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Research Article

Phylogenetic analysis of the genus *Conringia* Heist. Ex Fabr. (Brassicaceae) in Turkey based on nuclear (nrITS) and chloroplast (*trnL-F*) DNA sequences

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Abstract

In this study, phylogenetic analysis of Turkish *Conringia* (Brassicaceae) species was conducted based on nuclear ribosomal DNA (nrITS) and chloroplast DNA (*trn*L-F) sequences. In addition, the relationships between the sequences of some Brassicaceae family species retrieved from NCBI, and *Conringia* species were documented. All of the plant specimens were collected at their flowering and vegetation periods from different regions of Turkey, and brought to the laboratory. Total genomic DNA was extracted using the GeneMark kit. In PCR analyses, ITS4 and ITS5A primers were used for the amplification of the nrITS region, while the *trn*Le and *trn*Lf primers were used for the cpDNA *trn*L-F region. The DNA sequences obtained were then edited using BioEdit and FinchTV, and analyzed using MEGA 6.0 software. Neighbor joining (NJ) and bootstrap trees were constructed in order to identify the relationships among *Conringia* taxa. The nrITS sequences ranged between 573 and 672 nucleotides, while the *trn*L-F sequences ranged between 346 and 764 nucleotides. The divergence values of nrITS sequences differed between 0.177 and 0.00 and divergence values of *trn*L-F sequences differed between 0.902 and 0.00. NJ tree generated using nrITS and *trn*L-F sequences consisted of two clades. In trees generated with both the nrITS and *trn*L-F sequences, *C. orientalis, C. grandiflora* and *C. austriaca* appeared within the same group. In addition, according to the phylogenetic analysis results obtained with other Brassicaceae species, it is revealed that the *Conringia* genus is polyphyletic.

Keywords: *Conringia*, phylogenetic analysis; nrITS; *trn*L-F; Turkey

Introduction

The worldwide spread Brassicaceae family is a monophyletic group consisting of approximately 325 genera and over 3740 species. This cosmopolitan family has the highest diversity in the Western North America, Irano-Turanian region and the Mediterranean region (Bulbul *et al.*, 2018; Bulbul *et al.*, 2019). Turkey, the second richest country after the United States in terms of the Brassicaceae taxa, contains 91 genera and 686 species (Yılmaz-Çıtak and Dural, 2020; Dönmez *et al.*, 2021). Agronomically important Brassicaceae family also includes many economically important edible and industrial oilseeds, vegetables, flavors, spices,

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ethnobotanically and fodder plants (Koch *et al.*, 2001; Ahmed *et al.*, 2012; Polat *et al.*, 2017; Gidik *et al.*, 2019; Kawarty *et al.*, 2020). The most important edible oil plant is *Brassica napus* L. known as canola or oil turnip, while *Sinapis alba* L. and *Brassica nigra* (L.) Koch seeds are also used. Spice plants include *Brassica juncea* (L.) Czern*, Armoracia rusticana (*Lam.) Gaertner, Mey. Et Schreb., and *Erysimum* L. species (Sirali *et al.*, 2013). This family also includes *Arabidopsis thaliana* (L.) Heynh. which is a well-known model species in plant biology (Couvreur *et al.*, 2010; Özüdoğru *et al.*, 2017). *Conringia* Heist. Ex Fabr. is a Brassicaceae genus containing annual taxa with 6 species in Turkey. These species are; *C. grandiflora* Boiss. & Heldr., *C. orientalis* (L.) Dumort., *C. planisiliqua* Fisch. & Mey, *C. persica* Boiss., *C. austriaca* (Jacq.) Sweet, Hort. and *C. clavata* (C. A. Mey.) Busch. Of these 6 taxa, *C. grandiflora* is endemic with an endemism rate of 17%. *C. clavata* is not included in the Flora of Turkey (Hedge 1965) because the taxa of this species are known as *C. perfoliata* in the first volume of this book (Al-Shehbaz *et al.*, 2007; Selvi *et al.*, 2019).

