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INVESTIGATION OF THE GENETIC STRUCTURES AND PHYLOGENETIC RELATIONSHIPS FOR THE SPECIES OF THE GENUS *ANTHRISCUS* PERS. (APIACEAE) DISTRIBUTED IN TURKEY, BY USE OF THE NON - CODING "*TRN*" REGIONS OF THE CHLOROPLAST GENOME

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Abstract

The genus *Anthriscus* Pers. of the family Apiaceae is a small sized genus with 16 species, distributed in the world. In Turkey it is represented by 8 taxa, distributed in 4 sections. The aim of this study was to determine the genetic proximity and distances of taxa to each other and to identify interrelationships, systematic and phylogenetic relationships using the sequence analysis information of the non-coding trn region in the chloroplast genome of the *Anthriscus* species in Turkey.

The phylogenetic tree showed that the taxa *A. caucalis* var. *caucalis* and *A. tenerrima* var. *tenerrima* (belonging to sect. *Anthriscus*) with *A. cerefolium* var. *trichocarpa* (belonging to sect. *Cerefolium*) had completed their speciations and isolation with other species in terms of speciation was provided. It can be said that *A. kotschyi*, the only representative of the sect. Caroides in Turkey, is isolated having completed its speciation also. The presence of the continuing gene exchange between the taxa can be mentioned, while the taxonomy of the two taxa of *A. sylvestris* and *A. lamprocarpa*, two members of the sect. *Cacosciadium*, cannot be determined more clearly yet. For this reason, it can be said that the *A. sylvestris* subsp. *sylvestris* and *A. sylvestris* subsp. *nemorosa* taxa, previously identified as two subspecies belonging to *A. sylvestris*, should be raised again to *A. sylvestris* and *A. nemorosa* taxa. In addition, an infrageneric arrangement and subsequent taxonomic regulation need to be made for the subspecies belonging to the *A. lamprocarpa* taxa.

Key words: Apiaceae, *Anthriscus*, Chloroplast DNA, Molecular phylogeny, PCR, *trn*L3'-*trn*F region.

Introduction

The Apiaceae Lindley family of flowering plants is one of the most well-known plant families (Lindley, 1836). It is commonly found in moderate and tropical areas (Heywood, 1978). This cosmopolitan family is represented by 450 genera and 3,700 species in the world (Pimenov & Leonov, 1993). Turkey, with its different geomorphologic structures and climate diversity, has distinct biodiversity. Accordingly, our country has 104 genus and 486 species of plants belonging to the Apiaceae family, with the endemism ratio being rather high at 37% (Duran *et al.*, 2015). Due to its different volatile components, the Apiaceae family is used widely in both healthcare and food industries, but its taxonomy has not been clearly completed yet. Many species in the family have synonyms and there is no fixed overarching name (Tekin & Civelek, 2016).

Anthriscus Persoon (1805:320), a small genus closely related to *Chaerophyllum* Linnaeus (1753:258–259) and *Scandix* Linnaeus (1753:257), shares many synonyms with the two abovementioned genera (Spalik, 1997). Even though roughly 80 species of *Anthriscus* are described throughout the world, only 14 species are officially accepted (Spalik, 1997).

The *Anthriscus* genus in Turkey is represented by eight species (Hedge & Lamond, 1972; Güner *et al*., 2012). However, some species were reorganized within themselves and then the number of species in Turkey rose to 10 (Spalik, 1996).

Many molecular studies have been conducted on the *Anthriscus* species around the world. Many of these studies rely on the sequence alignment in regions like nrDNA ITS, cpDNA *rpl*16, *rps*16, *rpo*C1 and cpDNA *trn*. Downie *et al.,* conducted different molecular studies on the *Anthriscus* species and added many haplotypes to the GenBank database. Most of the conducted studies were comprehensive ones dealing with more genera within a family (including *Anthriscus*). They are mostly based on subfamilies and tribes in order to cast more light on the classification within the family by pointing out the differences between genera (Downie & Katz-Downie, 1996; Downie *et al.,* 1998; Downie, 2000; Downie *et al.*, 2000a; Downie *et al.*, 2000b; Downie *et al.*, 2002; Downie *et al.*, 2010). While studies relying on the molecular phylogenetic data of the *Anthriscus* genus were conducted abroad, a phylogenetic study related to the species widespread in Turkey was not found.

The use of sequence data in phylogenetics particularly from the chloroplast genome has found versatile applications in the field of plant molecular biology and evolution (Channa *et al.,* 2018). Many cpDNA phylogenetic studies carried out in recent years have yielded significant results (Palmer *et al.,* 1988). Non-coding sequences tend to evolve faster than coded ones and thus, they provide more information from the perspective of phylogenetic development (Wand *et al.,* 1999). Among these non-coding regions, the *trn* region is the most comprehensively studied part of cpDNA, because it is widely used to determine phylogenetic kinship within the subcategories of a family (Taberlet *et al.,* 1991; Kelchner, 2000). *trn* regions differ in most plant groups (Bayer &

Star, 1998; Bakker *et al.*, 2000; Mansion & Struwe, 2004). The *trn* region used in this study, *trn*L3'- *trn*F, is one of the three regions between the *trn*T and *trn*F regions and is particularly used in evolutionary studies. It is rather applicable because of its high mutation ratio in non-coding regions containing hundreds of base couples and in regions with only one copy (Wolfe *et al.*, 1987).

