Pak. J. Bot., 54(4): 1385-1390, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-4(37)

AN INVESTIGATION OF PHYLOGENETIC RELATIONSHIPS OF POPULATIONS OF *ARTEMISIA TAURICA* Willd. (ASTERACEAE) FROM TURKEY USING cpDNA "*trn*" REGIONS

PELIN YILMAZ SANCAR[1*], SUEDA DELIPOYRAZ[1], SEMSETTIN CIVELEK[1] AND MURAT KURSAT[2]

[1]*Fırat University, Faculty of Sciences, Department of Biology, 23119 Elazıg, Turkey*
[2]*Bitlis Eren University, Faculty of Arts and Sciences, Department of Biology, 13000 Bitlis, Turkey*
**Corresponding author's email: peyilmaz@firat.edu.tr*

Abstract

Artemisia L. is one of the biggest genera in the family Asteraceae, with around 500-600 taxa at specific and sub-specific levels diveded in five subgenera. The majority of the members of this genus have a high economic value. Due to the high number of taxa, lot of taxonomists are tried to solve the problem of its classification and phylogeny but its natural classification still has not been achieved.

In order to find the phylogenetic relations and kinships between individual samples, the present study investigated the base polymorphisms in sequences of non - coding *trn*T -*trn*L3' section of chloroplast DNA (cpDNA) of 54 individual samples of 22 different populations belonging to *A. taurica* Willd. in Turkey.

DNA was isolated from the leaves of the studied individual samples by the CTAB method, and isolated genomic DNA was multiplied in PCR by using *trn*a and *trn*d primaries of the *trn*T-*trn*L3' section. The obtained data were evaluated by the Mega X program, and phylogenetic trees were prepared by using the "Maximum Likelihood" method.

There are no molecular significant differences between populations of the species *A. taurica* in Turkey depending on absence of genetic isolation and still ongoing gene. The view that the morphological differences observed on the samples of the Zernek dam lake (Van) population of the species *A. taurica* were found to be related to the ecological conditions and ploidy level.

Key words: Asteraceae, *Artemisia taurica,* cpDNA *trn*T -*trn*L3', Phylogeny, PCR.

Introduction

Artemisia L. is a member of the Asteraceae family, which is a polymorphic genus and is very significant from a therapeutic and economic point of view. This genus is mostly distributed in the temperate sectors of northern hemisphere. A limited number of *Artemisia* species also occur in the southern hemisphere of the globe (Oberprieler *et al*., 2009). This genus contains five hundred plants including both shrubs and herbs (Valles & McArthur, 2001) and is designated as a prevalent and diverse genus of the Anthemideae community from Asteraceae (Martin *et al*., 2003).

A great number of the *Artemisia* species possess economic importance because they are utilized as fodder, therapeutics, aesthetics, and soil binder, whilst a few taxa have been shown to cause allergy and few are just noxious weeds damaging number of crops because of their poisonous properties (Hayat *et al*., 2009a; Hussain *et al*., 2019).

Twenty two species of *Artemisia* without any infraspecific taxa are reported in the 5th volume of the Flora of Turkey (Cullen, 1975; Davis, 1975; Davis *et al.*, 1988). Later, *Artemisia verlotiorum* Lamotte was added to the 10th volume of the Flora of Turkey as a new record for Turkey, so the number of the species in *Artemisia* in Turkey grew to 23 (Davis *et al*., 1988).

The genus *Artemisia* is divided into 4 subgenera as subgenus *Artemisia* Lessing, *Dracunculus* (Besser) Rydberg, *Seriphidium* Besser ex Lessing, and *Tridentatae* (Rydberg) McArthur (McArthur *et al*., 1981; Civelek *et al.*, 2010; Kursat, 2010; Kursat *et al*., 2015).

The general distribution areas of the species *Artemisia taurica* worldwide are Europe, Southern Russia (Caucasus), Crimea and Turkey. *Artemisia taurica* is widely distributed in the steppes of Central, Eastern, and Southeastern Anatolia in Turkey. It is one of the four species that have a wide distribution in Turkey. The other three species with a wide distribution in Turkey are *Artemisia absinthium* L., *Artemisia vulgaris* L., and *Artemisia campestris* L. (Civelek *et al*., 2010; Kursat, 2010).