Molecular phylogenetics has provided a new vision for understanding the origin of land plants, elucidating the relationships among them, and reconstructing phylogenetic history (Khan *et al.*, 2016). The ITS region of nuclear ribosomal DNA (nrDNA) is one of the most popular and effective genetic markers for inferring phylogenetic relationships and evolutionary studies in plants. Due to its high species distinction, technical amplification convenience, and high primer universality, it has been used in various organisms at genus and species levels (Sun *et al.*, 2012; Guo *et al.*, 2016; Demir *et al.*, 2020). In particular, ITS regions that are characterized by parental inheritance patterns develop more rapidly than coding regions, leading to higher levels of variation among closely related individuals (Najarian *et al.*, 2020). Advances in DNA sequence techniques have allowed the widespread use of short DNA fragments, particularly from the chloroplast genome, in phylogenetic relationships studies (Penjor *et al.*, 2010). Chloroplast DNA (cpDNA) regions are widely used for phylogenetic studies at all taxonomic levels (Drabkova *et al.*, 2004). *trn*L-*trn*F, one of these regions, is preferred for intra and interspecific levels in plants (Kaya *et al.*, 2018).

In this study, sequence analyses of Turkish *Conringia* taxa using nrITS and cpDNA *trn*L-F sequences were performed to elucidate phylogenetic relationships among the ingroup taxa. In addition, *Boreava orientalis* (GQ131309.1, DQ518353.1), *Isatis tinctoria* (KF022713.1, KJ765846.1)*, Fibigia clypeata* (KF022650.1, KM033543.1)*, Lepidium ruderale* (KJ623529.1, KJ623450.1)*, Sisymbrium altissimum* (HQ896628.1, AY958545.1)*, Arabis montbretiana* (KF547279.1, KF547548.1)*, Hesperis matronalis* (DQ357547.1, AY546166.1)*, Alyssoides utriculata* (KF022512.1, KM033540.1)*, Alyssum linifolium* (KJ623478.1, KJ623461.1)*, Brassica cretica* (GQ268075.1, Y15350.1)*, Brassica deflexa* (GQ268077.1, GQ268052.1)*, Calepina irregularis* (DQ249822.1, AY751760.1) and *Goldbachia laevigata* (DQ357546.1, KJ623408.1) were compared with *Conringia* species with respect to both nrITS and *trn*L-F sequences, after retrieving their sequence data from NCBI nucleotide database.

Materials and Methods

Plant samples and DNA isolations

The study materials included the specimens of *Conringia grandiflora, C. orientalis, C. persica, C. austriaca, C. perfoliata* and *C. planisiliqua* species. The collected specimens, by looking at the identification key from Flora of Turkey were identified by Dr. Mehmet Yavuz PAKSOY. All specimens and the locations they are collected from are presented in Table 1. *Conringia* species were brought to the laboratory, some of them were used for genomic DNA isolation and some of them were preserved as herbarium specimens. For genomic DNA isolation, a commercial kit (GeneMark Catalog No: DP022) was used.

Table 1. Location of *Conringia* species in Turkey

Taxa	Location	GenBank No (nrITS)	GenBank No (*trn*L-F)
Conringia perfoliata (Yıldızeli)	TURKEY/Sivas; Yıldızeli, Kümbet village, step, 1500 m, 15.05.2014, MY143	MZ723114	MZ726898
Conringia perfoliata (Ankara Ayaş)	TURKEY/Ankara; Ayaş- Beypazarı, Akkaya village, 650m, 19.05.2014, MY152	MZ723103	MZ726899
Conringia perfoliata (Kayseri)	TURKEY/Kayseri; Pınarbaşı, Şirvan mountain southern slopes, mountain steppe, 1700 m, 16.05.2014, MY146	MZ723115	MZ726900
Conringia grandiflora (Akseki Çukurköy)	TURKEY/Antalya; Akseki, between Murtiçi and Çukurköy, maquis span, 500 m, 21.03.2014, MY136	MZ734227	MZ726895
Conringia grandiflora (Alanya)	TURKEY/Antalya: Alanya, Hacımahmetli village, field side, 150-200 m, 21.03.2014, MY138	MZ734230	MZ726896
Conringia planisiliqua (Kırşehir)	TURKEY/Kırşehir;İnanç village opposite, Üçkuyu back, rocky places, 1450 m, 17.05.2014, MY120	MZ723083	MZ726887
Conringia planisiliqua (Bunyan)	TURKEY/Kayseri; Bunyan, Korumaz mountain, Bölünya locality, field edge, 1500 m, 16.05.2014, MY147	MZ723084	MZ726889
Conringia planisiliqua (Sivas)	TURKEY/Sivas- Zara road, Tödürge lake edge, 1300 m, 15.05.2014, MY144.	MZ723087	MZ726888
Conringia planisiliqua (Nallıhan-Çayırhan)	TURKEY/Ankara: Between Çayırhan and Nallıhan, step, 650 m, 19.05.2014, MY149	MZ723086	MZ726891
Conringia planisiliqua (Ankara Ayaş)	TURKEY/Ankara; Ayaş- Beypazarı, Akkaya village, 700 m, 19.05.2014, MY151	MZ723079	MZ726890
Conringia persica (Van)	TURKEY/Van: between Van-Başkale, Çuh passage, steppe, 2500 m, 29.05.2013, MY141	MZ734229	MZ726897
Conringia austriaca (Adana)	TURKEY/Adana: Kozan, Gürümze village upper sections, forest clearance, 1350 m, 18.04.2014, MY142	MZ734226	MZ726894
Conringia orientalis (Ankara Polatlı)	TURKEY/Ankara: Polatlı, Kavuncu bridge near, swamp places, 850 m, 19.05.2014, MY150	MZ723094	MZ726893
Conringia orientalis (Elazığ)	TURKEY/Elazig; Between Keban and Elazig, 5 km, steppe, 850 m, 13.05.2014, MY126	MZ723092	MZ726892