The purpose of this study is to re-classify the *Anthriscus* species grown in Turkey within themselves and to determine the systematic position of the genus members grown in our country. By determining the systematic place of the species, whose borders have not yet been specified using morphological, anatomical, palynological and karyological methods, the study provides another perspective on the taxonomy of the genus in Turkey. The molecular structure of the species of *Anthriscus* present in Turkey (*Anthriscus caucalis* Marschall von Bieberstein var. *caucalis, Anthriscus tenerrima* Boissier & Spruner var. *tenerrima, Anthriscus cerefolium* (Linnaeus, 1753:257) Hoffmann (1814:41) var. *trichocarpa* Neilreich, *Anthriscus kotschyi* Boiss. & Balansa in Boissier (1856:102), *Anthriscus lamprocarpa* Boissier (1844:59) subsp. *lamprocarpa*, *Anthriscus lamprocarpa* Boissier (1844:59) subsp. *chelikii* Tekin & Civelek (2016:253), *Anthriscus sylvestris* (Linnaeus 1753:258) Hoffmann (1814:40) subsp. *sylvestris*, *Anthriscus sylvestris* (Linnaeus, 1753:258) Hoffmann (1814:40) subsp. *nemorosa* (Marschall von Bieberstein, 1808:232) Koso-Poljansky (1920:103) were studied in this context. As the main goal of the study, the non-coding *trn*L3'-*trn*F region of cpDNA was replicated from genomic DNA using specific universal primers. The additional data provided will help to solve taxonomic problems and cast light on the evolutionary diversity and the problematic systematic relations of the *Anthriscus* species. Furthermore, the haplotypes of the abovementioned species were provided for the GenBank database.

Material and Methods

Plant material: Within the scope of this study, a total of 46 different individuals belonging to taxa at the species and subspecies levels of *Anthriscus* species were gathered from populations from 29 different regions in Turkey together with the *Chaerophyllum temulum* L. and *Chaerophyllum astrantia* Boiss. species belonging to the genus *Chaerophyllum* as an outgroup. These plants had been used in the Herbarium belonging to the Faculty of Sciences at Cumhuriyet University (CUFH), and they were gathered by the third author from natural habitats during the vegetative and flowering periods between April-September of 2010-2012, then pressed and deposited to the herbarium. The locations where the study's taxa were gathered are given in Table 1.

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

PCR amplification: In the PCR studies conducted using *trn*e and *trn*f primers, the *trn*L3'-*trn*F region had multiplied with 48 samples having a nucleotide length of 350-300 (two of them being outgroup). The sequence of primers used to amplify the *trn*L3'-*trn*F region are given in Table 2 (Taberlet *et al.*, 1991). Aiming for the final concentration for the PCR studies to be 25 µL, 5µL buffer, 1,5 µL $MgCl_2$, 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 DNA were mixed and the PCR device was repeated for 30 cycles consisting of 2 minutes at 95°C initial denaturation, 1 minute at 95°C denaturation, 40 seconds at 57°C annealing, 1 minutes at 72°C extension and 5 minutes at 72°C final extension. The PCR products were monitored in agarose gel with 1% ratio.

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. Those sequence alignments in the *trn*L3'-*trn*F region of the individual chloroplast DNA which were raised in different locations varying between three and nine for each taxon were selected for analysis. The Mega program 5.1 Version was used for sequence alignment and data analysis, and they coincide at the Clustal W step. To specify the phylogenetic relations among *Anthriscus* taxa that grow in different geographical regions, the Mega program was used and the molecular variability parameters among the taxa were determined. The sequence alignments that were obtained were recorded in the NCBI data bank and accession numbers were obtained (Table 1.).

DNA sequence alignments for 48 individuals (two of them being outgroup), were evaluated using the Mega Program 5.1 Version. DNA sequence alignments for all the individuals were subject to statistical analysis within the scope of this program.

Results

In this study of the non-coding *trn* L3'-*trn* F region of cpDNA belonging to the eight taxa of the genus *Anthriscus* (Fig. 1), the size of the *trn* L3'-*trn* F region ranged from 320-340 bp. A total of 315 bp was seen in the final data set, which with gaps and missing data including the outgroup (*Chaerophyllum temulum* and *Chaerophyllum astrantia*) was composed of 35 variable sites, 24 parsimony informative sites (PI) and 34.1% GC content (Table 3). 24 PI was indicated in Table 4.

