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SYSTEMATIC RELATIONSHIPS AND MOLECULAR PHYLOGENY OF SOME *EUPHORBIA* L. TAXA FROM TURKEY, INFERRED FROM CPDNA NON-CODING REGION (*TRNL*$^{(UAA)}$-*TRNF*$^{(GAA)}$ IGS)

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Abstract

In this study, cladistics analyses based on chloroplast *trnL–F* intergenic spacer sequence and combined nrDNA ITS and *trnL-F* data set were undertaken to estimate phylogenetic relationships in 22 *Euphorbia* taxa collected from their natural distribution areas in Turkey. Among the examined taxa, *E. grisophylla, E. rhytidosperma* and 11 *Euphorbia* taxa *trnL-F* sequences were newly generated and their first inclusion in a phylogenetic analysis based on cpDNA *trnL-F* loci. According to the phylogenetic data results obtained with the analyzed sequences, it was seen that the *E. chamaesyce* (in subgenus *Chamaesyce*) was clearly separated from other taxa in subgenus *Esula*. At the section level, the phylogenetic trees based on the combined data set of taxa belonging to *Chylogala*, *Cymatospermum* and *Paralias* sections are in a complex order, *Esula* and *Helioscopia* sections are generally compatible with the classification defined in the Flora of Turkey. Although phylogenetic results based on cpDNA are compatible with the combined data results in terms of section distributions, the groups containing some species appear contradictory. According to this new information, the need to reevaluate the systematic status of *E. gaillardotii*, *E. aleppica*, *E. denticulata*, *E. craspedia*, *E. macroclada*, *E. cheiradenia* and *E. seguieriana* subsp. *seguieriana* has arisen.

Key words: *Euphorbia*, cpDNA, *trnL-F*, Phylogeny.

Introduction

Turkey has one of the richest floras in the World. In addition to geological, geomorphological and different bioclimatic features, different habitats and gene centers in three different phytogeographic regions and the existence of the Anatolian Diagonal, which is a migration route of plants formed by high mountains, are the reasons why Turkey is rich in biodiversity compared to neighboring countries. According to the latest data, the Flora of Turkey is represented by a total of 11747 taxa belonging to 167 families, 1321 genera, and 3689 of these taxa are endemic, and their ratio to all plants in the flora is 31.82% (Güner *et al.*, 2012). Euphorbiaceae is one of the largest families of angiosperms. According to the description in the Flora of Turkey, Euphorbiaceae includes many different forms ranging from annual or perennial, mostly monoecious**,** some dioecious and some succulent, herbaceous plants to trees. Euphorbiaceae has approximately 340 genera and 7500 taxa in the world (Seçmen *et al.*, 2011). Euphorbia is a large genus plants in the Euphorbiaceae family contains more than 2000 taxa in the world *(*Erdoğan *et al.*, 2012). It is one of the most diverse groups of flowering plants on earth with wide tolerance and adaptation, distributed in Turkey, Cyprus, Greece, Italy, Western America, Japan and North Africa, and shows variety it in terms of shape characteristics and habitat diversity (Webster, 1994; Şenel *et al.*, 1996; Erülken, 2011). The genus *Euphorbia* is represented by 120 taxa in the Flora of Turkey, 18 of which are endemic (Güner *et al.*, 2012). Members of this genus are called "sütleğen" by local researchers due to the latex they carry in their mostly branched secretion tubes.

In recent years, due to the differences in micro morphological characters used in classical systematics, the correct identification of such problematic taxa is rather difficult. Therefore, in order to resolve the problem of DNA sequence analysis has been successfully tried for identification and determining the phylogenetic relationship. The regions for DNA sequence analysis must contain enough base differences to distinguish taxa from each other, as well as base similarities that will reveal the phylogenetic relationship between taxa. In phylogenetic studies, intergenic spacer sequences of uniparental inheritance chloroplast DNA (cpDNA) are mostly preferred in taxonomic classification and determination of evolutionary phylogenetic relationships. cpDNA sequence information is used by combining it with mitochondrial DNA (mtDNA) and especially with nuclear DNA (nrDNA) sequence information. cpDNA sequence variations are now widely used in cross-species studies to reveal relationships between angiosperms and the other plants. Non-coding regions show a very high mutation frequency (Taberlet *et al.*, 1991). The number of studies on angiosperm non-coding chloroplast DNA regions such as *trnL* $^{(UAA)}$ - *trnF* $^{(GAA)}$ is quite high and this region, especially called *trnL-F*, is used mostly for the purpose of redetermining phylogenetic relationships at the species level (Compton *et al.*, 1998; Bakker *et al.*, 1999; Bayer & Starr, 1999; McDade & Moody, 1999).

