

Original Article

The molecular investigations on the subgenus *Artemisia* Less. of the genus *Artemisia* L. (Asteraceae) in Turkey

Investigações moleculares sobre o subgênero *Artemisia* Less. do gênero *Artemisia* L. (Asteraceae) na Turquia

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Abstract

The genus *Artemisia* L. of the family Asteraceae is systematically very complex. The aim of this study was to evaluate taxonomic positions of taxa of the subgenus *Artemisia* belonging to the genus *Artemisia* in Turkey using some molecular techniques. In this molecular study, 44 individuals belong to 14 species of the subgenus *Artemisia* were examined. Analyses were performed on the combined dataset using maximum parsimony, maximum likelihood and Bayesian inference and Molecular parameters obtained from co-evaluations of sequences of the *psbA-trnH*, ITS and ETS regions of examined individuals were used in the phylogenetic tree drawing. According to the results of this study, two molecular groups have been formed based on the DNA sequence similarity of the species, but there are no obvious morphological characters corresponding to two molecular groups. There is no also agreement between the two molecular groups and the two morphological groups formed according to the hairiness condition of the receptacle of species. Due to the lack of molecular significance of their receptacles with or without hair, dividing of the subgenus *Artemisia* species into new subgenera or sections was not considered appropriate. Likewise, it has been found that with or without hair on the corolla lobes of the central hermaphrodite disc flowers have no molecular significance. It was found that there were no gene flow and hybridization between the 14 species of the subgenus *Artemisia* and these 14 species were found completed their speciation. This study is important as it is the first molecular based study relating with belong to subgenus *Artemisia* species growing naturally in Turkey. In addition, new haplotypes related to the populations of Turkey belonging to the subgenus *Artemisia* taxa were reported by us for the first time and added to the GenBank database.

Keywords: *Artemisia*, Turkey, *psbA-trnH*, ITS, ETS, phylogeny.

Resumo

O gênero *Artemisia* L. da família Asteraceae é sistematicamente muito complexo. O objetivo deste estudo foi avaliar as posições taxonômicas de táxons do subgênero *Artemisia* pertencentes ao gênero *Artemisia* na Turquia usando algumas técnicas moleculares. Neste estudo molecular, 44 indivíduos pertencentes a 14 espécies do subgênero *Artemisia* foram examinados. As análises foram realizadas no conjunto de dados combinado usando máxima parcimônia, máxima verossimilhança e inferência bayesiana e parâmetros moleculares obtidos a partir de coavaliações de sequências das regiões *psbA-trnH*, ITS e ETS de indivíduos examinados foram usados no desenho da árvore filogenética. De acordo com os resultados deste estudo, dois grupos moleculares foram formados com base na similaridade da sequência de DNA das espécies, mas não há caracteres morfológicos óbvios correspondentes a dois grupos moleculares. Também não há concordância entre os dois grupos moleculares e os dois grupos morfológicos formados de acordo com a condição de pilosidade do receptáculo da espécie. Devido à falta de significado molecular de seus receptáculos com ou sem cabelo, a divisão das espécies do subgênero *Artemisia* em novos subgêneros ou seções não foi considerada apropriada. Da mesma forma, verificou-se que com ou sem cabelo nos lobos da corola das flores do disco hermafrodita central não tem significado molecular. Constatou-se que não houve fluxo gênico e hibridização entre as 14 espécies do subgênero *Artemisia* e essas 14 espécies concluíram sua especiação. Este estudo é importante porque é o primeiro estudo de base molecular relacionado com espécies pertencentes ao subgênero *Artemisia* crescendo naturalmente na Turquia. Além disso, novos haplótipos relacionados às populações da Turquia pertencentes ao subgênero *Artemisia* taxa foram relatados por nós pela primeira vez e adicionados ao banco de dados do GenBank.

Palavras-chave: *Artemisia*, Turquia, *psbA-trnH*, ITS, ETS, filogenia.

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1. Introduction

Artemisia L. is one of the largest genera in the family Asteraceae and the largest genus in the tribe Anthemideae comprises from 200 to more than 500 taxa at the specific or infraspecific level (McArthur, 1979; Mabberley, 1990; Ling, 1991a, b; Bremer and Humphehries, 1993; Ling, 1991a, b; Torrell et al., 1999). Traditionally, the subgeneric classification of the genus *Artemisia* L. follows a system established by Besser (1829) where in he separated four sections based on various combinations of disc and ray flower occurrences and fertility. These sections are *Abrotanum* (Duhamel du Monceau) Besser, *Absinthium* (Miller) Candolle, *Dracunculus* Besser, and *Seriphidium* Besser. Besser's four sections have been modified by subsequent workers. Rydberg (1916) created subordinate sections including new section *Tridentatae* for the North American members of the section *Seriphidium* and elevated these sections to subgenera.

Nowadays, the most commonly accepted subdivisions of the genus *Artemisia* are separated into 4 subgenera as *Artemisia* Lessing, *Dracunculus* (Besser) Rydberg, *Seriphidium* Besser ex Lessing and *Tridentatae* (Rydberg) McArthur et al. (1981). The subgenus *Artemisia* is included traditional two subgenera *Abrotanum* Besser and *Absinthium* (Miller) Lessing. The first detailed phylogenetic interpretation of the sections or subgenera established *Artemisia* as the more primitive, while *Dracunculus*, *Seriphidium* and *Tridentatae* were considered to be more advanced (Hall and Clements, 1923). Notwithstanding, a major in-depth reclassification of the genus *Artemisia* (combining molecular and traditional data) has not yet been proposed.

There are 22 species of the genus *Artemisia* in the 5th volume of the Flora of Turkey (Cullen, 1975; Davis, 1975; Davis et al., 1988). Later, the species *A. verlotiorum* Lamotte was added to the 10th volume of the Flora of Turkey as a new record for Turkey, so species numbers of the genus *Artemisia* in Turkey became 23 in total (Kurşat et al., 2011a). At the same time, 23 species without any infraspecific taxa that belong to the genus *Artemisa* in Flora of Turkey are not classified levels of subgenus or section.