Civelek *et al*., (2010) revised the genus *Artemisia* in Turkey. During this study of the genus *Artemisia*, significant morphological differences were observed in the of *Artemisia taurica*, especially in the eastern Anatolia (Civelek *et al*., 2010; Kursat, 2010; Ayaz *et al.,* 2019). A molecular study was needed to determine if these differences had some genetic basis or just morphological polymorphisms. For this reason, the purpose of this study was to evaluate the genetic diversity and taxonomic position of the species *Artemisia taurica* in Turkey using some molecular techniques. No molecular systematic studies were conducted using the populations of *Artemisia* genus of Turkey. This study is important in that it is the first molecular-based study using *Artemisia taurica* taxa of Turkey. In addition, the first molecular data for the *trn*T-*trn*L3' gene region from Turkey were submitted to the international NCBI GenBank databases.

Material and Methods

Plant material: A total of 54 different individuals belonging to the taxa of *Artemisia taurica* species were gathered M. Kursat from the populations from the natural

habitat in 22 different regions in Turkey. These plants were kept in the Herbarium belonging to the Faculty of Sciences at Bitlis Eren University. The collection was made during the vegetative and flowering periods in the months of April-September in years of 2007-2010. The locations of the collections areas were given in (Table 1) and (Fig. 1).

DNA isolation: The leaf tissue of the samples were used for DNA isolation which was carried out manually using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle & Doyle, 1987). By measuring the concentrations of isolated DNAs using a nanodrop spectrophotometer, they were adjusted to 25 ng/ul. Stock DNA was preserved at -20 C.

PCR amplification: In the PCR studies conducted using *trn*T and *trn*L primers, the *trn*T - *trn*L3' region multiplied with the 54 samples had a nucleotide length of 750-800. The sequence of primers used to amplify the *trnT - trnL3'* region is given in Table 2 (Taberlet *et al.*, 2007). Aiming for the final concentration for the PCR studies to be 50 µL; 10µL buffer, 3 µL MgCl2, 1 µL dNTPs, 0,50 µL from each primer (forward and reverse), 0,50 µL taq polymerase, and nearly 6 ng (2,70 µL) template DNA were mixed. Amplifications were carried out in a Biorad T100 Gradient Thermal Cycler. The PCR device was repeated for 34X cycles, consisting of 2 minutes at 95°C initial denaturation, 1 minute at 95°C denaturation, 40 seconds at 57°C annealing, 1 minute at 72°C extension, and 5 minutes at 72°C final extension. The PCR products were monitored in agarose gel containing 2% ethidium bromide (0.5 µg/ml) and viewed with the UV translimünator.

Sequence analysis: Two-way reading was applied to the amplification products. The PCR purification process was carried out before the sequence analysis. The purification and sequencing processes were done by the Macrogen Company. Finch TV Version 1.4 was used to evaluate the data from the chromatogram. In order to specify the phylogenetic relations among *Artemisia taurica* taxa that grew in different geographical regions, Mega program was used and the molecular variability parameters among the taxa were determined. The sequence alignments were recorded in the NCBI data bank and accession numbers were obtained (Table 3). The DNA sequence alignments for 55 individuals (one of them being outgroup) were evaluated using the Mega Program X Version. The DNA sequence alignments for all the individuals were subjected to statistical analysis.

Results

In this research, 55 individuals taken from 22 different populations of *A. taurica* were examined. All the examined individuals from the same and different populations of *Artemisia taurica*, were used to determine the sequences of "*trn*" regions. The peak results of the bi-directional sequences sent to us from Macrogene were evaluated using Version 1.4 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 *Artemisia taurica*, no reference regions were obtained. For a more accurate visualization of the results of the alignment, we did not evaluate about 50-100 bases from the beginning and the end. For this reason, approximately 779 base pairs for the cpDNA *trn* regions were used. In the phylogenetic tree drawing, the DNA sequences of the *trn*T-*trn*L3' region in the chloroplast genome were evaluated using Version X of the Mega program.