PCR Amplifications and Sequencing

We amplified DNA of the nrITS and *trn*L-F regions of each genomic DNA. Amplification of nrITS region was performed using primers ITS5A (designed by Kenneth Wurdack) and ITS4 (White *et al.*, 1990) and *trn*L-F amplifications were performed using the primers designed by Taberlet *et al.* (1991). Both PCR

components and programs for nrITS and *trn*L-F region are given in Table 2. Gel electrophoresis in 0.1% agarose gel run in 10X Tris- Boric EDTA buffer was used to size fractionate amplicons. Subsequently, gels were stained with ethidium bromide and visualized over a UV trans-illuminator. ITS4 and ITS5A primers (for nuclear sequences) and *trn*Le and *trn*Ff primers (for chloroplast sequences) were used both for amplification and for sequencing which were conducted at TRIOGEN (İstanbul, Turkey). For each sample, forward and reverse sequencing reactions were performed and the sequences were analyzed via GenBank (NCBI) through (Basic Local Alignment Search Tool) BLASTn search. Later obtained DNA sequences were edited both manually and by using Bioedit (Hall, 1999) and FinchTV programs. Both nrITS and *trn*L-F sequences were uploaded into NCBI and GenBank numbers are given in Table 1.

Table 2. *nrITS* and cpDNA *trn*L-F primers and PCR components used for PCR amplification

Primer name	5' to 3' Primer sequence	PCR components	PCR amplification (35 cycle)
Forward ITS5A	CCTTATCATTTAGAGGAAGGAG	1 µL genomic DNA 1 µL primer (forward), 1 µL primer (reverse), 5 µL master mix (PCR buffer, 2 Mm $MgCl_2$, dNTP, 0.75 U Taq DNA polymerase) and 17 µL dH_2O	94 °C / 4 min 94 °C / 1 min 50 °C / 1 min 72 °C / 1 min 72 °C / 10 min.
Reverse ITS4	TCCTCCGCTTATTGATATGC		
Forward *trn*Le	GGTTCAAGTCCCTCTATCCC		
Reverse *trn*Ff	ATTTGAACTGGTGACACGAG		

Phylogenetic analysis

The phylogenetic tree was generated using the Neighbor joining (NJ) method (Saitou and Nei, 1987) and constructed using MEGA 6.0 software (Tamura *et al.*, 2013). The phylogenetic tree was evaluated with bootstrap test on 1000 resampling's (Felsenstein, 1985). Also, the genetic distance matrix between the species was also performed using the same software.

Results and Discussion

In recent years, many PCR based molecular markers such as nrDNA ITS, RAPD and ISSR markers have been developed and tested in genetic studies of various organisms among populations (Poyraz, 2016). The nrITS sequences obtained in this study ranged from 573 to 672 nucleotides among 14 samples. The highest nucleotide number for the nrITS sequence was recorded for *C. perfoliata* (Yıldızeli) (672 bases), and the lowest number of nucleotides for the nrITS sequence was observed in *C. orientalis* (Elazığ) (573 bases). The average nucleotide composition of nrITS was 21.4% T, 28.9% C, 22.8% A, and 26.8% G. The total length of the aligned nrITS sequence matrix was found to be 691 nucleotides. While the highest genetic distance between the species was 0.177, the lowest was found to be 0.000 (Table 3). NJ tree created with nrITS sequences consisted of two clades (Figure 1). Clade 1 is divided into two subclades within itself. Subclade A consisted of *C. planisiliqua* populations collected from different localities and received 100% bootstrap support. Subclade B, *C. perfoliata* populations, and *C. persica* species emerged as a monophyletic group with a bootstrap value of 99%. Clade 2 is divided into 2 subclades within itself. Subclade A was composed of *C. grandiflora* (Akseki) and *C. grandiflora* (Alanya) populations and received support with a bootstrap value of 99%. Subclade B consisted of *C. orientalis* populations and *C. austriaca* species and received a bootstrap support of 98%. nrITS analysis indicated that the populations belonging to the species came out in the same group. The *trn*L $^{(UAA)}$ -*trn*F $^{(GAA)}$ intergenic spacer (IGS) encoding transfer RNAs for leucine and phenylalanine is widely used for phylogenetic analysis of intraspecific variations in plants (Türktaş *et al.,* 2012; Filiz et al., 2018). For the *trn*L-F sequences, the length ranged from 346 to 769 nucleotides among 14 samples. For *trn*L-F sequences, the highest nucleotide number was observed in *C. grandiflora* (Alanya) (769 bases), and the lowest number of nucleotides was