Civelek et al. (2010) have carried out a revisionary study of the genus *Artemisia* in Turkey. According to the results of this revisionary study, there are a total of 21 species with 9 infraspecific taxa and 3 subgenera belonging to the genus *Artemisia* in Turkey (Civelek et al., 2010; Kurşat, 2010; Kurşat et al., 2011a, b, 2014, 2015, 2018). According to the results of this revisionary study of the genus *Artemisia*, subgenera *Artemisia*, *Dracunculus*, and *Seriphidium* have taxa in Turkey, but the subgenus *Tridentatae* which is endemic to North America has not taxa in Turkey (Civelek et al., 2010; Kurşat, 2010; Guner et al., 2012).

The subgenus *Artemisia* has the most species in Turkey that includes 14 species. During in the revisionary study, the species *A. alba* of the subgenus *Artemisia* Less. has been removed from Turkey's species list because its distribution is outside of Turkey's borders (Civelek et al., 2010; Kurşat, 2010; Kurşat et al., 2011a; Guner et al., 2012). No conclusive consensus has yet been provided on the classification at subgeneric and sectional levels of species belonging to the subgenus *Artemisia* which is the subject of this study.

During the revisionary study of the genus *Artemisia* L. in Turkey, the subgenus *Artemisia* did not divide into new subgenera or sections based on the morphological (phenotypic) character of receptacles which are hairy or glabrous (Civelek et al., 2010; Kurşat, 2010). Is this systematic arrangement of the subgenus *Artemisia* in this revisionary study that made according to only morphological data correct? The biggest question in the minds of the researchers doing the revisionary study is about whether the dividing at new subgeneric and sectional levels of species belonging to the subgenus *Artemisia* which is the subject of this study. The final decision on whether the subgenus *Artemisia* should be divided into new subgenera or sections will be made by this molecular study.

In this study, it will be also tried to determine whether this phenotypic variation has a molecular (genotypic) level response. The purposes of this research are as follows: For the species belonging to the subgenus *Artemisia*, to determine whether there is a molecular significance of the receptacles with or without hairs and accordingly to determine whether the subgenus *Artemisia* can be divided into new subgenera or sections. To determine whether the hairy and hairless corolla lobes of the central hermaphrodite disc flowers have a molecular significance for species belonging to the subgenus *Artemisia* and to find whether each species has completed its speciation. Also, obtain original data on species of subgenus *Artemisia* for use in new scientific molecular studies of Science World, and to provide the haplotypes of *psbA-trnH*, ITS and ETS regions of species belonging to the subgenus *Artemisia* in Turkey for the GenBank database.

2. Material and Methods

2.1. Plant material

Within the scope of this study, the plants that were gathered during the land survey conducted by M. Kurşat and S. Civelek were used. 44 individuals belong to 14 species of the subgenus *Artemisia* were examined. Depending on the large of populations, between 1 and 3 individuals with the same label information were used for each species. Localities and detailed population information the investigated taxa were gathered, were given in Table 1.

2.2. DNA isolation and PCR amplification

For the DNA isolation, was used herbarium material (dried plant leaf). The CTAB method was used in a modified form and DNA isolation was performed manually (Doyle and Doyle, 1987). By measuring the concentrations of the isolated DNAs with a nanodrop spectrophotometer, they were adjusted to 25 ng/ul. The Stock DNA was preserved at -20 C.

In the PCR studies conducted by using some specific universal primers, ITS, ETS and *psbA-trnH* region for 44 samples was multiplied. The sequence of primers that were used to amplify all of regions were given in Table 2 (Taberlet et al., 1991). Aiming to make the final concentration for the PCR studies to be 50 µL, by mixing

Table 1. The label information of examined individuals of specimens belonging to species of the subgenus *Artemisia* used in the study and detailed localities of populations (Civelek et al., 2010; Kurşat et al., 2011b).

