

Original Article

An investigation on the chloroplast and nuclear genomes of taxa belong to the subgenus *Dracunculus* (Bess.) Rydb. of *Artemisia* L. (Asteraceae) in Turkey

Uma investigação sobre o cloroplasto e os genomas nucleares de táxons pertencentes ao subgênero *Dracunculus* (Bess.) Rydb. de *Artemisia* L. (Asteraceae) na Turquia

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Abstract

Artemisia is one of the biggest genera in the family Asteraceae, with around 500-600 taxa at specific and sub-specific levels and organised in 5 subgenera. Due to the high number of taxa, a lot taxonomists are trying to solve the problem of its classification and phylogeny but its natural classification still hasn't been achieved. In this research, 60 individuals belonging to 4 taxa of the subgenus *Dracunculus* of *Artemisia* L. in Turkey were examined. For all the examined individuals from both the same and different populations belonging to the taxa of the subgenus *Dracunculus*, the sequences of the regions both *psbA-trnH* of chloroplast DNA and ITS of nuclear DNA were determined. Also, the gene regions obtained were recorded in the NCBI GenBank database and an accession number was taken. It was found that there was no gene flow and hybridization between the four studied taxa of the subgenus *Dracunculus*, and these 4 taxa also completed their speciation. According to the results of this molecular study, *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana* and *A. campestris* var. *araratica* were proposed to be raised from the variety level to the species level. This research is important as it is the first molecular based study relating with the subgenus *Dracunculus* growing in Turkey.

Keywords: *Artemisia*, Phylogeny, cpDNA *psbA-trnH*, r-DNA ITS, Turkey.

Resumo

Artemisia é um dos maiores gêneros da família Asteraceae, com cerca de 500 a 600 táxons em níveis específicos e subespecíficos e organizados em cinco subgêneros. Em razão do grande número de táxons, muitos taxonomistas estão tentando resolver o problema de sua classificação e filogenia, mas sua classificação natural ainda não foi alcançada. Nesta pesquisa, 60 indivíduos pertencentes a quatro táxons do subgênero *Dracunculus* de *Artemisia* L. na Turquia foram examinados. Para todos os indivíduos examinados de populações iguais e diferentes pertencentes aos táxons do subgênero *Dracunculus*, foram determinadas as sequências das regiões *psbA-trnH* do DNA do cloroplasto e ITS do DNA nuclear. Além disso, as regiões gênicas obtidas foram registradas no banco de dados do NCBI GenBank e um número de acesso foi obtido. Foi constatado que não houve fluxo gênico nem hibridização entre os quatro táxons estudados do subgênero *Dracunculus*, os quais também completaram sua especiação. De acordo com os resultados deste estudo molecular, *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana* e *A. campestris* var. *araratica* foram propostos para ser elevados do nível de variedade para o nível de espécie. Esta pesquisa é importante porque é o primeiro estudo de base molecular relacionado com o subgênero *Dracunculus* em crescimento na Turquia.

Palavras-chave: *Artemisia*, Filogenia, cpDNA *psbA-trnH*, r-DNA ITS, Turquia.

1. Introduction

Artemisia L. is one of the larger genera in the family Asteraceae and the largest genus in the tribe Anthemideae, and comprises of 200 to more than 500 taxa at the specific or subspecific level (McArthur, 1979; Mabberley, 1987; Ling, 1991a; Ling, 1991b; Bremer and Humphries, 1993; Ling, 1995a; Ling, 1995b; Torrell et al., 1999).

The genus *Artemisia* L. contains all life forms except trees: Annual, biennial and perennial herbs, suffruticoses (subshrubs), fruticose (shrubs), with some large in stature.

The genus *Artemisia* in the Flora of Turkey are not divided to sections or subgenera. At the same time, none of the species have infraspecific taxa.

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There are total of 23 species without any infraspecific taxa that belong to the genus *Artemisia* in the 5th and 10th volumes of the Flora of Turkey. The species *A. campestris* L., *A. marschalliana* Sprengel, *A. araratica* Krasch and *A. scoparia* Waldst. & Kit. are four of 23 independent species (Davis, 1975; Davis et al., 1988). In fact, these four species belong to the subgenus *Dracunculus*, but the genus *Artemisia* in the Flora of Turkey did not divide into subgenera.