The aim of this study is to determine the phylogenetic relationships between *Euphorbia* taxa indigenous to Turkey. In Turkey, there is no comprehensive study based on cpDNA sequences on the evolutionary relationships between *Euphorbia* taxa and infrageneric groups (subgenus, section, subsection and group) and of the sequences used in the analyses, *E. grisophylla, E. rhytidosperma* and *E. sanasunitensis* are endemic species. *trnL-F* region sequences of *E. sanasunitensis*, *E. erubescens*, *E. heteradena*, *E. denticulata*, *E. craspedia*, *E. seguieriana* subsp. *seguieriana*,

E. orientalis, *E. macrocarpa*, *E. grisophylla*, *E. altissima var. altissima* and *E. rhytidosperma* were newly generated and their first inclusion in a phylogenetic analysis. This study also aims to evaluate the *Euphorbia* taxa, which are morphologically similar to each other, such species are quite high in number, and whose morphological characters and systematics is difficult, and their placement in subgeneric classification varied from time to time based on molecular studies on DNA sequencing data and thus, it was aimed to eliminate the confusion in the taxonomy of the genus by combining our data with the literature from different localities in the following years.

Material and Methods

Plant material: Plant materials were obtained from silica-gel dried leaves of collected specimens in the habitats. The plant materials were identified by Prof. Dr. M. Kürşat according to Flora of Turkey and East Aegean Islands (Davis, 1965-1985). Voucher specimens were deposited at the Biology Laboratory of Bitlis Eren University. Plant taxa used in this study is shown in Table 1 and pictures of representative species of some of the subgenus of *Euphorbia* is shown in Fig. 1.

DNA extraction: Total genomic DNA was extracted from dried leaves of collected specimens in the wild by protocol of the Hibrigen® plant genomic DNA isolation kit. According to the procedure, 100 mg of plant sample was homogenized with liquid nitrogen. The buffer solutions provided in the kit were used in accordance with the procedure. The obtained DNA samples were stored at -20°C until used.

PCR amplification and sequencing: Amplification of intergenic spacer *trnL-F* with B49317 (5'CGAAATCGGTAGACGCTACG 3') and A49855 (5'GGGGATAGAGGGACTTGAAC 3') primers was performed according to the protocols of Taberlet *et al.*, (1991). In the PCR product purification stage, MAGBIO "HighPrep™ PCR Clean-up System" (AC-60005) purification kit was used for the single band samples obtained and purified by following the kit's procedures. Sanger Sequencing sample analyses were performed using ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Alignment and phylogenetic analyses: Phylogenetic analyses were undertaken using data set of samples aligned using ClustalW (Thompson, 1994) software and subsequently checked visually. Indels were not treated in final datasets. Variable sites, number of parsimony-informative sites, transition, transversion, genetic distance, nucleotide diversity, and divergence within species were computed as molecular diversity statistics for each dataset using Molecular Evolutionary Genetics Analysis software (MEGA 11.0) (Tamura *et al.*, 2021). Ultimately, phylogenetic tree was constructed by Maximum Likelihood Method.

Table 1. Voucher specimens of investigated *Euphorbia* taxa.