Species of The Subgenus <i>Artemisia</i>	Collector and number of specimens	Collection date	Detailed localities of specimens' populations
<i>A. vulgaris</i>	S.C. and M.K. 1166.	19.09.2007	B9 Bitlis : Between Tatvan and Van, 54 km after Tatvan, Besparmak village, stream edge and roadside, 1718 m, N 38° 21 E 42° 38
<i>A. verlotiorum</i>	S.C. and M.K. 1103	9.10.2007	A6 Ordu : Boztepe, around the recreation facilities of Governor Kemal Yazıcıoğlu, 505 m
	S.C. and M.K. 1104	29.10.2007	A7 Giresun : Espiye, Gelevare stream edge, 2m, N 40° 56 E 38° 43
	S.C. and M.K. 1105	30.10.2007	A8 Rize : Ardeşen - Çamlıhemşin highway, 2 nd km, roadside and stream edge, 9m, N 41° 10 E 40° 57
<i>A. abrotanum</i>	S.C. and M.K. 1014	01.09.2007	B6 Kahramanmaraş : Elbistan - Afşin highway, Ekinözü road junction, Bölek locality, gardens, 1147 m, N 38° 11 E 37° 0
	S.C. and M.K. 1044	19.09.2007	B8 Mus : City center entrance, after vocational school, roadside, 1266 m, N 38° 47 E 41° 29
<i>A. austriaca</i>	S.C. and M.K. 1161	27.07.2008	A9 Kars : Kars - Susuz highway, the entrance of Susuz, roadside 1770 m, N40° 46 E 43° 07
	S.C. and M.K. 1051	20.09.2007	B9 Van : Between Çavuştepe castle - Zerneik irrigation dam lake, field edges, 1851 m, N 38° 21 E 43° 33
	S.C. and M.K. 1158	26.07.2008	B10 Agri : Dogubeyazit, Iğdır highway, between Cengelli pass and Ararat mountain, Karabulak, Girasor mountain, north slopes of Bardaklı village, steppe, 2565m, N 39° 43 E 44° 03
<i>A. incana</i>	S.C. and M.K. 1075	23.09.2007	B9 Bitlis : Adilcevaz, Adilcevaz - Ahlat highway, the exit of Adilcevaz, rocky places, 1720 m, N 38° 47 E 42° 43
	S.C. and M.K. 1079	24.09.2007	B9 Mus : Malazgirt, between Aktuzla - Karıncalı villages, 3 km before Karıncalı village, roadside, rocky places, 1550 m, N 39° 21 E 42° 15
	S.C. and M.K. 1065	22.09.2007	B10 Agri : Dogubeyazit, Iğdır highway, between Cengelli pass and Ararat mountain, Karabulak, Girasor mountain, north slopes of Bardaklı village, rocky places, 1728 m, N 39° 42 E 44° 05
<i>A. armeniaca</i>	S.C. and M.K. 1162	27.07.2008	A9 Kars : Susuz, Kısır Mountain, north slopes of Kızıroğlu Village, meadows, 2450 m, N 40° 54 E 43° 02
	S.C. and M.K. 1144	23.09.2007	B10 Agri : Dogubeyazit, Iğdır highway, between Cengelli pass and Ararat mountain, Karabulak, Girasor mountain, north slopes of Bardaklı village, 2565m, N 39° 43 E 44° 03
<i>A. chamaemelifolia</i>	S.C. and M.K. 1069	23.09.2007	B10 Agri : Dogubeyazit, Iğdır highway, between Cengelli pass and Ararat mountain, Karabulak, Girasor mountain, north slopes of Bardaklı village, 2565m, N 39° 43 E 44° 03
	S.C. and M.K. 1163	27.07.2008	A9 Kars : Susuz, Kısır Mountain, north slopes of Kızıroğlu Village, meadows, 2450 m, N 40° 54 E 43° 02
<i>A. annua</i>	S.C. and M.K. 1025	08.09.2007	A2 (E) Istanbul : North of Terkos lake, wooded area, around the settlement area and roadside, 20 m
	S.C. and M.K. 1106	30.10.2007	A7 Trabzon : Maçka highway, Esiroğlu Kenan Oltan high School, roadside, 164 m, N 41° 10 E 40° 57
	S.C. and M.K. 1024	06.09.2007	B1 Balıkesir : Around Atköy village, 110 m, N 39° 34 E 27° 59
<i>A. tournefortiana</i>	S.C. and M.K. 1155	26.07.2008	B10 Agri : Doğubeyazit, around İshakpaşa palace, ruins, 1953 m, N 39° 31 E 44° 07
	S.C. and M.K. 1074	23.09.2007	B9 Bitlis : Adilcevaz, Aydınlar sub-district center, 1891m, N 38° 53 E 42° 56
<i>A. absinthium</i>	S.C. and M.K. 1179	27.08.2008	B8 Erzurum : Pasinler - Erzurum highway, 20 km before Erzurum, slopes at the side of the road, 1915 m, N 40° 38 E 43° 04
	S.C. and M.K. 1080	24.09.2007	B9 Mus : Malazgirt, between Aktuzla - Karıncalı villages, 3 km before Karıncalı village, roadside, rocky places, 1550 m, N 39° 21 E 42° 15
	S.C. and M.K. 1063	22.09.2007	B10 Agri : Dogubeyazit, Suluçem, Zor Mountain, north slopes of the water reservoir, creek, 2054 m, N 39° 42 E 43° 5
<i>A. arborescens</i>	S.C. and M.K. 1040	12.09.2007	C5 Hatay : Samandağı district, Musa mountain, Çevlik location, Titus tunnel area, 53 m, N 36° 07 E 38° 55
<i>A. splendens</i>	S.C. and M.K. 1142	21.09.2007	B9 Van : Gurpınar, Sopakonak village, slopes, 2692 m, N 38° 12 E 43° 37
	S.C. and M.K. 1072	03.09.2007	B9 Bitlis : Adilcevaz, Suphan mount, Aydınlar village, upper slopes of Kığgiller hamlet, 2810 m, N 38° 55 E 42° 52
<i>A. alpina</i>	S.C. and M.K. 1149	13.07.2008	B6 Sivas : Ulaş district, Tecer mount, lower parts of the oak community, around the water resources, rocky places, 1817 m, N 39° 25 E 37° 07
	S.C. and M.K. 1006	06.07.2007	C5 Niğde : Çamardı district, lower parts of Demirkazık village, steppe areas, 1560 m, N 37° 51 E 35° 04
<i>A. haussknechtii</i>	S.C. and M.K. 1059	21.09.2007	C9 Hakkari : Hakkari - Van highway, after 20 th km, Kırıkdağ village, Kirbaş castle, the upper part of Ovaspi stream, rock places, 1624m, N 37° 34 E 43° 5

10 µL buffer, 3 µL MgCl₂, 1 µL dNTPs, 0.50 µL from each primer (forward and reverse), 0.50 µL taq polymerase, 32.30 dH₂O, and nearly 12 ng (2.70 µL) template of DNA were mixed and the PCR device was repeated for 30 cycles, having 2 min 94 °C initial denaturation, 1 min 94 °C denaturation, 35 s 50 °C (for *psbA* - *trnH* region), and 55 °C (for ITS and ETS region) annealing, 2 min 72 °C extension and 10 min 72 °C final extension. PCR products were monitored in agarose gel with a 2% ratio.

2.3. Sequence and phylogenetic 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., 2018). The DNA sequence alignments of

the 44 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 100 bootstrap replicates. The GenBank accession numbers, where the investigated taxa were gathered, are given in Table 3.

3. Results

In this research, 44 individuals belong to 14 species of the subgenus *Artemisia* were examined. Depending on the numbers of individuals in the herbarium specimens, between 1 and 3 individuals with the same label information were used for each specimen. The sequences of the cpDNA *psbA-trnH*, r-DNA ITS and ETS regions for all individuals examined, were used to determine phylogenetic kinship relationships.

The peak results of the bi-directional sequences sent from the Macrogen were evaluated using Version 1.4 of the Finch TV program. Using the "Multiple Alignment Blast System" of the automatic sequencing systems, sequences were aligned. The differences in the noticeable were manually corrected.