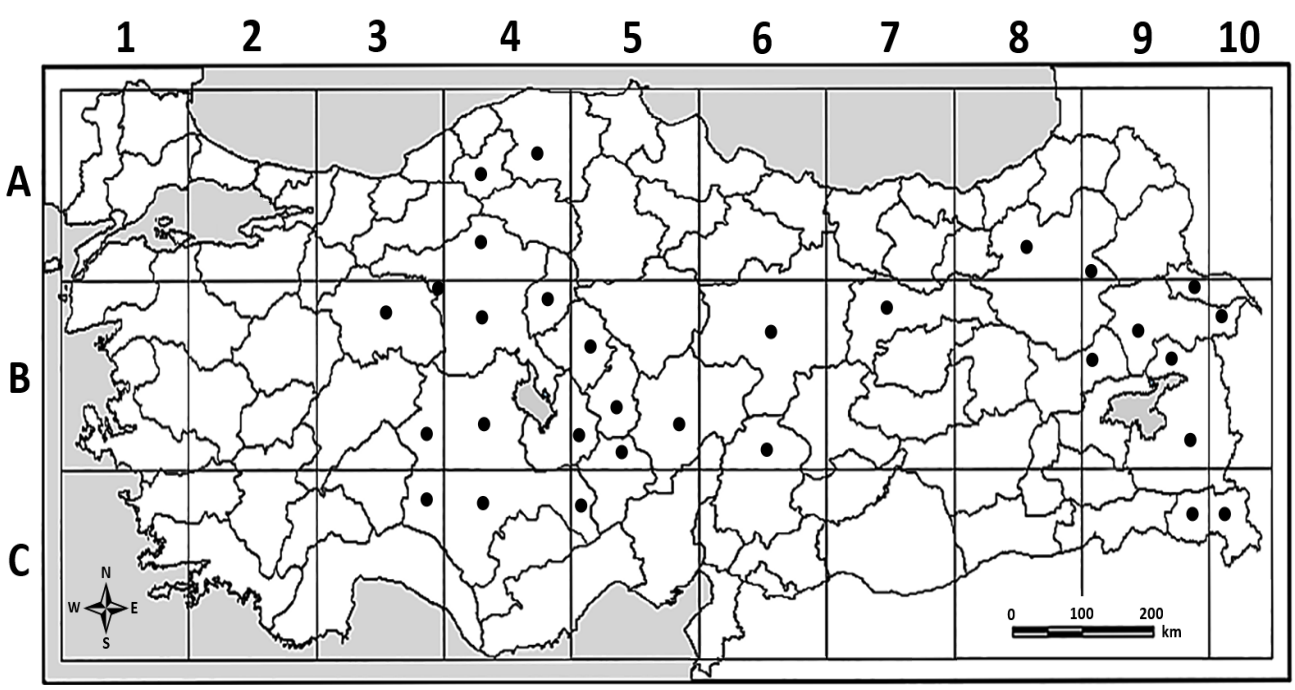

Fig. 1. Geographic distribution of species *A. taurica* (●) in Turkey (Civelek *et al*., 2010; Kursat, 2010).

Table 1. Information of *Artemisia taurica* location in field.