Civelek et al. (2010) carried out a revisionary study of the genus *Artemisia* in Turkey. According to results of this revisionary study, there are 3 subgenera, 22 species, and 26 taxa, which also include 8 infraspecific taxa belonging to the genus *Artemisia* in Turkey (Civelek et al., 2010; Kursat, 2010; Kursat et al., 2011a; Kursat et al., 2011b; Kursat et al., 2014; Kursat et al., 2015; Kursat et al., 2018).

The taxa of genus *Artemisia* were divided into subgenera during the revisionary study. Subgenera *Artemisia* Less., *Dracunculus* (Bess.) Rydb. and *Seriphidium* (Bess.) Rouy. have taxa in Turkey, but the subgenus *Tridentatae* (Rydb.) McArthur which is endemic to North America has no taxa in Turkey (Civelek et al., 2010; Kursat, 2010; Guner et al., 2012). During the revisionary study of the genus *Artemisia*, at the same time, it was also observed that the distribution areas of three closely related independent species *A. campestris*, *A. marschalliana* and *A. araratica* in Flora of Turkey have a sympatric distribution, which are partially mixed together (Civelek et al., 2010; Kursat, 2010).

With their distributions in Turkey and with their morphological features in mind, the three closely related independent species *A. campestris*, *A. marschalliana* and *A. araratica* in Flora of Turkey were reduced to a variety levels and these varieties were linked to the species *A. campestris*.

The taxonomic positions and combinations of the closely related independent three species *A. campestris*, *A. marschalliana* and *A. araratica* in Flora of Turkey changed in the revisionary study of the genus *Artemisia* in Turkey are as follows: *A. campestris* L. var. *campestris*, *A. campestris* L. var. *marschalliana* (Spreng.) Poljak. and *A. campestris*

L. var. *araratica* (Novopokr.) Poljak. However, because they were needed for the molecular data, the accuracy of the classification based on the morphological data in the revisionary study could not be guaranteed.

For this reason, the purpose of this study was to evaluate the genetic diversity and taxonomic position of the subgenus *Dracunculus* species in Turkey using some molecular techniques. In addition, the first molecular data for the rDNA ITS and *psbA-trnH* gene region from Turkey were submitted to the international NCBI GenBank databases.

2. Material and Methods

2.1. Plant Material

The plant materials used in this study were samples that were collected and diagnosed in the project named "The taxonomic revision of the genus *Artemisia* L. (Asteraceae) growing in Turkey" conducted by Murat Kursat and Semsettin Civelek in 2010 (Kursat, 2010). Within the scope of this study, the plants that were gathered during the land survey conducted by M. Kursat and S. Civelek, which were turned into herbarium materials, were used. 60 different individuals belonging to the 4 taxa of the subgenus *Dracunculus* from 17 different populations in Turkey were used. These plants were kept as herbarium materials in the Herbarium in the Faculty of Sciences at Bitlis Eren University. Depending on the with of the populations belonging to the taxa, between 1 and 3 individuals with the same label information were used for each taxon. Localities and geographic distribution where the investigated taxa were gathered, were given in Table 1 and Figure 1.

2.2. DNA Isolation

For the DNA isolation, leaf texture of plants that were gathered during the land survey conducted by us (M.K. and