The phylogenetic tree was constructed with the sequences from the current study and the sequences from the NCBI database using the maximum-parsimony (MP) method. One of the nine most common parsimonious trees is shown in Fig. 1. All sequences of *A. cerefolium* var. *trichocarpa* coming from eight populations and one sequence of *A. cerefolium* (GU456628.1) obtained from the NCBI database were grouped in the same clade with high bootstrap values (99%). When the base slice of *A. tenerrima* var. *tenerrima* and *A. caucalis* var. *caucalis* taxa was investigated, it was observed that the bases in their informative regions showed changes in the same direction. Because of this, these two taxa branch off from the same place in the phylogenetic tree and they exist as two very similar types having a bootstrap value of 89%

(Fig. 1). The *A. kotschyi* taxon revealed harmonious base arrangements with its individuals that were collected from different regions during the studies we carried out. As a result of achieving isolation among the species, it appeared independently on the phylogenetic tree (Fig. 1) having a bootstrap value of 98%. Two sub-types of *A. sylvestris* taxon have been noted within the borders of our country. These are *A. sylvestris* subsp. *sylvestris* and *A. sylvestris* subsp. *nemorosa* taxa (Spalik, 1997; Tekin & Civelek, 2013). As the informative regions of these two taxa were evaluated, it was observed that they had not changed in a like fashion. As a result, they have branched off at separate places on the phylogenetic family tree (Fig. 1). While the *A. sylvestris* subsp. *slyvestris* had branched off together with its own populations having a bootstrap value of 80%, a break down indicative of polytomy was observed on the branches belonging to the individuals of the *A. sylvestris* subsp. *nemorosa*. Because there were no distinguishing differences with respect to the base slice of the individuals belonging to the said taxon, no significant branching has occurred, and the bootstrap value has remained below 50%. The *A. lamprocarpa* species splits into two subspecies within the borders of Turkey and these are *A. lamprocarpa* subsp. *lamprocarpa* and *A. lamprocarpa* subsp. *chelikii* (Tekin & Civelek, 2016). On the phylogenetic family tree *A. lamprocarpa* and *A. slyvestris* subsp. *nemorosa* taxa were found on the same branches that collapsed.

Table 1. Information about taxa that are analysed within the scope of the study and genbank accesion numbers.

Specimens	Location (Altitude)-Voucher	GenBank Acc. No.
A. cerefolium var. *trichocarpa*	Konya (1127 m), -MT 1082	KY710804
A. cerefolium var. *trichocarpa*	Aksaray (1184 m), -MT 1089	KY710806
A. cerefolium var. *trichocarpa*	Erzincan (1265 m), -MT 1096	KY710803
A. cerefolium var. *trichocarpa*	Amasya (404 m), -MT 1105	KY710805
A. cerefolium var. *trichocarpa*	Eskişehir (1320 m), -MT 1086	KY710807
A. cerefolium var. *trichocarpa*	Amasya (726 m), -MT 1075	KY710810
A. cerefolium var. *trichocarpa*	Isparta (972 m), -MT 1204	KY710808
A. cerefolium var. *trichocarpa*	Amasya (726 m), -MT 1192	KY710809
A. caucalis var. *caucalis*	Aksaray, (1184 m) –MT 1090	KY710784
A. caucalis var. *caucalis*	Eskişehir, (866 m) –MT 1085	KY710785
A. caucalis var. *caucalis*	Bolu, (1373 m) –MT 1113	KY710786
A. caucalis var. *caucalis*	Kocaeli, (210 m) –MT 1196	KY710787
A. caucalis var. *caucalis*	Afyonkarahisar, (1153 m) –MT 1083	KY710788
A. caucalis var. *caucalis*	Tekirdağ, (168 m) –MT 1077	KY710789
A. caucalis var. *caucalis*	Isparta, (937 m) –MT 1080	KY710790
A. caucalis var. *caucalis*	Bursa, (311 m) –MT 1197	KY710791
A. caucalis var. *caucalis*	Afyonkarahisar, (1130 m) –MT 1084	KY710792
A. caucalis var. *caucalis*	Edirne, (50 m) –MT 1078	KY710793
A. sylvestrsis subsp. *sylvestris*	Kars, (2114 m) –MT 1130	KY710797
A. sylvestrsis subsp. *sylvestris*	Iğdır, (2008 m) –MT 1126	KY710798
A. sylvestrsis subsp. *sylvestris*	Kars, (2108 m) –MT 1129	KY710799
A. sylvestrsis subsp. *sylvestris*	Artvin, (1078 m) –MT 1143	KY710800
A. sylvestrsis subsp. *sylvestris*	Ardahan, (1849 m) –MT 1156	KY710801
A. sylvestrsis subsp. *sylvestris*	Bolu, (1379 m) –MT 1115	KY710802
A. sylvestrsis subsp. *nemorosa*	Sivas, (1447 m) –MT 1227	KY710818
A. sylvestrsis subsp. *nemorosa*	Artvin, (2380 m) –MT 1142	KY710819
A. sylvestrsis subsp. *nemorosa*	Artvin, (1435 m) –MT 1140	KY710820
A. sylvestrsis subsp. *nemorosa*	Erzincan, (1865 m) –MT 1099	KY710821
A. sylvestrsis subsp. *nemorosa*	Ardahan, (1849 m) –MT 1157	KY710822
A. sylvestrsis subsp. *nemorosa*	Artvin, (1556 m) –MT 1166	KY710817
A. sylvestrsis subsp. *nemorosa*	Bayburt, (2375 m) –MT 1015	KY710823
A. sylvestrsis subsp. *nemorosa*	Bilecik, (1251 m) –MT 1226	KY710824
A. sylvestrsis subsp. *nemorosa*	Ankara, (1130 m) –MT 1195	KY710825
A. sylvestrsis subsp. *nemorosa*	Konya, (1481 m) –MT 1205	KY710826
A. sylvestrsis subsp. *nemorosa*	Amasya, (1233 m) –MT 1106	KY710827
A. sylvestrsis subsp. *nemorosa*	Kütahya, (1342 m) –MT 1199	KY710828
A. kotschyi	Niğde (2584 m), -MT 1164	KY710794
A. kotschyi	Niğde (2666 m), -MT 1163	KY710796
A. kotschyi	Kastamonu (2430 m), -MT 1161	KY710795
A. tenerrima var. *tenerrima*	Aydın, (759 m), -MT 1095	KY710814
A. tenerrima var. *tenerrima*	Aydın, (759 m), -MT 1069	KY710815
A. tenerrima var. *tenerrima*	Manisa, (670 m), -MT 1200	KY710816
A. lamprocarpa subsp. *lamprocarpa*	Hatay, (958 m) -MT 1218	KY710811
A. lamprocarpa subsp. *cheliki*	Osmaniye, (1265 m) -MT 1224	KY710812
A. lamprocarpa subsp. *cheliki*	Karaman, (1158 m) - MT 1212	KY710813
Chaerophyllum astrantia (outgroup)	Artvin, (2312 m) - MT 1167	KY710830
Chaeropyllum temulum (outgroup)	Kastamonu, (1040 m) - MT 1107	KY710829