As a result of scans performed in the NCBI (National Center for Biotechnology Information) database site, for seven species of the subgenus *Artemisia*, three reference regions were obtained. These reference regions were an ITS with 700-750 bases (ITS1-5.8 gene - ITS2), ETS with 400-450 bases and *psbA-trnH* with 450-500 bases. For a more accurate visualization of the results of the alignment, about 50-100 base from the head and the end was not evaluated by us. For this reason, approximately 684 base pairs for the ITS regions, 506 base pairs for the ETS regions

Table 2. The base sequences of the primers used (Taberlet et al., 1991).

Primers	Base sequences (5' - 3')
ITS5	5' GAA AGT AAA AGT CGT AAC AAG G 3'
ITS4	5' TCC TCC GCT TAT TGA TAT GC 3'
ETS1f	5' CTT TTT GTG CAT AAT GTA TAT ATA GGG GG 3'
18S-2L	5' TGA CTA CTG GCA GGA TCA ACC AG 3'
<i>psbA</i>	5' GTT ATG CAT GAA CGT AAT GCT C 3'
<i>trnH</i>	5' CGC GCA TGG TGG ATT CAC AAT CC 3'

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

Taxa of the Subgenus <i>Artemisia</i>	GenBank Accession Numbers		
	ITS region	<i>psbA-trnH</i> region	ETS region
<i>A. vulgaris</i> 1-3	MT734913- MT734915	MT758494- MT758496	MT758538- MT758540
<i>A. verlotorum</i> 1-4	MT734916- MT734919	MT758497- MT758500	MT758541- MT758544
<i>A. abrotanum</i> 1-3	MT734920- MT734922	MT758501- MT758503	MT758545- MT758547
<i>A. austriaca</i> 1-3	MT734923- MT734925	MT758504- MT758506	MT758548- MT758550
<i>A. incana</i> 1-3	MT734926- MT734928	MT758507- MT758509	MT758551- MT758553
<i>A. armeniaca</i> 1-2	MT734929- MT734930	MT758510- MT758511	MT758554- MT758555
<i>A. chamaemelifolia</i> 1-4	MT734931- MT734934	MT758512- MT758515	MT758556- MT758559
<i>A. annua</i> 1-3	MT734935- MT734937	MT758516- MT758518	MT758560- MT758562
<i>A. tournefortiana</i> 1-3	MT734938- MT734940	MT758519- MT758521	MT758563- MT758565
<i>A. absinthium</i> 1-3	MT734941- MT734943	MT758522- MT758524	MT758566- MT758568
<i>A. arborescens</i> 1-2	MT734944- MT734945	MT758525- MT758526	MT758569- MT758570
<i>A. splendens</i> 1-4	MT734946- MT734949	MT758527- MT758530	MT758571- MT758574
<i>A. alpina</i> 1-4	MT734950- MT734953	MT758531- MT758534	MT758575- MT758578
<i>A. haussknechtii</i> 1-3	MT734954- MT734956	MT758535- MT758537	MT758579- MT758581

and 466 base pairs for the *psbA-trnH* regions were used and the Genbank accession number of these regions were given in the Table 3.

The DNA sequences of the ITS, ETS and the *psbA-trnH* regions of 44 individuals belong to subgenus *Artemisia* were analyzed statistically. In the both separate and co-evaluations of sequences of the ITS, ETS and *psbA-trnH* regions of examined individuals, some parameters of molecular diversity like 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 are given in Table 4. These parameters determine the distribution of individuals in the phylogenetic tree and thus gave us information about their phylogenetic relationships.

The nucleotide composition 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. When the values given in Table 4 are examined, it is seen that individuals are not very different in terms of the ratio of T, C, A, and G bases. At the same time, it was found that when the average values of the base contents of the individuals were calculated, the A-T ratio was 56% and the G-C ratio was 44%, and the A-T base pair was richer than G-C base pair.

In order to determine the phylogenetic relationships between individuals belonging to subgenus *Artemisia* species from both the same and different populations, in total 52 individuals were studied, 7 of them were control group and 1 of which was outgroup were arranged in Mega program.

Then, by using the Best DNA / Protein step in the Models menu of this program, we determined the methods that best express the phylogenetic relationship between individuals. In the list of methods given, the lowest value of BIC (Bayesian Information Criterion) was found in the T92 + G (Tamura-3-parameter) method. According to T92 + G, to see phylogenetic relationships between individuals, we

have seen that phylogenetic trees can be plotted using any of the methods such as Maximum Parsimony, Neighbor-Joining, UPGMA, and Maximum Likelihood.

Maximum Likelihood, Neighbor-Joining, UPGMA, and Maximum Parsimony methods were applied separately, but it was decided that the method that best illustrates the evolutionary and phylogenetic relations among the examined individuals, we work with is the Maximum Likelihood method. In the phylogenetic tree drawing, the DNA sequences of ITS and ETS regions in the nuclear genome and *psbA-trnH* in the chloroplast genome were co-evaluated by using version 7.1 of the Mega program. In the Maximum Likelihood method, by entering the bootstrap value 100, a single phylogenetic tree for a total of 52 individuals were obtained (Figure 1).

4. Discussions

There are 3 subgenera, 24 species with 5 infraspecific taxa belong to the genus *Artemisia* in Turkey. The subgenus *Artemisia* includes 14 species, the subgenus *Dracunculus* includes 4 species and the subgenus *Seriphidium* includes 6 species with 5 taxa that settled in the categories of subspecies and variety. Three species (*A. santonicum*, *A. taurica*, and *A. sieberi*) of the subgenus *Seriphidium* have five infraspecific taxa that settled in the categories of subspecies and variety (Table 5) (Civelek et al., 2010; Kurşat, 2010; Kurşat et al., 2011a, b, 2014, 2015, 2018). Although the subgenus *Artemisia* has been divided into new second subgenera or sections by some authors (Bremer and Humpheires, 1993; Schinskin and Bobrov, 1995; Ling et al., 2006; Shultz, 2006a, b), the subgenus *Artemisia* did not divide to new subgenera or sections during the revisionary study of the genus *Artemisia* in Turkey (Civelek et al., 2010; Kurşat, 2010). The most important of the purposes of this molecular study is to make the final decision whether the subgenus *Artemisia* should be divided into new subgenera or sections.