Taxa of the *Artemisia taurica*	Number of populations and collector	Collection date	Detailed localities of specimens populations	Lat- Lon
A. taurica 1 (4 individual)	1007 M. KURŞAT	06.07.2007	**B5 Niğde:** Kayseri highway, after 5 km, the area between the railway and the highway, 1250-1300m.	38.15 N 34.17 E
A. taurica 2 *(3 individual)*	1033 M. KURŞAT	10.09.2007	**B4 Ankara:** Between Lalahan and Elmadağ, around garrison and slopes, field edges, 1225 m	39.22 N 31.01 E
A. taurica 3 (4 individual)	1031 M. KURŞAT	06.12.2007	**B4 Ankara:** Polatlı highway, 10 km before Temelli, roadsides, 843 m	40.43 N 32.20 E
A. taurica 4 (2 individual)	1098 M. KURŞAT	23.10.2007	**B3 Eskişehir:** Polatlı - Sivrihisar, 35 km before Sivrihisar, roadsides, stepper, 837 m.	40.33 N 29.31 E
A. taurica 5 (1 individual)	1114 M. KURŞAT	26.11.2007	**B10 Ağrı:** Between Habur-Tutak, 4 km after Habur, roadsides, stepper, 1605 m.	38.31 N 30.32 E
A. taurica 6 (3 individual)	1032 M. KURŞAT	10.09.2007	**B4 Ankara:** Between Gölbaşı and Bayındır Dam, stepper 994 m.	41.16 N 27.59 E
A. taurica 7 (3 individual)	1034 M. KURŞAT	10.09.2007	**B4 Ankara:** Between Elmadag and Kirikkale, 1000 m.	38.08 N 30.56 E
A. taurica 8 (2 individual)	1035 M. KURŞAT	11.09.2007	**B5 Kırşehir:** Kaman - Hirfanlı dam road, roadsides, 10 km after Kaman, 975 m.	40.31 N 29.32 E
A. taurica 9 *(3 individual)*	1011 M. KURŞAT	07.07.2007	**B4 Ankara:** Şereflikoçhisar, South slopes of Akinler village, stepper, 989 m.	38.27 N 30.16 E
A. taurica (var. *nova*) 10 (3 individual)	1056 M. KURŞAT	20.09.2007	**B9 Van:** Van - Hakkari highway, Northern slopes of Zernek dam, roadside, mountain steppe, 1960m.	41.37 N 26.38 E
A. taurica 11 (2 individual)	1185 M. KURŞAT	02.11.2008	**B9 Van:** Erçiş, Zernaki Mountain, Irşat region, slopes, steppe, 1712 m.	39.03 N 43.20 E
A. taurica12 (1 individual)	1119 M. KURŞAT	26.11.2007	**B8 Muş:** Malazgirt, between Aktuzla-Karıncalı villages, 3 km before Karıncalı village, slopes, 1550 m.	39.21 N 42.15 E
A. taurica 13 (3 individual)	1071 M. KURŞAT	23.09.2007	**B9 Van:** Highway between Erçiş-Adilcevaz, 21. km, roadsides, slopes, 1750m.	38.57 N 43.13 E
A. taurica 14 (1 individual)	1089 M. KURŞAT	22.10.2007	**B5 Kayseri:** Between Kayseri and Avanos, 39 km after Kayseri, roadsides, slopes, steppes, 1121 m	38.43 N 35.04 E
A. taurica 15 (1 individual)	1036 M. KURŞAT	11.09.2007	**B5 Kırşehir:** Between Hirfanlı Dam and Şereflikoçhisar, from Hirfanlı Dam to the West, hills and roadsides, 972m.	39.16 N 33.30 E
A. taurica 16 (4 individual)	1028 M. KURŞAT	10.09.2007	**B4 Ankara:** Polatlı highway, 37 km before Polatlı, roadsides, 843m.	39.45 N 32.28 E
A. taurica 17 *(3 individual)*	1005 M. KURŞAT	04.07.2007	**B6 Kahramanmaraş:** Göksun, Upper parts of Fındıklıkoyak village, forest area, summit, open area, 640 m.	37.60 N 36.32 E
A. taurica 18 (3 individual)	1090 M. KURŞAT	22.10.2007	**B6 Kahramanmaraş:** Göksun, Fındıklıkoyak village entrance and surroundings, 1450 m	37.60 N 36.32 E
A. taurica 19 (1 individual)	1097 M. KURŞAT	23.10.2007	**B4 Ankara:** Polatlı highway, roadsides, 843m.	39.43 N 32.23 E
A. taurica 20 (2 individual)	1009 M. KURŞAT	07.07.2007	**B4 Ankara:** Şereflikoçhisar, Hamzalı village, Kayacık (Mutlucan) salt factory area, 933 m.	38.50 N 33.26 E
A. taurica 21 (3 individual)	1172 M. KURŞAT	26.08.2008	**B10 Ağrı:** Doğubeyazıt, Bottom of İshakpaşa Palace, 1935 m	39.31 N 44.07 E
A. taurica 22 (2 individual)	1058 M. KURŞAT	20.09.2007	**C10 Hakkari:** Hakkari highway, 37 km before Hakkari, roadsides, slopes, steppes, 1496 m.	37.41 N 43.58 E