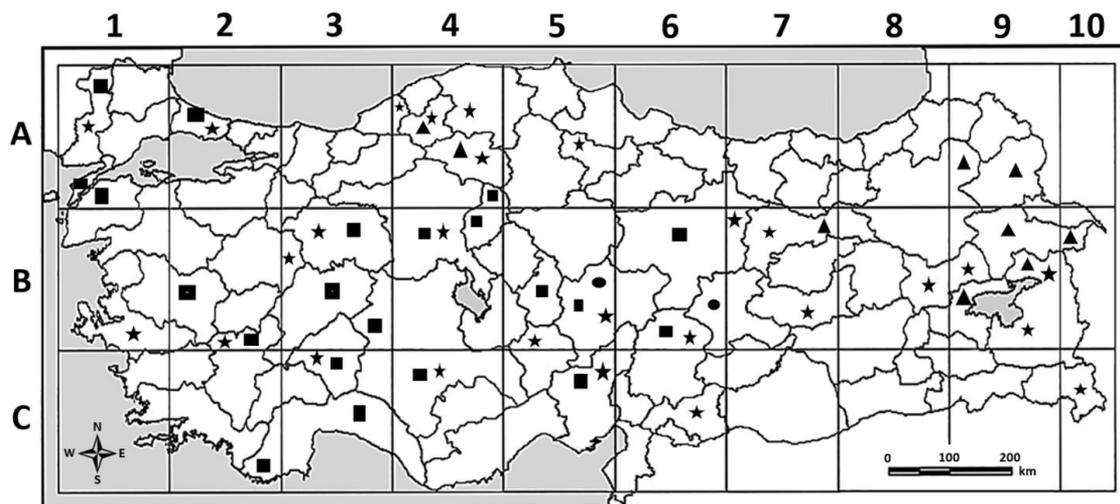


Figure 1. Geographic distribution of four species of the subgenus *Dracunculus* in Turkey (*A. campestris* (■), *A. marschalliana* (▲), *A. araratica* (●) and *A. scoparia* (★) (Civelek et al., 2010, Kursat 2010).

Table 1. The label information of examined individuals of specimens belong to 4 taxa of the subgenus *Dracunculus* used in the study.

Taxa of The Subgenus <i>Dracunculus</i>	Number of specimens	Collector	Collection date	Detailed localities of specimens populations
<i>A. campestris</i>	1096	M. Kursat	10.09.2007	B4 Ankara: N 39° 43.997, E 32° 23.860, 843m
<i>A. campestris</i>	1015	S. Civelek and M. Kursat	01.09.2007	B6 Kahramanmaras: N 38° 12.495, E 36° 56.488, 1231 m
<i>A. campestris</i>	1017	M. Kursat	02.09.2007	B6 Kahramanmaras: N 37° 56.285, E 36° 34.540, 1380 m
<i>A. campestris</i>	1018	S. Civelek and M. Kursat	02.09.2007	B6 Kahramanmaras: N 37° 56.285, E 36° 34.540, 1380 m
<i>A. campestris</i>	1022	M. Kursat	05.09.2007	C2 Antalya: N 37° 02.114, E 29° 53.304, 1430 m
<i>A. campestris</i>	1039	M. Kursat	11.09.2007	C5 Adana: N 37° 31.449, E 34° 39.159, 1185 m
<i>A. marschaliana</i>	1102	M. Kursat	28.10.2007	A4 Çankırı: N 40° 50.364, E 32° 42.350, 1070 m
<i>A. marschaliana</i>	1176	M. Kursat	27.08.2008	A9 Kars: N 40° 38.480 E 43° 04.524, 2010m
<i>A. marschaliana</i>	1046	S. Civelek and M. Kursat	19.09.2007	B9 Mus: N 38° 38.904, E 42° 47.182, 1284 m
<i>A. marschaliana</i>	1187	M. Kursat	02.11.2008	B9 Bitlis: N 38° 50.396, E 42° 46.917, 1975 m
<i>A. marschaliana</i>	1147	M. Kursat	22.06.2008	B9 Bitlis: N 38° 32.486, E 42° 21.303, 1715 m
<i>A. marschaliana</i>	1066	S. Civelek and M. Kursat	22.09.2007	B10 Agri: N 39° 42.618, 1728 m
<i>A. marschaliana</i>	1114	M. Kursat	26.11.2007	B10 Agri: N 39° 35.994, E 42° 55.698, 1605 m
<i>A. araratica</i>	1001	M. Kursat	04.07.2007	B6 Malatya: N 38° 11.942, E 37° 50.622, 1495 m
<i>A. araratica</i>	1002	M. Kursat	04.07.2007	B6 Malatya: N 38° 01.335, E 37° 55.289, 1280 m
<i>A. scoparia</i>	1030	S. Civelek and M. Kursat	10.09.2007	B4 Ankara: N 39° 42.876, E 32° 17.941, 796 m
<i>A. scoparia</i>	1003	M. Kursat	04.07.2007	B6 Kahramanmaras: N 37° 56. 285, E 36° 34.540, 1380 m
<i>A. scoparia</i>	1189	M. Kursat	03.11.2008	B7 Elazığ: N 38° 37.031, E 39° 02.095, 1160 m
<i>A. scoparia</i>	1169	M. Kursat	26.08.2008	B9 Van: N 38° 34.300, E 43° 38.770, 1750 m
<i>A. scoparia</i>	1038	M. Kursat	11.09.2007	C5 Adana: N 37° 31.449, E 34° 39.159, 1185 m
<i>A. scoparia</i>	1078	S. Civelek and M. Kursat	24.09.2007	B9 Mus: N 39° 14.210, E 42° 25.223, 1620 m

Civelek et al. (2010), Kursat (2010).