Table 4. Molecular diversity parameters obtained from both separate and co-evaluations of sequences of the ITS, ETS and *psbA-trnH* regions of examined individuals.

Parameters of Molecular Diversity	ITS region	ETS region	<i>psbA-trnH</i> region	Co-evaluated of ITS, ETS and <i>psbA-trnH</i>
Total individuals	44	44	44	44
Total band Length	~ 684	~504	~ 466	~ 1716
The ratio of G-C base pair (%)	54.1	49.6	29.5	44
Conserved regions (C)	410	429	392	763
Variation regions (V)	285	75	85	917
Single parts (S)	85	1	44	141
Parsimony informative regions (Pi)	200	74	41	776
Homologous base pairs (ii)	633	483	450	1305
Transitional base pairs (si)	27	14	3	117
Transversional base pairs (sv)	15	6	5	201
R value (si/sv)	1.8	2.3	0.5	0.6

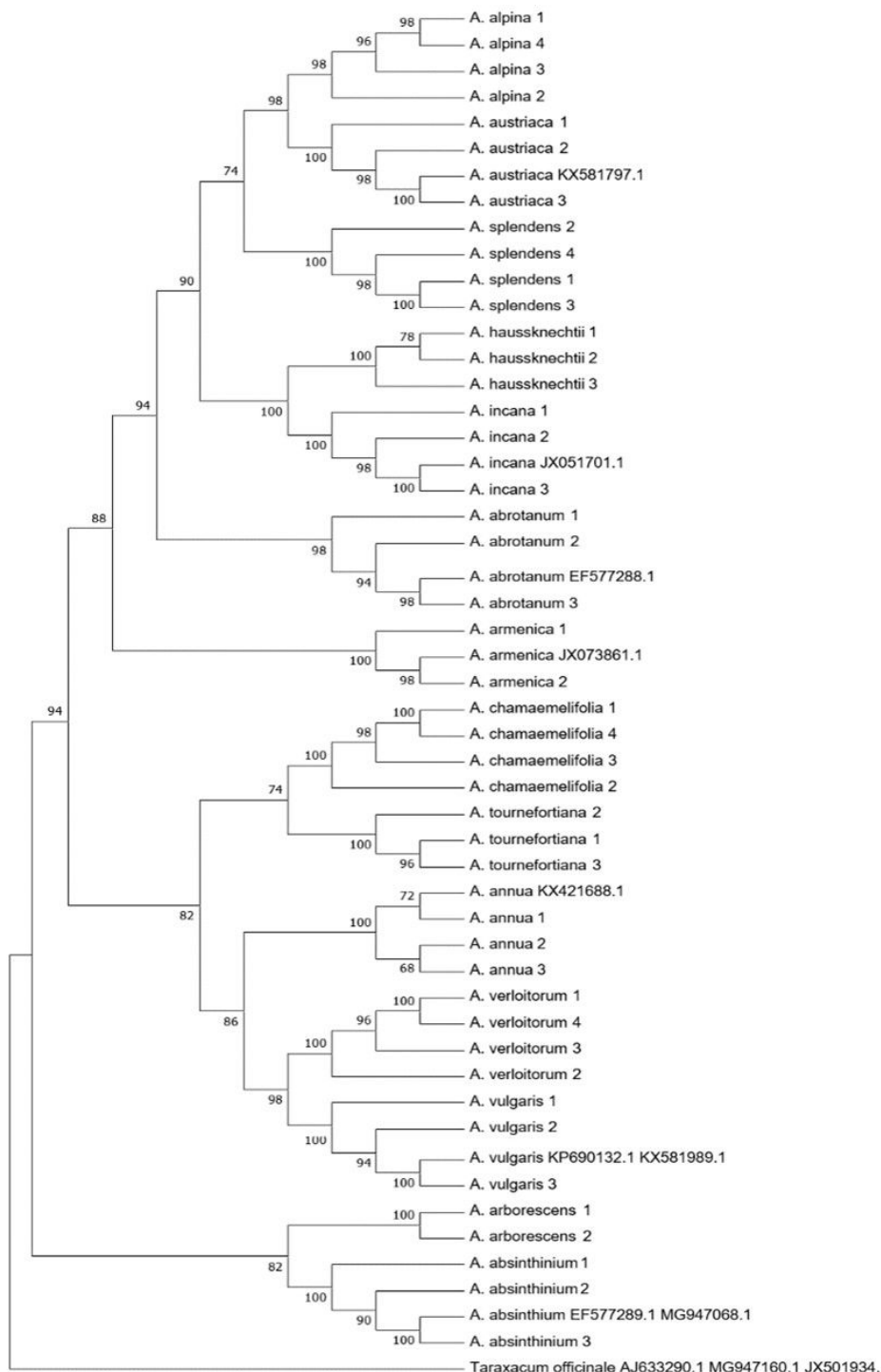


Figure 1. Phylogenetic tree obtained from the co-evaluation of sequences of the *psbA-trnH*, ETS and ITS regions of individuals.

During the revisionary study of the genus, *Artemisia* in Turkey, a new diagnostic character (a new phenotypic variation) had been determined for taxa belonging to subgenus *Artemisia*. This diagnostic character is that with or without hair on the corolla lobes of the central hermaphrodite disc flowers. Four species *A. arborescens*,

A. armeniaca, *A. austriaca*, and *A. incana* of the subgenus *Artemisia* have hairs on the corolla lobes of central hermaphrodite disc florets. However, ten species *A. vulgaris*, *A. verlotorum*, *A. abrotanum*, *A. chamaemelifolia*, *A. annua*, *A. tournefortiana*, *A. absinthium*, *A. splendens*, *A. alpina* and *A. haussknechtii* of the subgenus *Artemisia* have not hairs

on the corolla lobes of central hermaphrodite disc florets. Different researchers have taxonomically classified of the subgenus *Artemisia* at subgeneric and sectional levels according to whether receptacle has hairy or glabrous (Table 5).