The nucleotide composition and other features of the individuals were determined as a result of the statistical analyses performed by cutting the excess parts at the head and end of the DNA sequences. In the DNA sequences of the *trn*T-*trn*L3' region in the chloroplast genome of the examined individuals, some of the parameters of the molecular diversity such as the conservated 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 obtained values were given in (Table 4).

Using the Best DNA / Protein step in the Models menu of the MEGA 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 (Kumar *et al*., 2019). The Maximum Likelihood, Neighbor-Joining, UPGMA, and Maximum Parsimony methods were applied separately. The method that illustrated best the evolutionary and phylogenetic relationship between the examined individuals was the Maximum Likelihood method.

Many of the phylogenetic trees were drawn by trying many methods, and the most useful tree was chosen. In the Maximum Likelihood method, by entering the bootstrap value as 100, a single phylogenetic tree for a total of 55 individuals, 54 of which were examined and one of which was an outgroup, was obtained (Fig. 2). The species *Haplocarpa scaposa* L. as an outgroup was used (Sancar, 2017, Kursat *et al*., 2018, Sancar *et al*., 2019).

Table 2. The base sequences of the primers used.

Primers	Base sequences (5' – 3')
***trn*a (Forward):**	5' CAT TAC AAA TGC GAT GCT CT 3'
***trn*d (Reverse):**	5' GGG GAT AGA GGA CTT GAA C 3'

Table 3. Genbank accession numbers for the *trn*T-*trn*L3' regions of the studied samples.

Specimens	GenBank Accesion Numbers
Artemisia taurica 1	MT637781
Artemisia taurica 2	MT637782
Artemisia taurica 3	MT637783
Artemisia taurica 4	MT637784
Artemisia taurica 5	MT637785
Artemisia taurica 6	MT637786
Artemisia taurica 7	MT637787
Artemisia taurica 8	MT637788
Artemisia taurica 9	MT637789
Artemisia taurica 10	MT637790
Artemisia taurica 11	MT637791
Artemisia taurica 12	MT637792
Artemisia taurica 13	MT637793
Artemisia taurica 14	MT637794
Artemisia taurica 15	MT637795
Artemisia taurica 16	MT637796
Artemisia taurica 17	MT637797
Artemisia taurica 18	MT637798
Artemisia taurica 19	MT637799
Artemisia taurica 20	MT637800
Artemisia taurica 21	MT637801
Artemisia taurica 22	MT637802

Table 4. PCR amplified summary statistics of the cpDNA *trn* region of the genus *A. taurica*.

Parameters of molecular diversity	*trn*T - *trn*L3' region
Total population number	22
Total individuals	54
Total band Length	779
The ratio of G-C base pair (%)	35,5
Conserved regions (C)	719
Variation regions (V)	37
Single parts (S)	32
Parsimony informative regions (Pi)	5
Homologous base pairs (ii)	752
Transitional base pairs (si)	1.00
Transversional base pairs (sv)	1.00
R value (si/sv)	1.00

Discussion

In this research, a phylogenic systematic study was conducted by using the molecular data of *A. taurica*. 54 individuals taken from 22 different populations belonging to this species were examined by analyzing the base slice of the regions. An attempt to find some information about the populations' closeness with and distance from each other. This research is important as it is the first molecular-based study of the species *A. taurica* growing naturally in Turkey.

Molecular diversity parameters for the individuals of the *A. taurica* species are shown in Table 4. As seen in the table, the number of informative regions is very low, which indicates that the sequences between populations do not differ much. However, when the positions of the variable bases were examined in terms of the variation we obtained in our studies, it was seen that the individuals of the *A. taurica* species in both close and same localities generally showed similar base sequences and were placed sideways in the phylogenetic tree in Figure 2.