Taxa	Locality	Voucher and specimen code
E. chamaesyce L.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 03.09.2019.	M.Kurşat, 6114
E. rhytidosperma Boiss. & Balansa	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M.Kurşat, 6125
E. grisophylla M.L.S.Khan	Bitlis: Northern Hillside of the Mount Kambos, 1650m. 29.07.2019.	M.Kürşat, 6113
E. macrocarpa Boiss. & Buhse	Van: Artos mountain, Northern slopes, 2200 m, 26.07.2020.	M.Kurşat, 6112
E. orientalis L.	Van: 30 km of highway from Van to Hakkari, slopes, Zernek Irrigation Dam Lake, mountain steppe, 1960 m, 27.07.2019.	M.Kurşat, 6101
E. altissima Boiss. var. *altissima*	Elazığ: Baskil, Nazaruşağı neighborhood surroundings, meadow lands, 28.07.2020.	M.Kurşat, 6107
E. stricta L.	Artvin: Konaklı/Ardanuç- Lahşet plateau, 1900m, 30.06.2021.	M.Kurşat, 6124
E. gaillardotii Boiss. & Blanche	Elazığ: Freeway, Meryem Mountain, in the field, 08.08.2019.	M.Kurşat, 6110
E. helioscopia L.	Siirt: Tillo, Around Ismail Fakirullah Tomb, 1100 m, 09.04.2021.	M.Kurşat, 6121
E. aleppica L.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M.Kurşat, 6105
E. falcata L. subsp. *falcata*	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M.Kurşat, 6111
E. denticulata Lam.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 01.08.2019.	M.Kurşat, 6102
E. craspedia Boiss.	Mardin: Savur, Pınardere neighborhood, Stony land,899 m, 08.04.2020.	M. Ayaz, 6070
E. macroclada Boiss.	Van:Gevaş, Roadside, Slopes, 1750 m, 28.07.2019.	M.Kurşat, 6103
E. cheiradenia Boiss. & Hohen.	Van: Kuzgun Kıran Pass, 2240 m, 22.07.2019.	M.Kurşat, 6106
E. seguieriana Neck. subsp. *seguieriana*	Van: Gevaş to Edremit, Roadside, Slopes, 1750 m, 28.07.2019.	M.Kurşat, 6109
E. heteradena Jaub. & Spach.	Van: Gevaş to Edremit, in the field, 1750 m, 28.07.2019.	M.Kurşat, 6108
E. esula subsp. *tommasiniana* (Bertol.) Kuzmanov	Van: Edremit, roadside, 1650 m, 28.07.2019.	M.Kurşat, 6100
E. sanasunitensis Hand.-Mazz.	Bitlis: Northern Hillside of the Mount Kambos, 1650m. 29.07.2019.	M.Kurşat, 6104
E. iberica Boiss. (1)	Hakkari: Cilo plateau, Avaspi glaciers, 2540 m, 28.06.2021.	M.Kurşat, 6117
E. iberica Boiss. (2)	Bitlis: Northern Hillside of the Mount Kambos, 1650m. 29.07.2019.	M.Kurşat, 6128
E. oblongifolia (K.Koch) K.Koch	Artvin: Murgul-Damar, Kabaca plateau, Öküzyatağı location, 2200 m, 0.06.2021.	M.Kurşat, 6123
E. erubescens Boiss.	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M.Kurşat, 6126