S.C.) and which were turned into herbarium materials were used. By modifying the CTAB (Cetyl Trimethyl Ammonium Bromide) method, the DNA isolation was realized manually (Doyle and Doyle, 1987). By measuring the concentrations of the isolated DNAs with a nanodrop spectrophotometer, they were adjusted to 20 ng/ul. The Stock DNA was preserved at -20 °C.

2.3. PCR amplification

In the PCR studies conducted by using *psbA* - *trnH* primers and ITS4-ITS5 primers, *psbA-trnH* and ITS region for 60 samples was multiplied. The sequence of primers that were used to amplify both *psbA* - *trnH* region and ITS region were given in Table 2 (Taberlet et al., 1991). Aiming to make the final concentration for the PCR studies to be 25 µL, by mixing 5µL buffer, 1,5 µL MgCl₂, 0,5 µL dNTPs, 0,25 µL from each primer (forward and reverse), 0,25 µL taq polymerase and nearly 6 ng (1,35 µL) template of DNA were mixed and the PCR device was repeated for 30 cycles, having 2 min 95 °C initial denaturation, 1 min 95 °C denaturation, 40 s 50 °C (for *psbA* - *trnH* region), and

Table 2. The base sequences of the primers used.

Primers	Base sequences (5' – 3')
ITS5	5' GAAAGTAAAAGTCGTAACAAG 3'
ITS4	5' TCCTCCGCTTATTGATATGC 3'
<i>psbA</i>	5' GTTATGCATGAACGTAATGCTC 3'
<i>trnH</i>	5' CGCGCATGGTGGATTCAACAATCC 3'

Taberlet et al. (1991).

55 °C (for ITS region) annealing, 1 min 72 °C extension and 5 min 72 °C final extension. PCR products were monitored in agarose gel with a 1% ratio.

2.4. Sequence Analysis

A two-way reading was applied to the amplification products. The PCR purification process was realized before the sequence analysis. The purification and sequencing process was realized by the Macrogen Company. In order to evaluate the data of the chromatograms, a Finch TV 1.4

version was used. The variable sites, number of parsimony informative sites, genetic distance, nucleotide diversity, and divergence within the species were computed as molecular diversity statistics for each dataset using Molecular Evolutionary Genetics Analysis software (MEGA X) (Kumar et al., 2019). The DNA sequence alignments of the 60 individuals were evaluated by using the X version of the

MEGA program. The DNA sequence alignments of all the individuals was subject to the statistical analysis within the scope of this program. Ultimately, the phylogenetic trees were constructed by using the Maximum Likelihood Method with 1000 bootstrap replicates. The GenBank accession numbers, where the investigated taxa were gathered, are given in Table 3.

Table 3. Genbank accession numbers for the r-DNA ITS and *psbA-trnH* regions of the studied samples.