It was reported that the apomictic reproduction pattern was very common in the autopolyploid species and populations of the genus *Artemisia* (Czapik, 1996; Noyes, 2007; Melanie *et al*., 2014). According to Gustavsson (1947), the apomictic breeding pattern guarantees individual genotypes a long-lasting existence in large areas (Gustavsson, 1947). Apomictic reproduction may cause high similarities and fewer variations at the molecular level between samples of different populations studied. This is because apomictic seed development is a form of reproduction that generates offspring genetically identical to the parent plant. In this study, considering the high numerical values of the conserved region and homologous base pair and the low numerical values of regions with variation and informative regions; we can say that the studied populations of the *A. taurica* species have a high probability of apomictic reproduction.

It has been reported that the populations of the *A. taurica* species in the world and in Turkey are autotetraploid (2n=4x=36), and the only Van (Zernek Dam) population was autohexaploid (2n=6x=54) (Civelek *et al*., 2010; Kursat, 2010). We believe that the differences in morphological structures in Van (Zernek Dam) population samples belonging to the *A. taurica* species (Civelek *et al.*, 2010; Kursat, 2010) were caused by adaptation to different ecological conditions and higher ploidy levels.

In addition, according to the phylogenetic tree in Figure 2, samples belonging to the Zernek dam (Van) population, together with samples of other Van populations in close localities, sitting side by side on neighboring branches that can be explained in two ways:

i. It can show that different ploidy levels do not inhibit gene flow between populations and do not result in genetic isolation. However, the fact that autopolyploid species generally have apomictic reproduction thus making this less possible. Apomixis produces populations of plants of the same genotype with high levels and good adaptation.

ii. 2. Populations of the *A. taurica* species reproduce apomictically because they are autopolyploid and autohexaploid; due to apomictic reproduction (asexual seed production), populations cannot find the opportunity to differentiate genetically. This is because meiosis and crossing-over do not occur in apomictic reproduction. For this reason, newly formed embryos are genetically identical to the parent (mother) plant, as variations do not occur.

For this reason, the Van (Zernek dam) population of the *A. taurica* species were evaluated within the framework of the evolutionary species concept and intra-specific variation (micro-species) was accepted and a new variety has published (Kursat *et al*., 2018).