After the revisionary study, new research projects have been carried out for problematic taxa of the two subgenera *Seriphidium* and *Drancunculus* of the genus *Artemisia* in Turkey (Civelek and Sancar, 2012; Civelek et al., 2018, 2019). Taking into account the results of these new research projects, some new arrangements have been made for taxa of the genus *Artemisia* in Turkey. The species *A. fragrans* Willd. is published as a new record for Turkey. *A. taurica* var. *vanensis* Kursat & Civelek is published as a new variety globally (Kurşat et al., 2014, 2018). *A. campestris* var. *campestris*, *A. campestris* var. *marschalliana* and *A. campestris* var. *araratica* that are new combinations in the revisionary study was proposed to raise to species level again (Civelek et al., 2019). Thus, like in the Flora of Turkey, the new systematic positions and combinations of the three varieties of the species *A. campestris* became independent species *A. campestris*, *A. marschalliana*, and *A. araratica*, like the Flora of Turkey (Table 6) (Civelek et al., 2019; Sancar et al., 2022).

In the scope of this molecular study, 44 individuals belong to 14 species of the subgenus *Artemisia* were examined. For all examined individuals, belonging to species of the subgenus *Artemisia*, the sequences of regions with cpDNA *psbA-trnH* region, r-DNA ITS and ETS region, have been used to determine the phylogenetic relationship between 44 individual.

If we carefully look the phylogenetic tree in Figure 1, the species are divided into two primary branches. In the first primary branch, it is seen that the species *A. arborescens*

and *A. absinthium* differentiate with 82% strong bootstrap value from all other species of the subgenus.

The second primary branch carries the remaining 12 species with a strong bootstrap value of 94%. The second primary branch is also divided into two secondary level branches. One of these secondary level branches carries the species *A. chamaemelifolia*, *A. tournefortiana*, *A. annua*, *A. vulgaris*, *A. verlotiorum*, while the other secondary level branch carries the species *A. abrotanum*, *A. austriaca*, *A. incana*, *A. armeniaca*, *A. splendens*, *A. alpina*, *A. haussknechtii* (Table 7).

The first primary branch carries two species whose receptacles are only hairy, while other primary branch carries the species whose receptacles are both hairy and hairless. One of the secondary level two branches of other primary branch carries five species whose receptacles are only hairless, other secondary level branch carries seven species whose receptacles are both hairy (three species) and hairless (four species). This molecular grouping may be related to the polyphyletic origin of the subgenus *Artemisia*. It is stated in the literature that the subgenus *Artemisia* is polyphyletic while the other subgenera are monophyletic (Hussain et al., 2019). Also, this subgenus must to be recircumscribed stated, indicated by Hussain et al. (2019).

There is no agreement between two molecular groups in the phylogenetic tree created on the basis of the DNA sequence similarity of the species and the two morphological groups formed according to the hairiness condition of the receptacles. In this study, as it was determined that lack of molecular significance of their receptacles with or without hair, dividing of the subgenus *Artemisia* species into new subgenera or sections was not considered appropriate. As known, even during the revisionary study of the genus *Artemisia* in Turkey which

Table 5. Different taxonomic classifications of the subgenus *Artemisia* at subgeneric and sectional levels according to receptacle with or without hair (Kursat 2010).

Receptacle	Species	Ling et al. 2006; Schinskin and Bobrov 1995	Shultz 2006a;b	Bremer and Humphries, 1993	Civelek et al. 2010
Glabrous (Naked)	<i>A. vulgaris</i>	Section <i>Artemisia</i>	Section <i>Artemisia</i> (= <i>Abrotanum</i>)	Subgenus <i>Artemisia</i> (= <i>Abrotanum</i>)	Subgenus <i>Artemisia</i>
	<i>A. verlotiorum</i>				
	<i>A. abrotanum</i>				
	<i>A. austriaca</i>				
	<i>A. incana</i>				
	<i>A. armeniaca</i>	Section <i>Abrotanum</i>			
	<i>A. chamaemelifolia</i>				
	<i>A. annua</i>				
	<i>A. tournefortiana</i>				
	Hairy	<i>A. absinthium</i>			
<i>A. arborescens</i>					
<i>A. splendens</i>					
<i>A. alpina</i>					
<i>A. haussknechtii</i>					

Table 6. The final status of the all taxa of the genus *Artemisia* in Turkey.

Genus <i>Artemisia</i> in Turkey	
Subgenus <i>Artemisia</i> Less.	
1.	<i>A. vulgaris</i> L.
2.	<i>A. verlotorum</i> Lamotte
3.	<i>A. abrotanum</i> L.
4.	<i>A. austriaca</i> Jacq.
5.	<i>A. incana</i> (L.) Druce
6.	<i>A. armeniaca</i> Lam.
7.	<i>A. chamaemelifolia</i> Vill.
8.	<i>A. annua</i> L.
9.	<i>A. tournefortiana</i> Reich.
10.	<i>A. absinthium</i> L.
11.	<i>A. arborescent</i> L.
12.	<i>A. splendens</i> Willd.
13.	<i>A. alpina</i> Pall. ex Willd.
14.	<i>A. haussknechtii</i> Boiss.
Subgenus <i>Dracunculus</i> (Bess.) Rydb.	
15.	<i>A. campestris</i> L.
16.	<i>A. marschalliana</i> Sprengel
17.	<i>A. araratca</i> Krasch.
18.	<i>A. scoparia</i> Waldst. & Kit.
Subgenus <i>Seriphidium</i> (Bess.) Rouy.	
19.	<i>A. santonicum</i> L. subsp. santonicum subsp. patens (Neilr.) K. Persson
20.	<i>A. taurica</i> Willd. var. taurica var. vanensis Kursat & Civelek
21.	<i>A. spicigera</i> K. Koch
22.	<i>A. sieberi</i> Bess. subsp. sieberi
23.	<i>A. fragrans</i> Willd.
24.	<i>A. bashkalensis</i> Kursat & Civelek

based on morphological characters, the subgenus *Artemisia* did not divide into subgenera and sections (Civelek et al., 2010; Kurşat, 2010). The correctness of this classification was determined by the current molecular study. Although the receptacle with or without hair can be appropriately not used for classification in molecular systematics of the subgenus *Artemisia*, it is known that it is extremely useful in the morphological identification of species.