Specimens	GenBank Accession Numbers	
	ITS	<i>psbA-trnH</i>
<i>A. campestris</i>	MT161431	MT637764
<i>A. campestris</i>	MT161432	MT637765
<i>A. campestris</i>	MT161433	MT637766
<i>A. campestris</i>	MT161434	MT637767
<i>A. campestris</i>	MT161435	MT637768
<i>A. campestris</i>	MT161436	MT637769
<i>A. campestris</i>	MT161437	MT637770
<i>A. campestris</i>	MT161438	MT637771
<i>A. campestris</i>	MT161439	MT637772
<i>A. campestris</i>	MT161440	MT637773
<i>A. campestris</i>	MT161441	MT637774
<i>A. campestris</i>	MT161442	MT637775
<i>A. campestris</i>	MT161443	MT637776
<i>A. campestris</i>	MT161444	MT637777
<i>A. campestris</i>	MT161445	MT637778
<i>A. campestris</i>	MT161446	MT637779
<i>A. campestris</i>	MT161447	MT637780
<i>A. marschalliana</i>	MT161406	MT637745
<i>A. marschalliana</i>	MT161407	MT637746
<i>A. marschalliana</i>	MT161408	MT637747
<i>A. marschalliana</i>	MT161409	MT637748
<i>A. marschalliana</i>	MT161410	MT637749
<i>A. marschalliana</i>	MT161411	MT637750
<i>A. marschalliana</i>	MT161412	MT637751
<i>A. marschalliana</i>	MT161413	MT637752
<i>A. marschalliana</i>	MT161414	MT637753
<i>A. marschalliana</i>	MT161415	MT637754
<i>A. marschalliana</i>	MT161416	MT637755
<i>A. marschalliana</i>	MT161417	MT637756
<i>A. marschalliana</i>	MT161418	MT637757
<i>A. marschalliana</i>	MT161419	MT637758
<i>A. marschalliana</i>	MT161420	MT637759
<i>A. marschalliana</i>	MT161421	MT637760
<i>A. marschalliana</i>	MT161422	MT637761
<i>A. marschalliana</i>	MT161423	MT637762
<i>A. marschalliana</i>	MT161424	MT637763

Table 3. Continuation.

Specimens	GenBank Accession Numbers	
	ITS	psbA-trnH
<i>A. araratica</i>	MT161425	MT648000
<i>A. araratica</i>	MT161426	MT648001
<i>A. araratica</i>	MT161427	MT648002
<i>A. araratica</i>	MT161428	MT648003
<i>A. araratica</i>	MT161429	MT648004
<i>A. araratica</i>	MT161430	MT648005
<i>A. scoparia</i>	MT161388	MT637727
<i>A. scoparia</i>	MT161389	MT637728
<i>A. scoparia</i>	MT161390	MT637729
<i>A. scoparia</i>	MT161391	MT637730
<i>A. scoparia</i>	MT161392	MT637731
<i>A. scoparia</i>	MT161393	MT637732
<i>A. scoparia</i>	MT161394	MT637733
<i>A. scoparia</i>	MT161395	MT637734
<i>A. scoparia</i>	MT161396	MT637735
<i>A. scoparia</i>	MT161397	MT637736
<i>A. scoparia</i>	MT161398	MT637737
<i>A. scoparia</i>	MT161399	MT637738
<i>A. scoparia</i>	MT161400	MT637739
<i>A. scoparia</i>	MT161401	MT637740
<i>A. scoparia</i>	MT161402	MT637741
<i>A. scoparia</i>	MT161403	MT637742
<i>A. scoparia</i>	MT161404	MT637743
<i>A. scoparia</i>	MT161405	MT637744

3. Results

In this research, 60 individuals of 21 specimens taken from 17 different populations belong to 4 taxa of the subgenus *Dracunculus* were examined. In all the examined individuals from the same and different populations belonging to taxa of the subgenus *Dracunculus*, were used to determine the sequences of regions both *psbA-trnH* of the chloroplast DNA and ITS of the nuclear DNA (ITS1-5.8 gene - ITS2). The peak results of the bi-directional sequences sent to us from Macrogen were evaluated using the Version 1 of the Finch TV program. Using the “Multiple Alignment Blast System” of the automatic sequencing systems, the sequences were aligned. The noticeable differences were manually corrected.

As a result of the scans performed in the NCBI (National Center for Biotechnology Information) database, for two taxa of the genus *Artemisia*, two reference regions were obtained. The reference base sequences of the two individuals belonging to species *A. campestris* (JX051736.1 and JX073894.1) and *A. scoparia* (KX581818.1 and KX581973.1) were also included in our analysis to demonstrate the accuracy of the study (Hobbs and Baldwin,

2013; Liu et al., 2017). For a more accurate visualization of the results of the alignment, about 50-100 base from the beginning and the end were not evaluated by us. For this reason, approximately 686 base pairs for the ITS regions and 464 base pairs for the *psbA-trnH* regions were used.

In the phylogenetic tree drawing, the DNA sequences of the ITS (ITS1-5.8gene-ITS2) regions in the nuclear genome and the DNA sequences of the *psbA-trnH* region in the chloroplast genome were co-evaluated using version X of the Mega program.

