig. 4. Maximum parsimony phylogenetic tree reconstructed from the analysis of *mat*K sequences of nine local, twenty-one GenBank *Artemisia* species and three outgroup taxa. The subgenera classifications are given according to Poljakov, 1961. The lengths of branches are based on maximum composite likelihood and numbers at nodes shows a probability bootstrap. * denotes GenBank species with reference numbers from the NCBI database. Black arrows indicate endemic species.

subgenus *Seriphidum*. The next clade combined three species (*A. gmelinii*, *A. santolinifolia*, and *A. roxburghiana*) from the subg. *Artemisia*. The third subclade corresponded to the subg. *Dracunculus* and included all six species of this subgenus from GenBank. The local endemic species *A. kotuchovii* was also placed in this subclade. The last subclade was heterogeneous and represented four subgenera — *Artemisia, Absinthium, Seriphidum*, and *Tridentatae*.

The optimal MP tree was chosen from ten replicates (bootstrap 1000) for the same 33 species (Fig. 4) that had been studied using the NJ method. The generated tree showed different topology when it was compared to the NJ tree. In the MP tree all *Artemisia* species formed one major clade with two subclades apart from the outgroups. The first subclade was subgenus *Dracunculus,* which included *A. kotuchovii*. The second subclade was heterogeneous and included all other subgenera except *Dracunculus*.

The *mat*K dataset, which combined both the GenBank and local accessions, was used for the network association analysis. The Network incorporated 33 species with 16 haplotypes clustering in six major haplotype lineages, which corresponded to five *Artemisia* subgenera and the outgroup (Table 2). Mean haplotype diversity for the set was relatively high (Hd = 0.841), but nucleotide diversity was rather low (π = 0.0029, k = 2.211).

In order to compare topology of phylogenetic trees with the haplotype network, the studied nucleotide sequences combined in 16 haplotypes, including the outgroup, were analysed using Network 4.6. The obtained diagram shows consensus network with six groups of haplotypes corresponded to five subgenera and the outgroup cluster (Fig. 5). The largest haplotype H_6 in the centre of the diagram included 11 species from subg. *Artemisia*, *Absinthium*, and *Seriphidum*. The second largest haplotype was H_3, which contained five species from the subgenus *Dracunculus*, closely connected to *A. kotuchovii* in H_12. The haplotype H_9 was represented by *A. tridentatea* originated from the subg. *Artemisia* group, not supporting the theory of *Seriphidum* as the ancestor of the *Tridentatae* subg.

Phylogenetic and haplotype network analyses of local species. The second dataset was restricted to only nine Kazakhstan species and three outgroup taxa. This restricted dataset was also used for phylogenetic reconstruction by NJ and MP methods. Two trees showed very similar profiles for this dataset (Fig. 6 A, B). In both cases, subg. *Artemisia* species *A. santolinifolia* and *A. gmelinii* formed a separate subclade. *A. kotuchovii* was also placed apart from the others.

Local species sequences formed five haplotypes and were also used for median-joining network reconstruction (Table

Table 2

LIST OF HAPLOTYPES FORMED FROM THE ANALYSIS OF *MAT*K GENE SEQUENCES OF LOCAL *ARTEMISIA* SPECIES, GENBANK SPECIMENS AND THE OUTGROUP

Haplotype	Number of species	Species
H_1	1	KJ372399.1 *Artemisia roxburghiana**
H_2	1	KF648716.1 *Artemisia vulgaris**
H_3	5	HM989797.1 *Artemisia scoparia** JN894047.1 *Artemisia campestris** JQ173388.1 *Artemisia capillaris** KC474124.1 *Artemisia borealis* subsp. *richardsoniana** KF530805.1 *Artemisia japonica**
H_4	3	JQ173387.1 *Artemisia annua** JQ173390.1 *Artemisia sacrorum** KC474133.1 *Artemisia tilesii**
H_5	1	KC474129.1 *Artemisia hyperborea** (*Artemisia furcata*)
H_6	11	FN668458.1 *Artemisia arctisibirica** GQ434109.1 *Artemisia gmelinii** HM989726.1 *Artemisia argyi** HM989729.1 *Artemisia lactiflora** JQ173389.1 *Artemisia igniaria** JQ173391.1 *Artemisia sieversiana** JQ412200.1 *Artemisia afra** *Artemisia radicans* *Artemisia scopaeformis* ***Artemisia sublessingiana*** ***Artemisia transiliensis***
H_7	1	JN894044.1 *Artemisia absinthium**
H_8	1	HQ593182.1 *Artemisia dracunculus**
H_9	1	AF456776.1 *Artemisia tridentata**
H_10	2	*Artemisia gurganica* (*Artemisia fragrans* subsp. *gurganica* Krasch.) *Artemisia terrae-albae*
H_11	1	*Artemisia santolinifolia*
H_12	1	*Artemisia kotuchovii*
H_13	1	*Artemisia gmelinii*
H_14	1	JN895338.1 *Tanacetum parthenium**
H_15	1	JN895745.1 *Achillea ptarmica**
H_16	1	JN895749.1 *Anthemis cotula**

* indicates specimens taken from the GenBank with their accession numbers. Endemic species for Kazakhstan are indicated in bold.