Table 2. Sequences of the universal primer-pairs used to amplify non-coding *trn*L-F region of cpDNA.

Region name	Primer name	DNA sequence
*trn*L 3'-*trn*F	e (forward)	5' GGT TCA AGT CCC TCT ATC CC 3'
	f (reverse)	5' ATT TGA ACT GGT GAC ACG AG 3'

Table 3. Molecular diversity parameters of individuals.

Molecular diversity parameters	*trn*L3'-*trn*F Region
Number of taxa	10
Number of sequences	48
Total length (bp)	315
GC content (%)	34.1
Variable sites (V)	35
Parsimony informative sites (PI)	24

Discussion

In this study, a phylogenic systematic study was carried out using the molecular data of the *Anthriscus* taxa that grow in Turkey. By making a DNA isolation for the leaf structure of the 10 different taxa of the said type growing within the borders of our country, the non-coding *trn* L3'-*trn*F region of chloroplast DNA was reproduced in PCR. An attempt was made to determine the proximity and distance of the taxa with respect to one another by analyzing the base slice of the region obtained. This research is important as it is the first molecular-based study involving the *Anthriscus* taxa growing naturally in Turkey.

To date, only two taxonomic revision studies of the genus *Anthriscus* have been carried out. The first one was conducted in 1997 by Krzysztof Spalik (Spalik, 1997) and the other one was conducted in 2013 by Tekin & Civelek. The sects of the genus *Anthriscus* and the taxa they contain according to the *Flora of Turkey* (Davis, 1972) and the studies that have been conducted are given in Table 5.

According to the revision study of Spalik (1997), *A. tenerrima* var. *leicocarpa*, *A. cerefolium* var. *cerefolium* and *A. ruprechtii* are said to exist in Turkey, but since they do not exist in the locations used in this study, they were not included.

As per the phylogenetic tree is drawn in accordance with the base slice of non-coding *trn*L3'-*trn*F region of chloroplast DNA (Fig. 1), all the members of the three-taxa sect. of *Anthriscus* (*A. cerefolium* var. *trichocarpa*, *A. caucalis* var. *caucalis*, *A. tenerrima*. var. *tenerrima*) are completely isolated from the rest of the taxa. When the base slice for the taxa *A. caucalis* var. *caucalis* and *A. tenerrima* var. *tenerrima* taxa are investigated, it is observed that the bases in their informative regions show changes in the same direction. As a result of this, these two taxa branch off from the same place in the phylogenetic tree and they exist as two very similar types having a bootstrap value of 89% (Fig. 1). In the previous study by Spalik (1996), he stated that the chromosome numbers for the *A. caucalis* and *A. tenerrima* taxa were $2n=14$ and he also stated in the phenogram that was drawn according to morphological characteristics that these two taxa were the most similar taxa. Furthermore, Spalik stated that *A. caucalis* and *A. tenerrima* taxa could have appeared as a result of the separation of a Mediterranean taxon in the North (Spalik, 1996).