The nucleotide composition and other features of the individuals was determined as a result of the statistical analyzes performed by cutting the excess parts at the head and end of the DNA sequences. In both the separate and co-evaluations of the sequences of the *psbA-trnH* and ITS regions of the examined individuals, some of the parameters of the molecular diversity such as the conserved regions (C), variation regions (V), parsimony informative regions (Pi), single parts (S), homologous base pairs (ii), transitional base pairs (si), transversional base pairs (sv), and R value (si / sv) were calculated and the values obtained were given in Table 4.

Table 4. PCR amplified region length and summary statistics from the r-DNA (ITS) and the cpDNA (*psbA-trnH*) dataset of subgenus *Dracunculus*.

Parameters of Molecular Diversity	ITS region	<i>psbA-trnH</i> region	Co-evaluated of ITS and <i>psbA-trnH</i>
Total individuals	60	60	60
Total band Length	686	464	1150
The ratio of G-C base pair (%)	55	28	44.3
Conserved regions (C)	594	415	1009
Variation regions (V)	95	49	144
Single parts (S)	85	33	119
Parsimony informative regions (Pi)	10	16	25
Homologous base pairs (ii)	675	446	1122
Transitional base pairs (si)	3.00	1.00	4.00
Transversional base pairs (sv)	3.00	5.00	7.00
R value (si/sv)	1.2	0.2	0.5

Using the Best DNA / Protein step in the Models menu of this program, the methods that best expressed the phylogenetic relationship between the individuals were determined. In the list of the methods given, the lowest value of the BIC (Bayesian Information Criterion) was found in the T92 + G (Tamura-3-parameter) method.

The Maximum Likelihood, Neighbor-Joining, UPGMA, and Maximum Parsimony methods were applied separately, but it was decided that the method that best illustrated the evolutionary and phylogenetic relationship between the examined individuals, we worked with, is the Maximum Likelihood method. In the phylogenetic tree drawing, the DNA sequences of the regions both the ITS in the nuclear genome and *psbA-trnH* in the chloroplast genome were co-evaluated by using version X of the Mega program.

Many of the phylogenetic trees were drawn by trying out many methods, and the most useful tree was chosen. In the Maximum Likelihood method, by entering the bootstrap value 100, a single phylogenetic tree for total of 63 individuals, 60 of which were examined, 2 of which were the control group, and 1 of which was an external group was obtained (see Figure 2). The species *A. campestris* and *A. scoparia* as control group, and the species *Anthemis cotula* L. as an external group were used (Sancar, 2017; Kursat et al., 2018; Sancar et al., 2019).

4. Discussion

In this study a phylogenetic systematic study was conducted by using the molecular data of subgenus *Dracunculus* that grow in Turkey. 60 individuals of 21 specimens taken from 17 different populations belonging to 4 taxa of the subgenus *Dracunculus* were examined by analyzing the base slice of the regions being obtained, in an attempt to find some information about the closeness and distance of the taxa with each other. This research is important as it is the first molecular based study relating to subgenus *Dracunculus* growing naturally in Turkey.

In different parts of the world, various research has been carried out to better understand the systematic position of the genus *Artemisia* and relationships among its (four) subgenera. The phylogenetic relationship among the different *Artemisia* species collected from different regions of Pakistan based on the chloroplast gene RPS11 was investigated by Mahmood et al. (2011). The molecular phylogenetic analyses of the Hawaiian *Artemisia* and its worldwide divergence based on nuclear and chloroplast DNA markers were reported by Hobbs and Baldwin (2013). As discussed by Haghghi et al. (2014), the phylogenetic relationships among *Artemisia* species based on nuclear ITS and chloroplast *psbA-trnH* DNA markers using three sections of *Artemisia*, *Dracunculus* and *Serphidium* propose that the r-DNA ITS and cpDNA *psbA-trnH* markers are practicable in the systematic revision of troubled taxa at the intra-genus level in plants. Furthermore, Pellicer et al. (2014) performed phylogenetic analysis of the annual *Artemisia* within its major lineages and suggested that annual *Artemisia* have been specially misidentified at a subgeneric level and verified that they are phylogenetically restricted to basal grades. However, to date, very few *Artemisia* species have been verified with molecular phylogenetic studies based on the nucleotide sequence data in Turkey (Koloren et al., 2016).