3). Haplotype diversity for this set of data is Hd = 0.806, nucleotide diversity is π = 0.024, k = 1.889. As a result, they were combined into two major groups corresponding to *Artemisia* and *Seriphidum* subgenera (Fig. 6 C). The largest haplotype H_1 includes four species, three of them belonged to *Seriphidum* and one's subgenus (*A. radicans*) is *Artemisia*. *A. kotuchovii* H_4 was placed separately from them, just like on phylogenetic trees.

DISCUSSION

Artemisia is one of the most complex genera and it is represented by the large number of species, diverse morphological types, ploidy and complicated genetic relationships

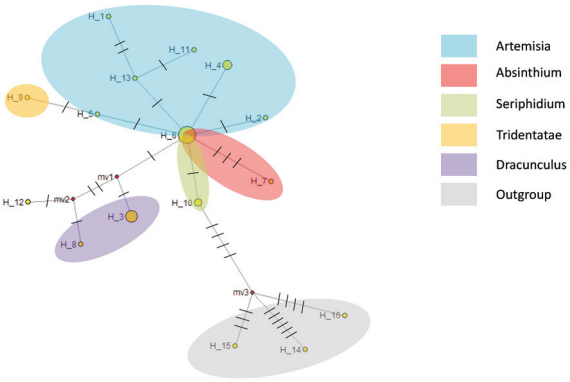

Fig. 5. Median-joining haplotype network. No MP criterion (ε = 0). Red small dots are median vectors presumed unsampled or missing intermediates. Yellow dots denote haplotypes; size is proportional to their frequencies. Number of perpendicular dashes on branches is equal to the number of mutations between two neighbouring dots. Colours denote the major groups based on subgeneric division. The subgenera classifications are given according to Poljakov, 1961.

(Winward and McArthur, 1995). Because of this, the clarification of the genus's taxonomy using classical botanical tools and morphological characteristics has many difficulties (Torrel *et al*., 1999). Therefore, usage of molecular markers is a valuable and promising addition to the traditional morphology-based classification. In this study, DNA barcoding approach based on the usage of *mat*K marker was applied for the assessment of the *Artemisia* taxonomy of nine species collected in Kazakhstan. The analysis of the *mat*K nucleotide sequences suggested that the marker provides sufficient information for differentiation of studied taxa. Generated NJ and MP trees of the studied taxa allowed to determine a single clade suggesting monophyletic origin of the genus, which was proposed earlier in the classical approaches (Torrel *et al.,* 1999; Watson *et al.*, 2002).

Previous molecular taxonomy studies with ITS using three main infrageneric groups of the genus (*Dracunculus*, *Seriphidium*, and *Tridentatae*) suggested that they have monophyletic origin, while the two remaining subgenera *Absinthium* and *Artemisia* appeared to be polyphyletic (Torrel *et al*., 1999; Sanz *et al*., 2008). Since the number of samples used in this study was limited, both NJ and MP phylogenetic trees only partially confirmed previously suggested taxonomic classification. For example, it is clearly visible, that the *Dracunculus* subclade is very distinct in both trees. *A. terrae-albae* and *A. gurganica* from subgenus *Seriphidium* formed the separate subclade distantly apart from the other species. Another outcome from the analysis of the phylogenetic trees was the taxonomy of less studied species — *A. kotuchovii.* The topology of both NJ and MP trees indicated that this species clearly belongs to the subgenus *Dracunculus.*

The haplotype networking diagram (Fig. 5) was rather more informative in comparison with the NJ and MP trees. First, the network analysis showed that haplotype H_6 was comprised from 11 species of subgenera *Artemisia, Absinthium,* and *Seriphidium.* Second, the H_6 was directly descendent