Table 5. *Anthriscus* taxa which are determined in Turkey through the revision studies (Tekin & Civelek, 2013) and that are specified in literature records (Davis, 1972, Spalik, 1997).

Flora of Turkey (Davis, 1972)	Revision of *Anthriscus* (Spalik, 1997)	A taxonomic revision of the genus *Anthriscus* (Apiaceae) in Turkey (Tekin & Civelek, 2013; 2017)
	Sect. Anthriscus	**Sect. Anthriscus**
A. caucalis	*A. caucalis* var. *caucalis*	*A. caucalis* var. *caucalis*
A. tenerrima	*A. tenerrima* var. *tenerrima*	*A. tenerrima* var. *tenerrima*
	A. tenerrima var. *leiocarpa*	-
		Sect. Cerefolium
A. cerefolium	*A. cerefolium* var. *cerefolium*	-
	A. cerefolium var. *trichocarpa*	*A. cerefolium* var. *trichocarpa*
	Sect. Caroides	**Sect. Caroides**
A. kotschyi	*A. kotschyi*	*A. kotschyi*
A. ruprechtii	*A. ruprechtii*	-
	Sect. Cacosciadium	**Sect. Cacosciadium**
A. lamprocarpa	*A. lamprocarpa*	*A. lamprocarpa* subsp. *lamprocarpa*
	-	*A. lamprocarpa* subsp. *chelikii*
A. sylvestris	*A. sylvestris* subsp. *sylvestris*	*A. sylvestris* subsp. *sylvestris*
A. nemorosa	*A. sylvestris* subsp. *nemorosa*	*A. sylvestris* subsp. *nemorosa*

Table 4. The Parsimony-informative (PI) and Variable (V) sites in the non-coding *trn*L 3'-*trn*F sequence of the examined *Anthriscus* populations in Turkey.

Taxa	2	6	7	22	23	24	25	26	27	29	30	31	37	43	44	54	60	61	74	75	77	88	154	161	231	244	254	292	293	309
A. kotschyii (MT 1164)	T	C	T	T	T	T	T	C	G	T	A	G	C	T	A	A	-	A	A	A	C	G	C	T	G	G	G	A	G	G
A. kotschyii (MT 1163)	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.
A. kotschyii (MT 1161)	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.
A. cerefolium var. *trichocarpa* (MT 1096)	.	-	-	.	.	.	.	.	.	.	.	.	.	C	C	C	A	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1082)	.	-	-	.	.	.	.	.	.	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1105)	.	-	-	.	.	.	.	.	.	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A.cerefolium var. *trichocarpa* (MT 1089)	.	-	-	.	.	.	.	.	T	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1086)	.	-	-	C	.	.	.	T	T	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1204)	.	-	-	C	.	.	.	T	T	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1074)	.	-	-	C	.	.	.	T	T	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. cerefolium var. *trichocarpa* (MT 1192)	.	-	-	.	.	.	.	T	T	.	.	.	.	C	C	C	.	C	C	.	A	A	T	.	A	A	T	.	.	.
A. caucalis var. *caucalis* (MT 1090)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1085)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1113)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1096)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1083)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A.caucalis var. *caucalis* (MT 1077)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1080)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1197)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1084)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. caucalis var. *caucalis* (MT 1078)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	T	.	.	.	.	.	.	C	A	.
A. tenerrima var. *tenerrima* (MT 1095)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	A	.	.	C	.	.	.	C	A	.
A. tenerrima var. *tenerrima* (MT 1069)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	A	.	.	C	.	.	.	C	A	.
A. tenerrima var. *tenerrima* (MT 1200)	.	-	-	.	.	.	.	G	T	A	G	.	A	.	C	.	.	C	C	.	A	.	.	C	.	.	.	C	A	.
A. sylvestrsis subsp. *sylvestris* (MT 1129)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	T	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *sylvestris* (MT 1126)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	T	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *sylvestris* (MT 1130)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	T	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *sylvestris* (MT 1143)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	T	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *sylvestris* (MT 1156)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	A	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *sylvestris* (MT 1115)	.	-	-	-	-	-	-	G	T	A	G	.	.	.	C	.	.	C	C	A	C	.	.	.	.	.	.	.	.	.
A.sylvestrsis subsp. *nemorosa* (MT 1142)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1227)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1140)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1166)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1099)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1157)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1015)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	C	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1226)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1195)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	.
A. sylvestrsis subsp. *nemorosa* (MT 1205)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	C
A. sylvestrsis subsp. *nemorosa* (MT 1106)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	C
A. sylvestrsis subsp. *nemorosa* (MT 1199)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	.
A. lamprocarpa var. *lamprocarpa* (MT 1218)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	C
A. lamprocarpa var. *cheliki* (MT 1224)	.	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	C
A. lamprocarpa var. *cheliki* (MT 1212)	G	-	-	.	.	.	.	G	T	A	G	-	.	.	C	.	.	C	C	.	T	.	.	.	.	.	.	.	.	..