observed in *C. persica* (346 bases). The average nucleotide composition of *trn*L-F was 33.1% T, 19.1 C, 29.3% A, and 18.6% G. The total length of the aligned *trn*L-F sequence matrix was found to be 830 nucleotides. The highest genetic distance between species was found to be 0.902, while the lowest was 0.000 (Table 4).

Table 3. Pairwise genetic distance matrix obtained from *nrITS* sequences

C.orientalis **(Elazig)**	-												
C.orientalis **(Ankara)**	0.081												
C.austrica **(Adana)**	0.094	0.081											
C.grandiflora **(Akseki)**	0.085	0.047	0.068										
C.grandiflora **(Alanya)**	0.085	0.047	0.068	0.000									
C.perfoliata **(Ankara)**	0.158	0.118	0.138	0.102	0.102								
C.perfoliata **(Kayseri)**	0.158	0.118	0.138	0.102	0.102	0.000							
C.perfoliata **(Yıldızeli)**	0.158	0.118	0.138	0.102	0.102	0.000	0.000						
C. ***planisiliqua*** **(Ankara)**	0.177	0.135	0.158	0.107	0.107	0.152	0.152	0.152					
C ***planisiliqua*** **(Kirsehir)**	0.177	0.135	0.158	0.107	0.107	0.152	0.152	0.152	0.000				
C. planisiliqua **(Bunyan)**	0.177	0.135	0.158	0.107	0.107	0.152	0.152	0.152	0.000	0.000			
C. planisiliqua **(Nallıhan)**	0.177	0.135	0.158	0.107	0.107	0.152	0.152	0.152	0.000	0.000	0.000		
C. planisiliqua **(Sivas)**	0.177	0.135	0.158	0.107	0.107	0.152	0.152	0.152	0.000	0.000	0.000	0.000	
C.persica **(Van)**	0.126	0.083	0.105	0.064	0.064	0.066	0.066	0.066	0.114	0.114	0.114	0.114	0.114

Table 4. Pairwise genetic distance matrix obtained from cpDNA *trn*L-F sequences

C.austrica **(Adana)**	-												
C. planisiliqua **(Ankara)**	0.083												
C. planisiliqua **(Nallıhan)**	0.083	0.000											
C. planisiliqua **(Sivas)**	0.083	0.000	0.000										
C.orientalis **(Ankara)**	0.017	0.063	0.063	0.063									
C.grandiflora **(Akseki)**	0.022	0.059	0.059	0.059	0.004								
C.persica **(Van)**	0.892	0.811	0.811	0.811	0.837	0.819							
C.perfoliata **(Ankara)**	0.902	0.852	0.852	0.852	0.847	0.862	0.044						
C.perfoliata **(Kayseri)**	0.902	0.852	0.852	0.852	0.847	0.862	0.044	0.000					
C.perfoliata **(Yıldızeli)**	0.902	0.852	0.852	0.852	0.847	0.862	0.044	0.000	0.000				
C.grandiflora **(Alanya)**	0.026	0.054	0.054	0.054	0.009	0.004	0.831	0.875	0.875	0.875			
C. planisiliqua **(Bunyan)**	0.078	0.004	0.004	0.004	0.059	0.063	0.829	0.837	0.837	0.837	0.059		
C planisiliqua **(Kirsehir)**	0.083	0.000	0.000	0.000	0.063	0.059	0.811	0.852	0.852	0.852	0.054	0.004	
C.orientalis **(Elazig)**	0.017	0.063	0.063	0.063	0.000	0.004	0.837	0.847	0.847	0.847	0.009	0.059	0.063

NJ phylogenetic tree created with cpDNA *trn*L-F sequences consisted of two clades (Figure 2).