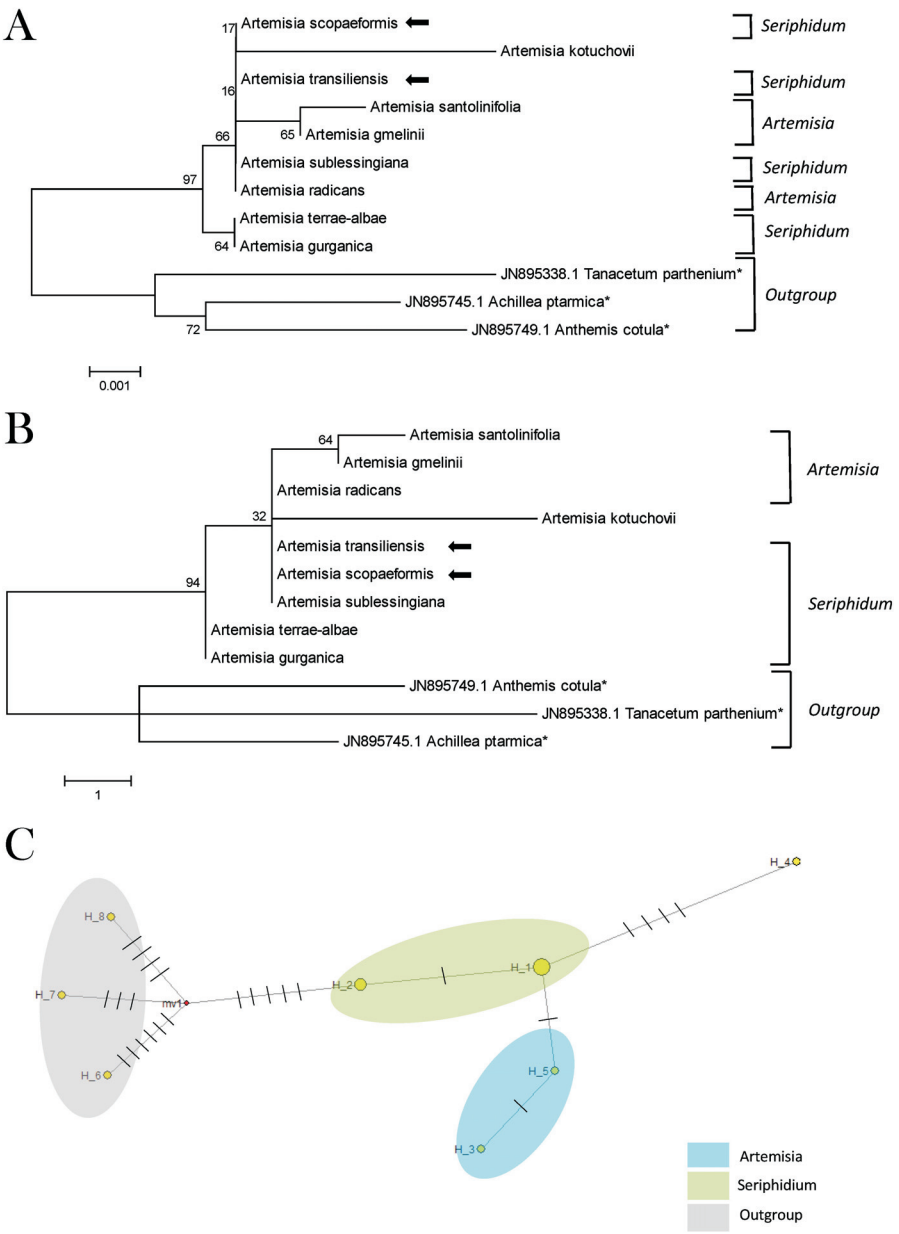

Fig. 6. Phylogenetic trees and the haplotype network based on the *matK* sequences of nine local *Artemisia* species and three outgroup taxa. **A.** Neighbour-joining phylogenetic tree. **B.** Maximum parsimony phylogenetic tree. **C.** Median-joining haplotype network. The subgenera classifications are given according to Poljakov, 1961. The lengths of branches are based on Maximum Composite Likelihood and numbers at nodes shows a probability bootstrap. * denotes GenBank species. Black arrows indicate endemic species. Red small dots are median vectors presumed unsampled or missing intermediates. Yellow dots denote haplotypes; size is proportional to their frequencies. Number of perpendicular dashes on branches is equal to the number of mutations between two neighbouring dots. Colours denote the major groups based on subgeneric division.

from H_10, consisting of two species of subg. *Seriphidium* (Table 2). The presence of *A. scopaeformis (Seriphidium)* in H_6 makes sense because it logically connects these two haplotypes. Third, the haplotype H_5 consisted of only one species (*A. hyperborea*) in this study, which descended from H_6. Fourth, the haplotype H_9 descended from H_5, which suggests that the subgenus *Tridentatae* originated directly from species of subgenus *Artemisia.*