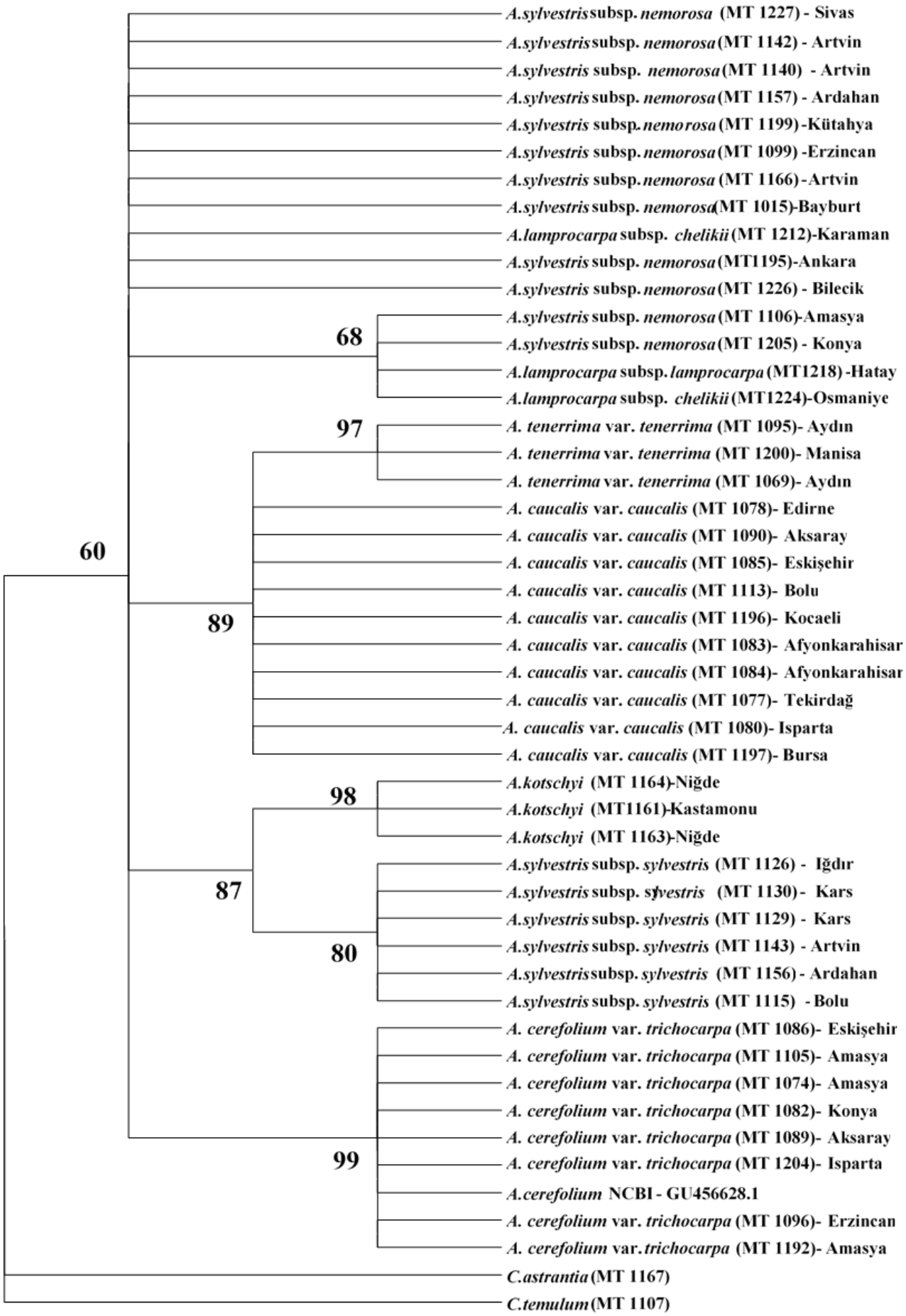

Fig. 1. The Phylogenetic Tree Constructed Using Maximum Parsimony Method.

A. cerefolium, which is the third type of sect. *Anthriscus*, being represented with only one variety in Turkey, *A. cerefolium* var. *trichocarpa*, has completed its speciation and has achieved its isolation from the other taxa. When the base slice of the individuals belonging to the *A. cerefolium* var. *trichocarpa* is investigated, it is observed as the only type with the highest differentiation ratio with its informative region having 13 pieces, and it has its own place on the phylogenetic tree (Fig. 1) with a bootstrap value of 99%. It does not occupy the same branch as any other species. In the previous study conducted by Spalik, among the *Anthriscus* taxa, only the *A.cerefolium* taxa appeared to have a chromosome number of 2*n*=18 (Spalik, 1996). Furthermore, the phenogram that is drawn is seen to be quite different from the taxa existing on the same section from a morphological aspect. It branched off from the same arm together with *A. caucalis* and *A. tenerrima* taxa but it existed alone on a different branch separate from them. In the study by Downie (2000), Downie observed that although *A. tenerrima*, *A. caucalis*, and *A. cerefolium* were annual species, *A. cerefolium* and *A. caucalis* were not closely related and it was asserted that *Anthriscus* could be polyphyletic. Similarly, in the study conducted by Tekin and Civelek, *A. cerefolium* are evaluated as a different section (Tekin & Civelek, 2017). Our studies support all of these previous studies.