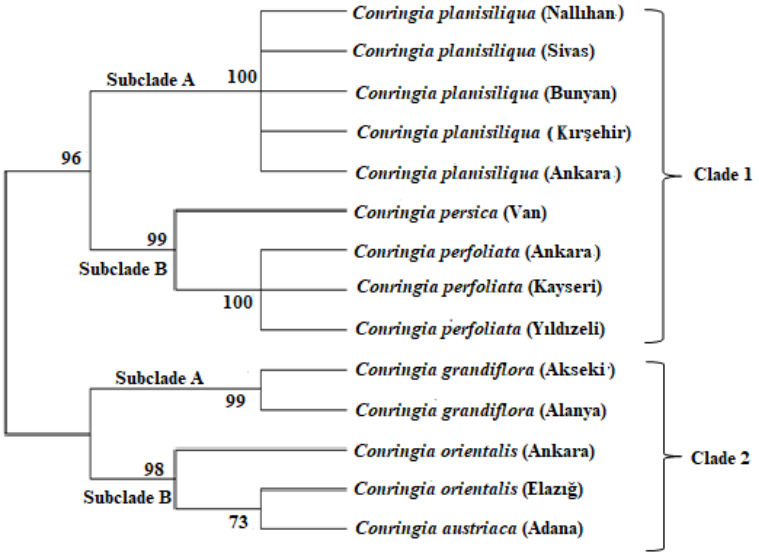

Figure 1. The Neighbour joining tree generated using nrITS sequences *Conringia* genus (Bootstrap values greater than 50% are given above branches)

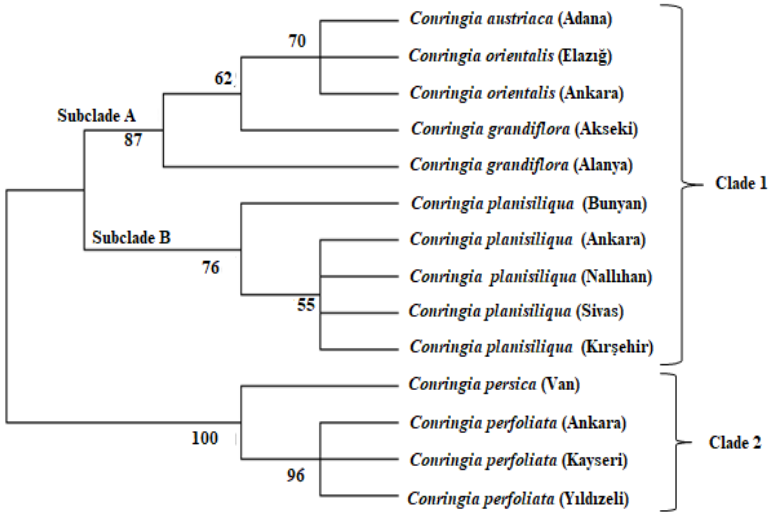

Figure 2. The Neighbour joining tree generated using cpDNA *trn*L-F sequences *Conringia* genus (Bootstrap values greater than 50% are given above branches)

Clade 1 is divided into two subclades within itself. Subclade A is composed of *C. austriaca*, *C. orientalis*, and *C. grandiflora* species and was supported with a bootstrap value of 87%. Subclade B consisted of *C. planisiliqua* populations and was supported by a 76% bootstrapped value. Clade 2 consisted of *C. perfoliata* and *C. persica* species. This clade is supported by a 100% bootstrap value. When we compare the results of nrITS with *trn*L-F, in both *nr*ITS and *trn*L-F analysis, *C. orientalis*, *C. grandifolora*, and *C. austriaca* were found to be in the same clade. Similarly, *C. perfoliata* and *C. persica* species also emerged together. However, in the nrITS results, while *C. planisiliqua* populations were found to be in Clade 1 with *C. persica* and *C. perfoliata* species; in *trn*L-F analyzes, *C. planisiliqua* populations were found to be in clade 1