Likewise, it has been found that with or without hair on the corolla lobes of the central hermaphrodite disc flowers have no molecular significance. Four species (*A. austriaca*, *A. incana*, *A. armeniaca*, *A. arborescens*) that have hairs on the corolla lobes of central hermaphrodite disc florets distributed into two molecular groups in half. *A. arborescens* is one of the two species found in the first primary branch. *A. austriaca*, *A. incana* and *A. armeniaca* species are three of the seven species in the second branch of the other primary branch. The secondary branch of the other primary branch with five species hasn't any species with hair on the corolla lobes of the central hermaphrodite disc flowers.

It is also an important finding that individuals of fourteen species belonging to the subgenus *Artemisia* are located only on the same branches of the phylogenetic tree with their species individuals and own control group members, and not to interfere with individuals belonging to other species. This situation is also a molecular indication that there is reproductive isolation among fourteen species and there is no gene flow and hybridization between them. Although some of the species of the subgenus *Artemisia* morphologically similar to each other in outline, the samples of each species belonging to the subgenus *Artemisia* are easily distinguishable from the samples of another species of this subgenus and there is no difficulty in their identification. In other words, even if only considering their morphological characters, it seems that the species belonging to the subgenus *Artemisia* have completed their

Table 7. The hairiness of both receptacle and corolla lobes in the species of subgenus *Artemisia*, molecular groups formed as a result of phylogenetic analysis

Species	Receptacle	Corolla Lobes of Central Hermaphrodite Disc Florets	Molecular Group
<i>A. alpina</i>	Hairy	Glabrous	
<i>A. austriaca</i>	Glabrous	Hairy	
<i>A. splendens</i>	Hairy	Glabrous	
<i>A. haussknechtii</i>	Hairy	Glabrous	II. Secondary Group
<i>A. incana</i>	Glabrous	Hairy	
<i>A. abrotanum</i>	Glabrous	Glabrous	
<i>A. armeniaca</i>	Glabrous	Hairy	II. Primary Group
<i>A. chamaemelifolia</i>	Glabrous	Glabrous	
<i>A. tournefortiana</i>	Glabrous	Glabrous	
<i>A. annua</i>	Glabrous	Glabrous	I. Secondary Group
<i>A. verlotorum</i>	Glabrous	Glabrous	
<i>A. vulgaris</i>	Glabrous	Glabrous	
<i>A. arborescens</i>	Hairy	Hairy	I. Primary Group
<i>A. absinthium</i>	Hairy	Glabrous	

speciation and there is no hybridization between them (Civelek et al., 2010; Kurşat, 2010). These molecular study data confirmed this generalization obtained only from morphological data.

The fact that the individuals of the seven species used as the control group are only in the same branches with individuals belonging to their own species makes our study 100% reliable.

As a result of this research, it was obtained original data for use in new scientific molecular studies on the taxa of genus *Artemisia* and provided the haplotypes of ETS, ITS and *psbA-trnH* regions for 14 species of the subgenus *Artemisia* in Turkey for the GenBank database (Table 3).

This study reports for the first time, molecular phylogeny of subgenus *Artemisia* from Turkey by using r-DNA (ITS and ETS) and cpDNA (*psbA-trnH*) sequences.

Based on the current data and all available in literature, it is concluded that the morphological studies coupled with modern molecular techniques may lead to the clear infrageneric classification of the genus *Artemisia*.