It is a group comprising of the sect. *Caroides*, *A. kotschyi*, and the *A. ruprechtii* species. Since no taxa were observed at the locations specified in the Revision of *Anthriscus* (Spalik, 1997) with respect to the *A. ruprechtii* species during the land surveys carried out by Tekin (Tekin & Civelek, 2013), it was not possible to include it in our analysis. *A. kotschyi*, being the only representative of the sect. *Caroides* in Turkey has revealed harmonious base arrangements with its individuals collected from different regions and by achieving isolation among the species, it appears independent on the phylogenetic tree (Fig. 1), having a bootstrap value of 98%.

Sect. *Cacosciadium* consists of the *A. sylvestris* and *A. lamprocarpa* taxa. *A. sylvestris* has been evaluated as two subspecies in Turkey. These are *A. sylvestris* subsp. *sylvestris* and *A. sylvestris* subsp. *nemorosa* taxa. (Spalik, 1997; Tekin & Civelek, 2013; 2017). The differentiation of said taxa on the phylogenetic tree from one another shows that as the molecular features of *A. sylvestris* subsp. *sylvestris* and the molecular features of *A. sylvestris* subsp. *nemorosa* have played differentiating roles in separating these two groups. Therefore, the borders of subspecies need to be identified well. Although these two groups, which are accepted as subspecies, have covered a good distance but their speciation is not yet complete. While reproductive barrier within the species continues, isolation among the subspecies has been achieved well. It was observed that these sibling species, which are very similar from a morphological point of view, have been isolated significantly well from each other and that there has been no hybridization among them. As the informative regions of these two taxa were evaluated, it was observed that they did not show a similar change. As a result of this, they have branched off at separate places on the phylogenetic family tree (Fig. 1). While *A. sylvestris* subsp. *sylvestris* has branched off together with its own populations having a bootstrap value of 80%, polytomy-indicating break down on the branches belonging to the individuals of *A. sylvestris* subsp. *nemorosa*. Because there were no distinguishing differences with respect to the base slice of individuals belonging to the *A. sylvestris* subsp. *nemorosa*, no significant branching had occurred, and the bootstrap value had remained below 50%. Thus, this situation has revealed that the speciation of *A. sylvestris* is not yet complete. In the study he conducted, Spalik (1996) has stated that *A. sylvestris* taxa were unable to demonstrate a good clustering in any of the methods that were tried. Furthermore, he specified in the same study that both of the taxa had the same chromosome number: 2*n*=16 (Spalik, 1996). However, Spalik emphasized that despite the analyses that were made, the taxonomy of *A. sylvestris* group could not be resolved (Spalik, 1996). In the study by Downie (2000), the researcher stated that the taxonomy of sect. *Cacosciadium* could still not be determined. In the taxonomic revision study they carried out, Tekin & Civelek (2017) evaluated the *A. sylvestris* group as *A. sylvestris* subsp. *sylvestris* and *A. sylvestris* subsp. *nemorosa.* According to our data, there may be two different types, as stated in *Flora of Turkey* (Davis, 1972). Because in the analysis made, it is observed that the two subspecies are genetically distant from one another and that they have branched off at different places on the phylogenetic family tree (Fig. 1). Furthermore, Downie stated that *A. sylvestris* taxa are paraphyletic (Downie, 2000).

We can explain the distribution of *A. lamprocarpa* taxa, which is a different member of the sect. *Cacosciadium*, with the line of mountain ranges known as the Anatolian Diagonal. *A. lamprocarpa* is an East Mediterranean element and it spreads beyond our borders reaching regions in Palestine, Israel, Syria, and Jordan. Turkey forms the northern edge of the area of this species' geographical footprint. It is thought that *A. lamprocarpa*, which probably entered Turkey from Syria, has differentiated due not only to the climate changes created by the Anatolian Diagonal as it came towards the country but also due to the different environmental conditions that are seen in the border area between the two countries. Therefore the *A. lamprocarpa* species divides into two subspecies within the borders of Turkey: *A. lamprocarpa* subsp. *lamprocarpa* and *A. lamprocarpa* subsp. *chelikii* (Tekin & Civelek, 2016). The population of *A. lamprocarpa* subsp. *lamprocarpa* exists in Hatay and one population of *A. lamprocarpa* subsp. *chelikii* exists in Osmaniye, whereas another population exists in Karaman, making three different populations in total. Since the *A. lamprocarpa* subsp. *lamprocarpa* population in Hatay and *A. lamprocarpa* subsp. *chelikii* population in Osmaniye remain within the region where the Anatolian Diagonal forks, they are found to be very close from a genetic standpoint. However, since the *A. lamprocarpa* subsp. *chelikii* population, which exists in Karaman, remained in the west side of the Anatolian Diagonal and since it has adapted to the different ecological conditions in this region, production isolation has appeared between itself and the other populations and it has progressed faster in speciation when compared with the others (Fig.