with *C. orientalis*, *C. austriaca* and *C. grandiflora*. *C. perfoliata* and *C. persica* species were found to be in clade 2. Sevindik *et al.* (2020) revealed the genetic variation and molecular relationship of *Conringia* species in Turkey using RAPD markers. According to the RAPD results, *C. perfoliata* populations and *C. grandiflora* (Akseki-Çukurköy) species were found in a clade. In our nrITS results, while *C. perfoliata* and *C. persica* species were found to be in Clade 1, and together with *C. persica* species, *C. grandiflora* appeared in the same group with *C. orientalis* in Clade 2. In *trn*L-F results, *C. grandiflora* were found to be in the same group as *C. orientalis*, while *C. perfoliata* populations appeared in Clade 2 with *C. persica* species. In RAPD analyzes, *C. orientalis*, *C. planisiliqua*, *C. austriaca*, and *C. persica* were found to be in the same clade. In nrITS results, while *C. orientalis*, *C. austriaca*, and *C. grandiflora* species were found to be in Clade 2 and *C. planisiliqua* populations in Clade 1. According to *trn*L-F analyses, *C. orientalis*, *C. austriaca*, *C. grandiflora*, and *C. planisiliqua* species appeared in Clade 1. Selvi *et al.* (2019) studied the micromorphological and anatomical characters to contribute to the systematic structure of the genus *Conringia* in Turkey. In the study, root, stem and leaf anatomy along with stem and leaf surfaces of the species were examined with micromorphological studies. Leaf and stem anatomies of *C. austriaca* and *C. persica* and *C. orientalis* and *C. planisiliqua* species were found to be close to each other. In the nrITS and *trn*L-F results, *C. austriaca* and *C. persica* appeared in different groups. While *C. orientalis* and *C. planisiliqua* species appeared in different groups in nrITS results, they were found to be in the same group according to *trn*L-F results. While the results were inconsistent with the nrITS results, they were consistent with the *trn*L-F results.

In our study, phylogenetic relationships of some taxa (belonging to Brassicaceae family whose nrITS and *trn*L-F sequences were retrieved from NCBI) with *Conringia* species have been revealed. In nrITS results, while *C. perfoliata*, *C. orientalis*, *C. austriaca*, *C. grandiflora*, and *C. persica* species were included in the same clade, populations of *C. planisiliqua* species were included in a different clade. According to the nrITS results, the genus *Conringia* is a polyphyletic species. According to cpDNA *trn*L-F analysis, *C. planisiliqua*, *C. orientalis*, *C. grandiflora*, and *C. austriaca* species are included in Clade 1, while *C. perfoliata* and *C. persica* species are included in Clade 2. Liu *et al.* (2012) revealed the phylogenetic relationship of Brassicaceae species by cpDNA *mat*K sequence analysis. They found that *C. planisiliqua* was in the same group with *Isatis minima* and *Isatis tinctoria*. Zhao *et al.* (2010) revealed the phylogenetic relationships of species belonging to the Brassicaceae family by sequence analysis of *chalcone synthase (chs)* genes. Their study also revealed that *C. planisiliqua* was a sister species with *Isatis tinctoria* and *Pachypterygium multicaule*. Koch *et al.* (2007) revealed that *C. planisiliqua* emerged in a sister group with *Isatis tinctoria* and *Boreava orientalis* in their Plastid *trn*L intron and *trn*L-F intergenic spacer sequences analysis. In both nrITS and *trn*L-F sequence analysis we performed, *C. planisiliqua* populations, *Isatis tinctoria* and *Boreava orientalis* were found in the same group. Our results support the studies by Liu *et al.* (2012), Zhao *et al.* (2010) and Koch *et al.* (2007). Warwick and Sauder, (2005) performed the phylogenetic analysis of the Brassiceae tribe using chloroplast restriction site polymorphisms and nuclear ribosomal ITS and chloroplast *trn*L intron sequences. According to the *trn*L intron and chloroplast restriction site polymorphism results of the study, *C. orientalis* and *C. perfoliata* species were found to be in the same group. In our study, these two species are included in Clade 2, according to the nrITS results (Figure 3). In the *trn*L-F results, *C. orientalis* was included in Clade 1 and *C. perfoliata* in Clade 2 (Figure 4). In addition, as a result of ITS, *trn*L intron, and chloroplast restriction site polymorphisms, *C. orientalis* and C. *perfoliata* species were found to coexist with *Calepina irregularis*. In our study, according to the ITS results, *Calepina irregularis* is separated from *C. orientalis*, and C. *perfoliata* and *C. planisiliqua* were included in Clade 1. In *trn*L-F results, *Calepina irregularis*, *C. orientalis*, and *C. planisiliqua* were included in Clade 1, while *C. perfoliata* was included in Clade 2. Khosravi *et al.* (2009) performed the phylogenetic analysis of the Brassicaceae family with nrDNA ITS sequences. According to nrDNA ITS results, *Conringia perfoliata, Conringia orientalis*, and *Conringia persica* species were in the same group. Their study revealed that *C. planisiliqua* and *Orychophragmus violaceus* species were found to be related to *Isatis* and *Tauscheria*. In our nrITS results, while *C. perfoliata, C. orientalis,* and *C. persica* species were found to be in clade 2, *C. planisiliqua* is related with *Boreava orientalis* and *Isatis tinctoria* in clade 1

(Figure 3). According to *trn*L-F results, while *C. orientalis* and *C. persica* species were found to be in Clade 1, *C. persica* was found to be in Clade 2. Similarly, *C. planisiliqua* was found to be in Clade 1, in the same group as *Boreava orientalis* and *Isatis tinctoria* (Figure 4).