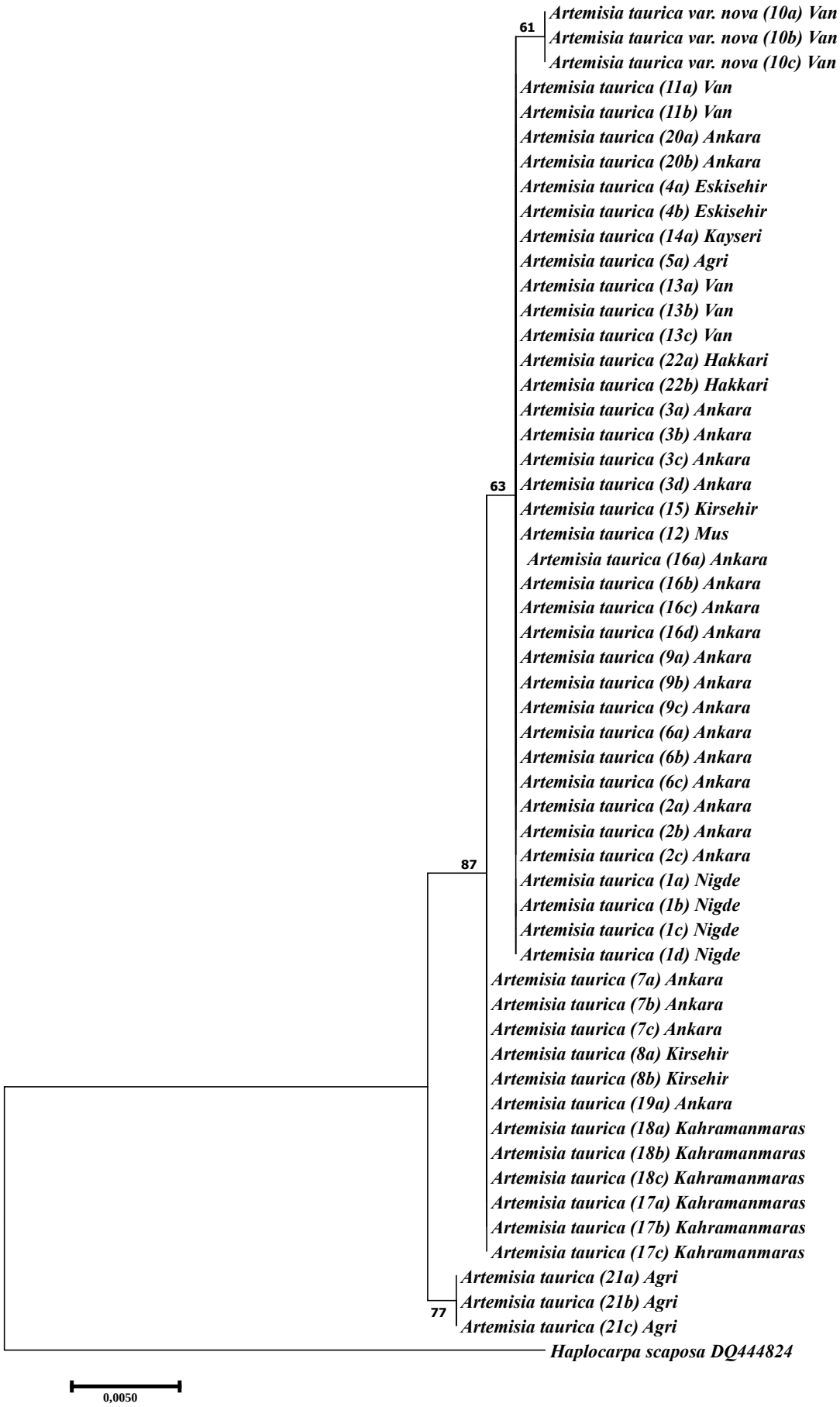

Fig. 2. Maximum Likelyhood tree showing the phylogenetic relationship between individuals.

References

Ayaz, S., M. Ahmad, M. Zafar, M.I. Ali, S. Sultana, M.R.U. Mustafa, O. Kilic, D.N. Çobanoğlu, A. Demirpolat, A. Ghani, R. Afza, K. Ahmad, M. Munir, N. Kalsoom and J. Raza. 2020. Taxonomic significance of cypsela morphology in tribe Cichoreae (Asteraceae) using light microscopy and scanning electron microscopy. *Microscopy Research and Technique Mar*., 83(3): 239-248. doi: 10.1002/jemt.23407. Epub 2019 Nov 12. PMID: 31713962.

Civelek, S., Ö. Yilmaz, E. Bagci, S. Kirbag, N. Gür, İ. Türkoglu, S. Tabur and M. Kurşat. 2010. The researches of taxonomical, chemical (Flavonoids and Essential oils), karyological, palynological and antimicrobial activities on taxa of the genus *Artemisia* L. (Asteraceae) growing in Turkey. TÜBİTAK, Project no. TBAG-106T559.

Cullen, J. 1975. *Artemisia* L. In: (Ed.): Davis, P.H. Flora of Turkey and the East Aegean Islands. *Edinburgh Univ Press.* 5: 311-324.

Czapik, R. 1996. Problems of apomictic reproduction in the families Compositae and Rosaceae. *Folia Geobot. Phytotax.,* 31: 381-387.

Davis, P.H. and I.C. Hedge. 1975. The Flora of Turkey: Past, Present and Future. *Candollea*, 30: 331-351.

Davis, P.H., R.R. Mill and K. Tan. 1988. Flora of Turkey and The East Aegean Islands. *Edinburgh University Press,* 10: 163-164.