Civelek et al. (2010) carried out a revisionary study of the genus *Artemisia* in Turkey. According to the results of the revisionary study based on the morphological features, there are two species and four taxa belonging to the subgenus *Dracunculus* in Turkey. These species in the subgenus *Dracunculus* in Turkey were determined as *A. scoparia*, *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana*, *A. campestris* var. *araratica* (Civelek et al., 2010; Kursat, 2010).

During the revisionary study of the genus *Artemisia* in Turkey, it was observed that the closely related independent three species *A. campestris*, *A. marschalliana* and *A. araratica* in the Flora of Turkey are quite approximate to one another in terms of morphological characters (Civelek et al., 2010;

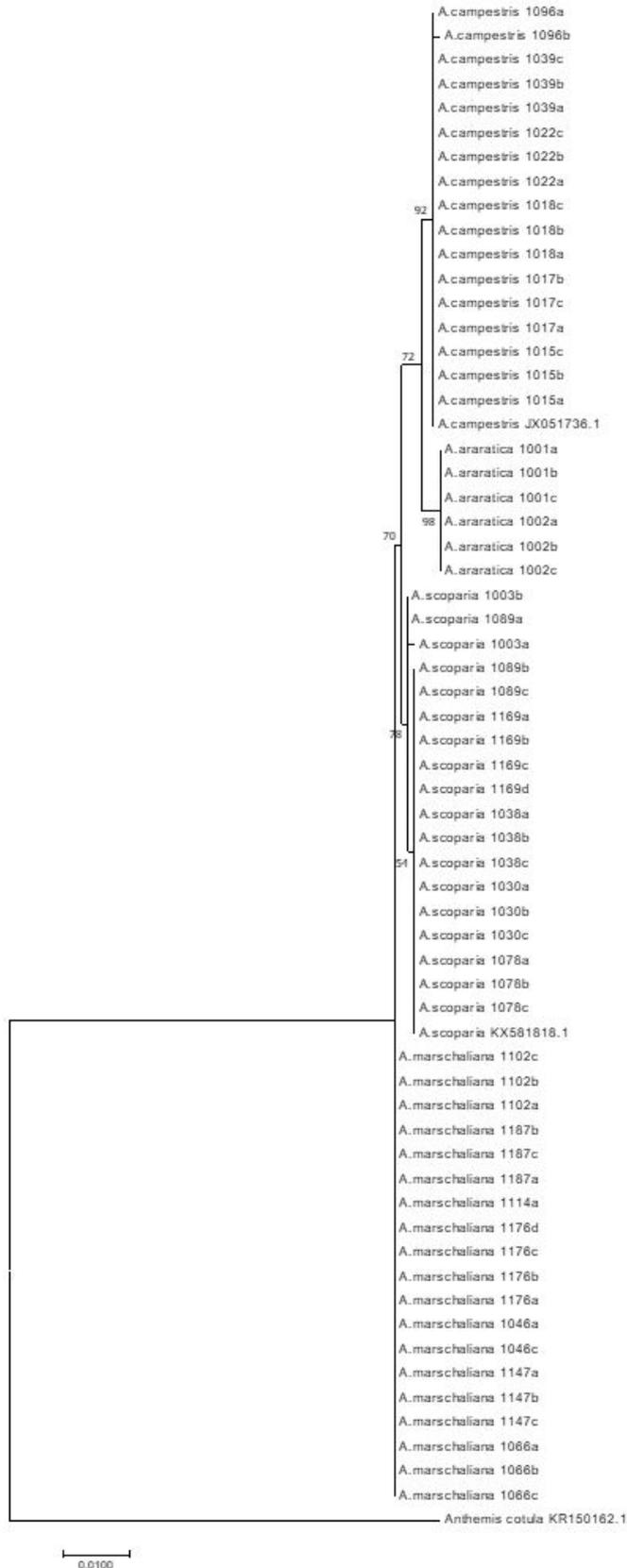


Figure 2. Maximum Likelihood tree showing the phylogenetic relationship between individuals.

Kursat, 2010). On the other hand, the two taxa *A. scoparia* and *A. campestris* var. *campestris* in the Flora of Turkey are also similar to each other morphologically.