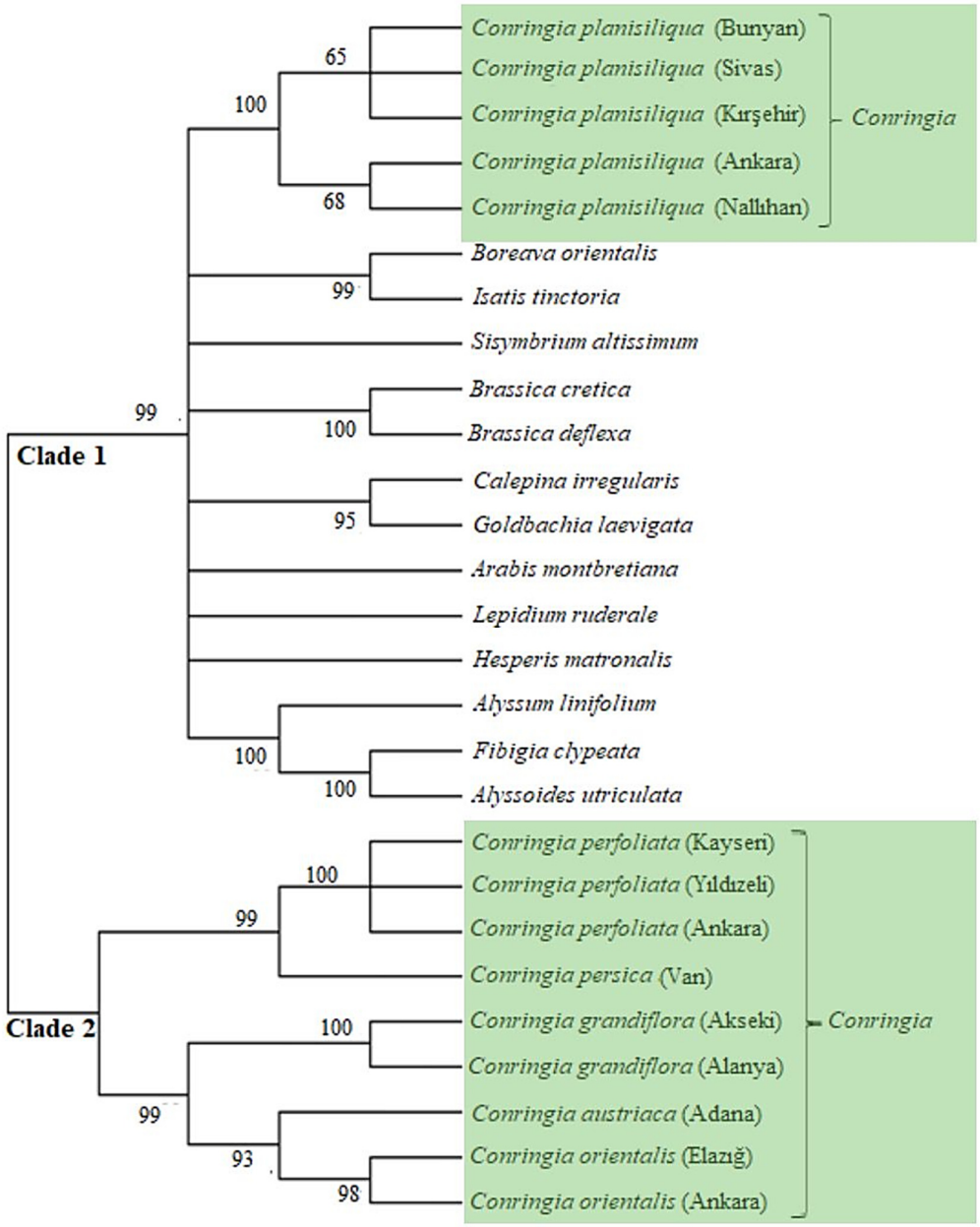

Figure 3. The Neighbour joining tree generated using nrITS sequences *Conringia* genus and other Brassicaceae species sequences retrieved form NCBI (Bootstrap values greater than 50% are given above branches)