Doyle, J.J. and J.L. Doyle. 1987. A Rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*., 19: 11-15.

Gustavsson, Å. 1947. Apomixis in higher plants. *III. Biotype and species formation. – Lunds Univ. Årsskrift*, *N. F., Avd*., 2: 43(12): 183-370.

Hayat, M.Q., M.A. Khan, M. Ashraf and S. Jabeen. 2009a. Ethnobotany of the Genus *Artemisia* L. (Asteraceae) in Pakistan. *Ethnobot. Res. & Appl.,* 7: 147-162.

Hussain, A., D. Potter, S. Kim, M.Q. Hayat and S. Bokhari. 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-*trn*H) sequences. *Plant Ecol. & Evol.,* 152: 507-520.

Kumar, S., G. Stecher, M. Lı, C. Knyaz and K. Tamura. 2019. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol*., 30: 2725-2729.

Kurşat, M. 2010. The taxonomic revision of *Artemisia* L. (Asteraceae) genus growing in Turkey Fırat University, Institute of Science and Technology, Biology Department, PhD thesis, Elazığ, Turkey.

Kurşat, M., S. Civelek, İ. Türkoğlu, S. Tabur and N. Gür. 2015. A new species of subgenus Seriphidium of *Artemisia* L. (Asteraceae) from Turkey. *Turk. J. Bot.,* 39: 88-95.

Kursat, M., S. Civelek, P.Y. Sancar and I. Turkoglu. 2018. *Artemisia taurica* Willd. var. *vanensis* Kursat & Civelek (Asteraceae: Anthemideae), a new variety from Eastern Anatolia of Turkey. *Biol. Div. & Cons.,* 11(3): 106-114.

Martin, J., M. Torrel, A.A. Korobkov and J. Valles. 2003. Palynological features as systematic marker in *Artemisia* L. and related genera (Asteraceae, Anthemideae)–II: implications for subtribe Artemisiinae delimitation. *Plant Biol.,* 5: 85-93.

McArthur, E.D., C.L. Pope and D.C. Freeman. 1981. Chromosomal studies of subgenus *Tridentatae* of *Artemisia*: evidence for autopolyploidy. *Amer. J. Bot.,* 68: 589-605. doi:10.2307/2442786.

Melanie, L. Hand and Anna M.G. Koltunow. 2014. The genetic control of apomixis: asexual seed formation. *Genetics.* 197: 441-450.

Noyes, R.D. 2007. Apomixis in the Asteraceae: Diomands in the Rough. *Functional Plant Science and Biotechnology*. Global Science Books, P. 217.

Oberprieler, C.H., S. Himmelreich, M. Källersjö, J. Vallès, L.E. Watson and R. Vogt. 2009. Tribe Anthemideae. In 'Systematics, Evolution and Biogeography of the Compositae'. (Eds.): Funk, V.A., A. Susanna, T. Stuessy and R. Bayer. pp. 632-666.

Sancar, P.Y. 2017. The investigation on the genetic structures and phylogenetic relationships of the species of the genus *Anthriscus* Pers. (Apiaceae) distribution in Turkey, by use of non - coding "*trn*" regions of chloroplast genome. Institute of Science and Technology of Fırat University Biology Department Ph.D. thesis Elazığ Turkey.

Sancar, P.Y., S. Civelek, M. Tekin and S.D. Dastan. 2019. İnvestigation of the genetic structures and phylogenetic relationships for the species of the genus Anthriscus pers. (Apiaceae) distributed in Turkey, using the non – codıng "*trn*" regions of the chloroplast genome. *Pak. J. Bot.*, 51(3): 1049-1057.

Taberlet, P., E. Coissac, F. Pompanon, L. Gielly, C. Miquel and A. Valentini. 2007. Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. *Nucl. Acids Res.*, 35: 3.

Valles, J. and E.D. McArthur. 2001. *Artemisia* systematics and phylogeny: cytogenetic and molecular in sights. US Department of Agriculture Forest Service, Rocky Mountain Research Station, Provo, UT Ogden, USA.

(Received for publication 13 January 2021)