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References

- BESSER, W., 1829. Synopsis Absinthium. *Bulletin de la Société Impériale des Naturalistes de Moscou*, vol. 1, pp. 219-265.
- BREMER, K. and HUMPEHRIES, C.J., 1993. Generic monograph of the Asteraceae-Anthemideae. *Bulletin of the British Museum (Natural History)*. Botany, vol. 23, no. 2, pp. 71-177.
- CIVELEK, S. and SANCAR, P.Y. 2012. *The morphological and Karyological Investigation of Artemisia Spicigera C.Koch Populations that Around Van Lake*. Turkey: Firat University. Research project FUBAP - 2090.
- CIVELEK, S., SANCAR, P.Y. and DELIPOYRAZ, S., 2018. *The investigation by using some molecular markers on the phylogenetic relationships of the populations of Artemisia taurica Willd. (Asteraceae) in Turkey*. Turkey: Firat University. Research project FUBAP- FF.16.17.
- CIVELEK, S., SANCAR, P.Y. and WAHID, C.F., 2019. *The investigation on the chloroplast and nuclear genomes of taxa belong to the subgenus Dracunculus (Bess.) Rydb. of Artemisia L. (Asteraceae) in Turkey*. Turkey: Firat University. Research project FUBAP -FF.17.28.
- CIVELEK, S., YILMAZ, O., BAGCI, E., KIRBAG, S., GUR, N., TURKOGLU, I., TABUR, S. and KURŞAT, M., 2010. *The researches of taxonomical, hemical (Flavonoids and Essential oils), karyological, palynological and antimicrobial activities on taxa of the genus Artemisia L. (Asteraceae) growing in Turkey*. Turkey. TUBITAK TBAG-106T559.
- CULLEN, J., 1975. *Artemisia L.* In: P.H. DAVIS, ed. *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh University Press, vol. 5, pp. 311-324.
- DAVIS, P.H., ed., 1975. *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh University Press, vol. 5, pp. 311-324.
- DAVIS, P.H., MILL, R.R. and TAN, K., 1988. *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh University Press, vol. 10, pp. 163-164.
- DOYLE, J.J. and DOYLE, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, vol. 19, pp. 11-15.
- GUNER, A., ASLAN, S., EKIM, T., VURAL, M. and BABAC, M.T., 2012. *Türkiye Damarlı Bitkiler Listesi*. Turkey: Nezahat Gökyigit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, pp. 118-120.
- HALL, H.M. and CLEMENTS, F.E., 1923. The phylogenetic method in taxonomy. The North American species of *Artemisia*, *Chrysothamnus* and *Atriplex*. *Carnegie Institution of Washington*, vol. 326, pp. 355. <http://dx.doi.org/10.5962/bhl.title.13922>.
- HUSSAIN, A., POTTER, D., KIM, S., HAYAT, M.Q. and BOKHARI, S.A.I., 2019. Molecular phylogeny of *Artemisia* (Asteraceae-Anthemideae) with emphasis on undescribed taxa from Gilgit-Baltistan (Pakistan) based on nrDNA (ITS and ETS) and cpDNA (*psbA-trnH*) sequences. *Plant Ecology and Evolution*, vol. 152, no. 3, pp. 507-520. <http://dx.doi.org/10.5091/plecevo.2019.1583>.
- KUMAR, S., STECHER, G., LI, M., KNYAZ, C. and TAMURA, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, vol. 35, no. 6, pp. 1547-1549. <http://dx.doi.org/10.1093/molbev/msy096>. PMID:29722887.
- KURŞAT, M., 2010. *The taxonomic revision of the genus Artemisia L. (Asteraceae) growing in Turkey*. Elazığ: Institute of Science and Technology, Firat University Biology Department. PhD thesis in Botany.
- KURŞAT, M., CIVELEK, S., TURKOGLU, I. and TABUR, S., 2011a. *Artemisia sieberi* Besser subsp. *sieberi* a new record for Turkey and a delete record for Turkey *Artemisia herba-alba* Asso. (Asteraceae). *Pakistan Journal of Botany*, vol. 43, no. 4, pp. 1819-1821.
- KURŞAT, M., TURKOGLU, I., CIVELEK, S. and TABUR, S., 2011b. A new subspecies records for the flora of Turkey: *Artemisia santonicum* L. subsp. *patens* (Neilr.) K.M. Perss. (Asteraceae). *Turkish Journal of Botany*, vol. 35, no. 1, pp. 89.
- KURŞAT, M., SANCAR, P.Y. and CIVELEK, S., 2014. New record for the flora of Turkey, *Artemisia fragrans* Willd. (Asteraceae). *The Herb Journal of Systematic Botany*, vol. 21, no. 2, pp. 49-58.
- KURŞAT, M., CIVELEK, S., TURKOGLU, I., TABUR, S. and GUR, N., 2015. A new species of the subgenus *Seriphidium* of the genus *Artemisia* L. (Asteraceae) from Turkey. *Turkish Journal of Botany*, vol. 39, pp. 88-95. <http://dx.doi.org/10.3906/bot-1311-33>.
- KURŞAT, M., CIVELEK, S., SANCAR, Y.P. and TURKOGLU, I., 2018. *Artemisia taurica* Willd. var. *vanensis* Kursat & Civelek (Asteraceae: Anthemideae), a new variety from Eastern Anatolia of Turkey. *Biological Diversity and Conservation*, vol. 11, no. 3, pp. 106-114.
- LING, Y.R., 1991a. The Old World *Seriphidium* (Compositae). *Bulletin of Botanical Research*, vol. 11, no. 4, pp. 1-40.
- LING, Y.R., 1991b. The Old World *Artemisia* (Compositae). *Bulletin of Botanical Research*, vol. 12, no. 1, pp. 1-108.
- LING, Y.R., HUMPHRIES, C.J. and SHULTZ, L., 2006. The genus *Artemisia* L. In: FLORA OF CHINA EDITORIAL COMMITTEE, ed. *Flora of China*. Beijing: Science Press, vol. 20, pp. 1151-1259.
- MABBERLEY, D., 1990. *The plant-book*. 2nd ed. Cambridge: Cambridge University Press.
- MCARTHUR, E.D., 1979. Sagebrush systematics and evolution. In: UTAH STATE UNIVERSITY, ed. *Sagebrush Ecosystem Symposium*. Logan: Utah State University, pp. 14-22.
- MCARTHUR, E.D., POPE, C.L. and FREEMAN, D.C., 1981. Chromosomal Studies of subgenus *Tridentatae* of *Artemisia*, Evidence for Auto polyploidy. *American Journal of Botany*, vol. 68, no. 5, pp. 589-605. <http://dx.doi.org/10.1002/j.1537-2197.1981.tb12391.x>.

- RYDBERG, P.A., 1916. *Artemisia* and *Artemisiastrum*. *North American Flora*, vol. 34, no. 3, pp. 244-285.
- SANCAR, P.Y., WAHI D, C.F., CIVELEK, S. and KURSAT, M., 2022. An investigation on the chloroplast and nuclear genomes of taxa belong to the subgenus *Dracunculus* (Bess.) Rydb. of *Artemisia* L. (Asteraceae) in Turkey. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e242403. <http://dx.doi.org/10.1590/1519-6984.242403>.
- SCHINSKIN, B.K. and BOBROV, E.G., 1995. Genus 1550 *Artemisia*. In: B.K. ŠIŠKIN, ed. *Flora of The USSR*. Dehra Dun: Bishen Singh Mahendra Pal Singh, vol. 27, pp. 404-600.
- SHULTZ, L.M., 2006a. Subgeneric concepts in *Artemisia* L. (Asteraceae: Anthemideae): the *Tridentatae* revisited. In: Y.R. LING, ed. *International Symposium on the genus Artemisia and allies*. Beijing: South China Institute of Botany, pp. 36-44.
- SHULTZ, L.M., 2006b. *Artemisia*. In: FLORA OF NORTH AMERICA EDITORIAL COMMITTEE, ed. *Flora of North America north of Mexico*. New York: Oxford University Press, vol. 21, pp. 503-534.
- TABERLET, P., GIELLY, L., PAUTOU, G. and BOUVET, J., 1991. Universal primers amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, vol. 17, no. 5, pp. 1105-1109. <http://dx.doi.org/10.1007/BF00037152>. PMID:1932684.
- TORRELL, M., GARCIA-JACAS, N., SUSANNA, A. and VALLÉS, J., 1999. Phylogeny in *Artemisia* (Asteraceae, Anthemideae) inferred from nuclear ribosomal DNA (ITS) sequences. *Taxon*, vol. 48, no. 4, pp. 721-736. <http://dx.doi.org/10.2307/1223643>.