It is interesting that the network suggests three subgroups within the subgenus *Artemisia.* As the topology of the network suggests that H_13 *(A. gmelinii)* is a predecessor of H_1 *(A. roxburghiana)* and H_11 *(A. santolinifolia),* it resembles the outcome from the MP tree (Fig. 4). The other outcome from the networking analysis is that haplotype H_7 (*A. absinthium, Absintium)* is directly connected to haplotype H_6, which is a reasonable connection because A. *sieversiana (Absintium)* is a part of this most frequent haplotype (H_6) in the study. Unlike in *Absinthium*, the haplotypes of *Dracunculus* (H_3 and H_8) are not connected to H_6 directly, but through intermediates mv1 and mv2, respectively (Fig. 5). As the mv2 connects *A. dracunculus* with *A. kotuchovii,* this confirms that this species belongs to the subg. *Dracunculus*.

In general, the analyses based on *mat*K indicated that *A. terrae-albae* and *A. gurganica* from the subg. *Seriphidium* are predecessors of all other taxa within the genus *Artemisia.* Therefore, the conclusions based on the usage of plastid genome marker is not completely congruent with outcomes based on nuclear genome markers ITS (Torrels *et al.*, 1999), as their parsimony analysis of 31 species resulted in a multifurcate type of the tree. Nevertheless, the authors indicated that the first clade of the tree was formed primarily from species of subg. *Seriphidium.* Later Watson with coauthors (Watson *et al.*, 2002) analysed a larger number of

Table 3

LIST OF HAPLOTYPES FORMED FROM THE ANALYSIS OF *MAT*K GENE SEQUENCES OF LOCAL *ARTEMISIA* SPECIES AND THE OUTGROUP TAXA

Haplotype	Number of species	Species
H_1	4	*Artemisia radicans* *Artemisia scopaeformis* ***Artemisia sublessingiana*** ***Artemisia transiliensis***
H_2	2	*Artemisia gurganica* (*Artemisia fragrans* subsp. *gurganica* Krasch.) *Artemisia terrae-albae*
H_3	1	*Artemisia santolinifolia*
H_4	1	*Artemisia kotuchovii*
H_5	1	*Artemisia gmelinii*
H_6	1	JN895338.1 *Tanacetum parthenium**
H_7	1	JN895745.1 *Achillea ptarmica**
H_8	1	JN895749.1 *Anthemis cotula**

* indicates specimens taken from the GenBank with their accession numbers. Endemic species for Kazakhstan are indicated in bold.

the genus *Artemisia* taxa consisting of 57 species of subtribe Artemisiinae from Old and New Worlds and pointed out on separation of subg. *Dracunculus* from all remaining *Artemisia* species. Therefore, one of the main conclusions in that study was the recognition of two subgenera within the *Artemisia* — subg. *Dracunculus* and an expanded subg. *Artemisia* (Watson *et al*., 2002). Phylogenetic trees and haplotype network generated using *mat*K in this study hinted that the *Dracunculus,* although it is genetically a distinct subclade, descended from subg. *Artemisia.*

CONCLUSIONS

The application of phylogeny and haplotype network analyses indicated that some of the species of subgenus *Seriphidium* can be predecessors of the genus *Artemisia*. Specifically, *A. terrae-albae* and *A. gurganica* of this subgenus were closest to outgroup species used in this study. The haplotype network analysis was more informative in comparison to generated NJ and MP trees, as it is suggested a hypothetical evolutionary pathway within the genus. The network showed that the most frequent haplotype H_6 was common for three subgenera *Artemisia, Absinthium,* and *Seriphidium.* The species of subgenera *Tridentatae* derived from the species of *Artemisia,* whereas species of subgenera *Dracunculus* were distantly apart from the remaining species of the genus but via intermediate median vectors associated with the major haplotype H_6 of the genus *Artemisia*. Also, it was shown that the earlier unstudied species *A. kotuchovii* is a part of the subgenus *Dracunculus.*

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KAZAHSTĀNAS *ARTEMISIA* L. SUGU FILOĢENĒTISKĀ TAKSONOMIJA, PAMATOJOTIES UZ *MAT*K ANALĪZI

Tika sekvencēti *mat*K gēni deviņām Kazahstānas *Artemisia* sugām, t.sk. endēmiskām. Tika apstiprināta monofiletiskā apakšģints *Dracunculus* izcelšanās. *A. kotuchovii* tuvums citām *Dracunculus* sugām norāda uz šīs sugas piederību minētai apakšģintij. *mat*K gēnu var veiksmīgi izmantot kā barkoda marķieri *Artemisia* ģints izpētē.