2). Furthermore, on the phylogenetic family tree that we drew, the *A. lamprocarpa* taxa and *A. slyvestris* subsp. *nemorosa* taxa were found on the same branches that collapsed. This points to the existence of a gene flow that continues among the taxa. In the study he conducted, Spalik stated that *A. lamprocarpa* has a chromosome number 2*n*=16 just like *A. sylvestris* taxa and that they were not a monophyletic group (Spalik, 1996). On the other hand, Downie emphasized that *A. lamprocarpa* species could be paraphyletic (Downie, 2000).

As a result of the analysis made, we can state that the genus *Anthriscus* is monophyletic, that its taxonomic structure is complex and even though it cannot be clearly revealed yet, that there is still a gene flow, especially between the *A. sylvestris* and *A. lamprocarpa* taxa, that its genetic structure is constantly changing due to this gene flow, and that an isolation mechanism with respect to production has not developed among them since their speciation is not yet complete. We can state that more studies are required to clarify the systematic placement of the genus.

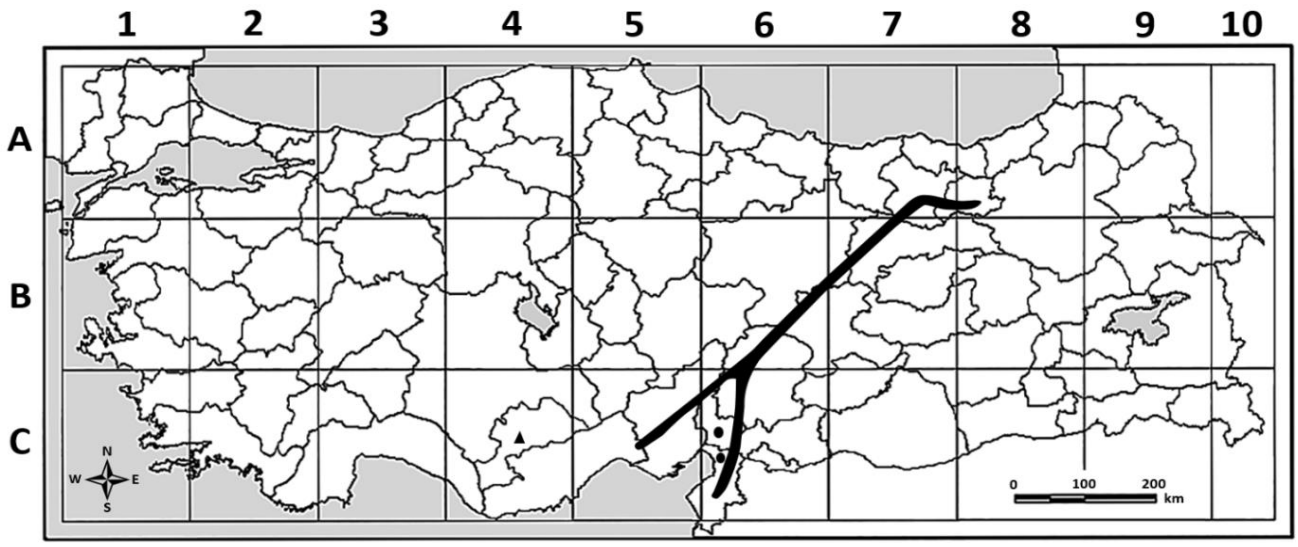

Fig. 2. Populations of *A. lamprocarpa* taxa in Turkey according to the position of the Anatolian Diagonal. (▲: *A. lamprocarpa* subsp. *chelikii* ●: *A. lamprocarpa* subsp. *lamprocarpa*) (Original).

Conclusion

According to this study, *A. cerefolium* var. *trichocarpa*, one of the three taxa of sect. *Anthriscus* in the phylogenetic tree must be excluded from this section and should be placed in the sect. *Cerefolium* because it is in a different branch from the other two taxa in the same section. Our findings support the results of the taxonomic revision done by Tekin & Civelek (2017).

The subspecies *A. sylvestris* subsp. *nemorosa ris* need to be upgraded to the species level *Anthriscus nemorosa*, as done in *Flora of Turkey* (Davis, 1972).

Given the area of spread and the existence of the Anatolian Diagonal, it is necessary that the three populations of *A. lamprocarpa* taxa in Turkey are rearranged from a systematic point of view. Because there are geographical, morphological, and genetic differences between the populations in Osmaniye and Hatay, which are included in the subspecies of *A. lamprocarpa* subsp. *lamprocarpa*, when the rearrangement of populations is made from a systematic perspective, it will be necessary to create two different varieties under the subspecies of *A. lamprocarpa* subsp. *lamprocarpa*. The population in Karaman will remain the only population representing the subspecies of *A. lamprocarpa* subsp. *chelikii.*

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