As a result, the taxonomic positions and combinations of the closely related independent three species *A. campestris*, *A. marschalliana* and *A. araratica* in the Flora of Turkey changed in the revisionary study of the genus *Artemisia* in Turkey as follows: *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana* and *A. campestris* var. *araratica*. Thus, it has been adapted to their taxonomical positions in the Russian Flora (Davis, 1975; Schinskin and Bobrov, 1995; Civelek et al., 2010; Kursat, 2010; Kursat et al., 2015). However, because they were needed for the molecular data, the accuracy of the classification based on the morphological data in the revisionary study could not be guaranteed. Therefore, this research was planned.

When we look at the phylogenetic tree carefully, it is seen that the phylogenetic tree is divided into two branches in the first stage. One of these branches carries only individuals of the species *A. scoparia*, while the other carries individuals of varieties belonging to the species *A. campestris* (see Figure 2). This situation shows that the species *A. scoparia* is an independent apart from the three varieties of the species *A. campestris* and is genetically differentiated from them.

The species *A. scoparia* is diploid in terms of the chromosome number, and varieties of the species of *A. campestris* are tetraploid (Civelek et al., 2010; Kursat, 2010). At the same time, the species *A. scoparia* and varieties of the species of *A. campestris* have different basic chromosome numbers (dysploidy) as $X = 8$ and $X = 9$ respectively. Genetically, no reproduction can occur between the species *A. scoparia* and varieties of the species *A. campestris* (Civelek et al., 2010; Kursat, 2010).

The second important finding is that the individuals of species both *A. campestris* and *A. scoparia* which are used as control groups are found together with the examined individuals of their own species, and makes the reliability of our study 100% (see Figure 2).

It is a third important issue that the individuals of the four taxa belonging to the subgenus *Dracunculus* are included only in the same branches with their own taxon individuals and do not interfere with individuals belonging to other taxa. This situation is also an indication that there is reproductive isolation among the four taxa, and there is no gene flow and hybridization between them.

It is very interesting that the individuals belonging to each variety of the species *A. campestris* do not interfere with the individuals belonging to the other two varieties of the species *A. campestris* and are only found together with their own individuals varieties. The following results can be deduced from this situation:

(i). Three varieties of the species *A. campestris* are very similar morphologically (Civelek et al., 2010; Kursat, 2010). However, these similarities are not dependent on the flow of genes between them because the grouping of the individuals of each taxon only among themselves showed that there is no gene flow between these taxa. In other words, the fact that a complete reproductive isolation between the tetraploid three varieties of the species *A. campestris*,

which have the same basic chromosome numbers, was found.

(ii). The tetraploid three varieties of the species *A. campestris* may claim that they have separately completed their speciations due to reproductive isolation. However, each variety is not sufficiently differentiated due to the apomictic reproduction in itself. Because a high percentage of the genus *Artemisia* species are polyploid, numerous apospory and diplospory apomictics were reported for the genus *Artemisia* (Gustaffson, 1946; Battaglia, 1951; Davis, 1967; Pullaiah, 1984; Czapik, 1996; Carman, 1997; Noyes and Rieseberg, 2000).

The of the classification of the taxa belonging to the subgenus *Dracunculus* based on the morphological characteristics during the revisionary study of the genus *Artemisia* in Turkey was tested, and the most accurate classification of these taxa was determined. The fact that the subgenus *Dracunculus* has four independent species in Turkey was found in this study.

According to the results of this molecular study, *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana* and *A. campestris* var. *araratica* were proposed to be raised from a variety level to species level. Thus, like in the Flora of Turkey, the new systematic positions and combinations of the three varieties of the species *A. campestris* will be independent species *A. campestris*, *A. marschalliana* and *A. araratica*, like the Flora of Turkey (Davis, 1975). Another basis for this new combination is the fact that the individual of the independent species *A. campestris* used as one of the control groups was aggregated side by side on the same branch of phylogenetic tree with the individuals of *A. campestris* var. *campestris*. *A. campestris*, *A. marschalliana* and *A. araratica* species are morphologically similar, but they are different genetically, it means that these species are sibling species.

As a result of this research, original data was obtained for use in new scientific molecular studies on the taxa of genus *Artemisia*, and provided the haplotypes of *psbA-trnH* and ITS regions for the subgenus *Dracunculus* taxa in Turkey for the first time in the GenBank database.

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