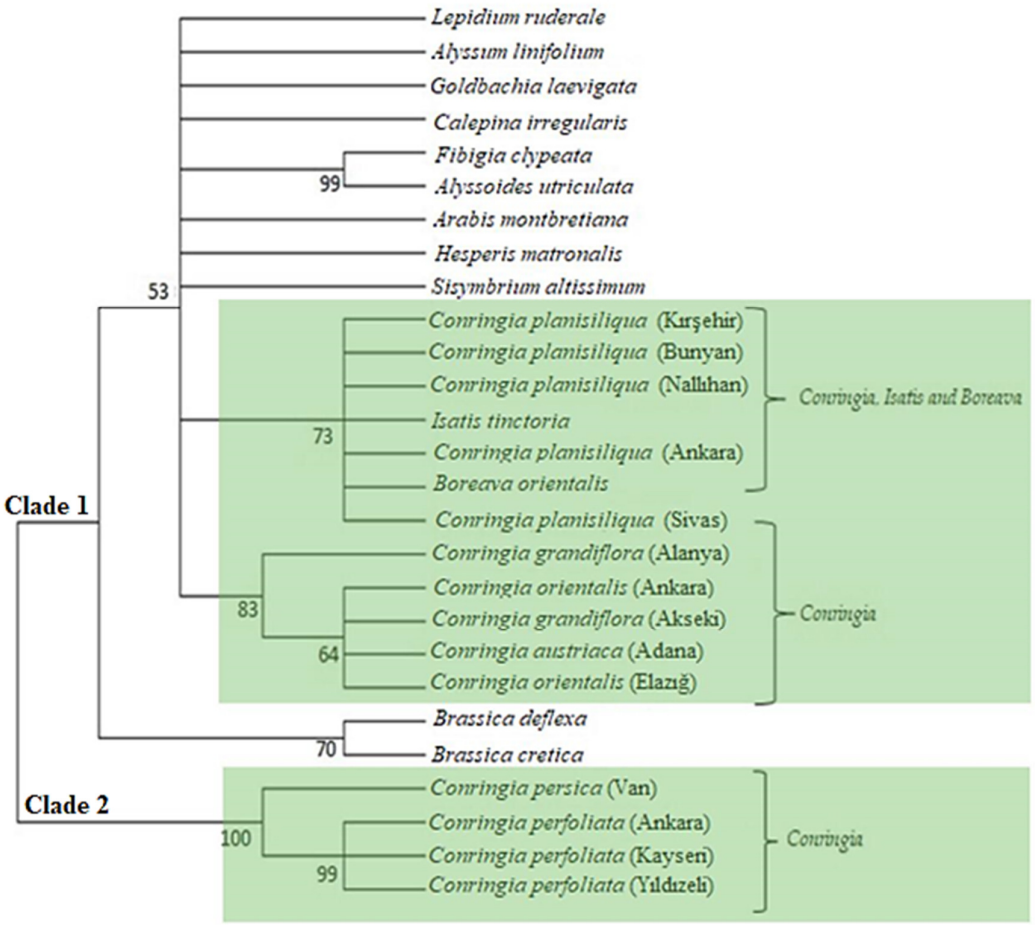

Figure 4. The Neighbor joining tree generated using *trn*L-F sequences *Conringia* genus and other Brassicaceae species sequences retrieved form NCBI (Bootstrap values greater than 50% are given above branches)

Conclusions

In this study, phylogenetic analysis was performed using nrITS and *trn*L-F sequences to elucidate the phylogenetic relationships of Turkish *Conringia* taxa. In trees generated with both the nrITS and *trn*L-F sequences, *C. orientalis, C. grandiflora* and *C. austriaca* appeared in the same group. However, while *C. orientalis, C. grandiflora* and *C. austriaca* were in separate clades (or sub-groups) in nrITS analyzes, *C. perfoliata* and *C. persica* appeared in the same clade in *trn*L-F results. Results obtained with other Brassicaceae species have revealed that the *Conringia* is polyphyletic, *C. planisiliqua* coexists with *Boreava orientalis* and *Isatis tinctoria*, and these findings are supported by previous studies.

Authors' Contributions

M.Y.P: collected plant species. Molecular and data analysis was done by M.A and E.S. The manuscript was drafted by E.S. and reviewed by all the authors before the submission.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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