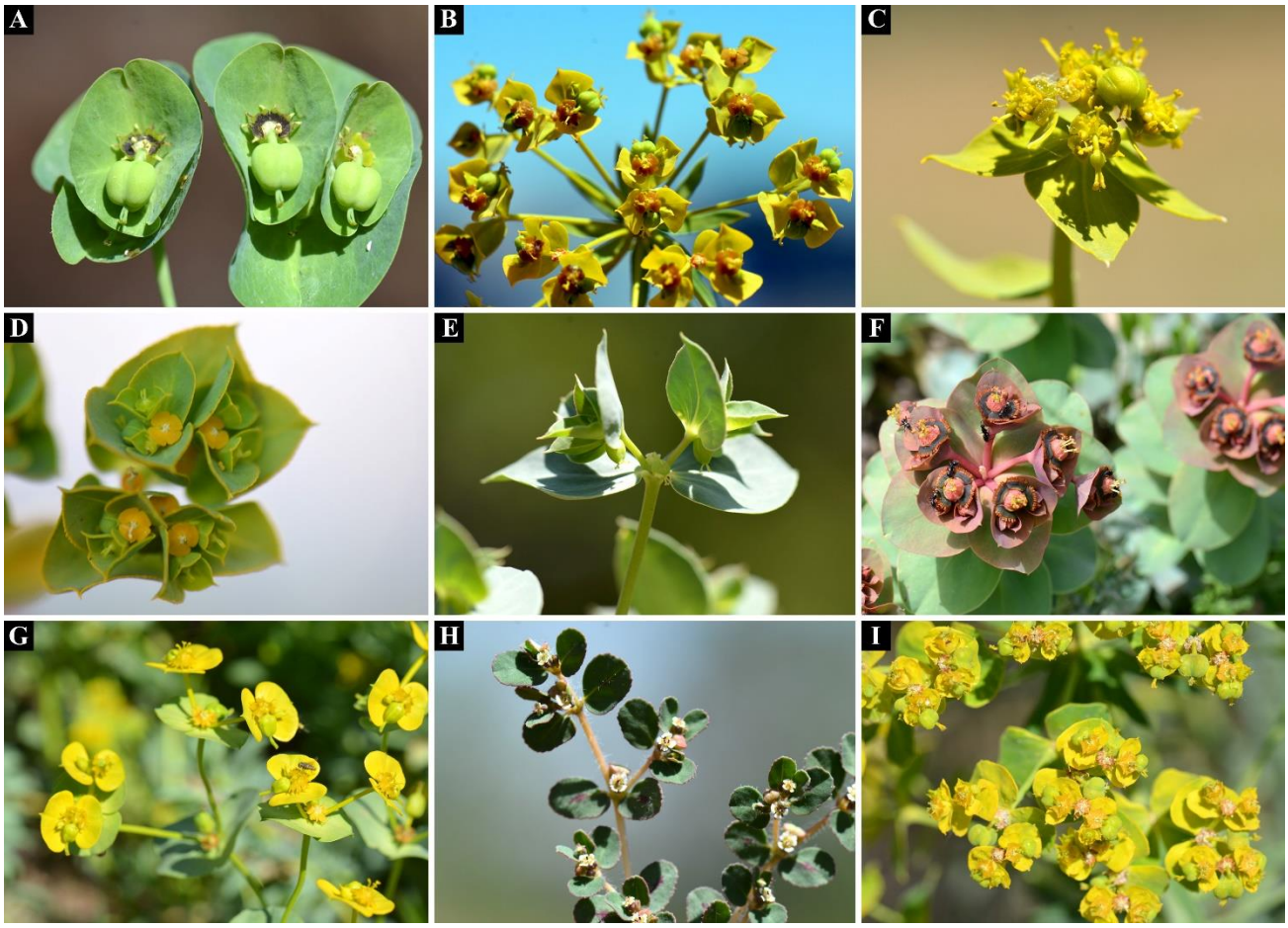

Fig. 1. Pictures of representative species of some of the subgenus of *Euphorbia*.
(A) *E. erubescens* Boiss.; (B) *E. seguieriana* Neck. subsp. *seguieriana*; (C) *E. heteradena* Jaub. & Spach.; (D) *E. gaillardotii* Boiss. & amp; Blanche; (E) *E. falcata* L. subsp. *falcata*; (F) *E. denticulata* Lam.; (G) *E. cheiradenia* Boiss. & Hohen; (H) *E. chamaesyce* L.; (I) *E. esula* subsp. *tommasiniana* (Bertol.) Kuzmanov

Results and Discussion

The characteristics of sequences

***trnL-F* data set:** The aligned data set of entire cpDNA *trnL-F* and combined cpDNA *trnL-F* and nrDNA ITS included 22 *Euphorbia* taxa collected from their natural distribution areas in Turkey (24 samples in total, including *E. iberica* collected from 2 different localities and *E. cheiradenia* sequence retrieved from GenBank). The total length of *trnL-F* region varied from 189bp (*E. grisophylla*) to 429bp (*E. chamaesyce*) with 3 major indels. Of the total 537 sites, 229 sites were variable and 274 were constant. Of the variable sites, 49 sites were singleton sites, and 175 sites were parsimonously informative (Table 2). The maximum pair-wise distance measured between individual sequences of cpDNA *trnL-F* was 0.3295 between *E. grisophylla* and *E. chamaesyce*. The overall mean distance was calculated as 0.15. The transition/transversion bias (*R*) recorded as 0.69. The estimated value of the shape parameter for the discrete Gamma Distribution is 0.4434. Mean evolutionary rates in these categories were 0.01, 0.13, 0.42, 1.07, 3.37 substitutions per site. The nucleotide frequencies are A = 28.74%, T/U = 37.88%, C = 17.38%, and G = 16.00%.

Table 2. Parameters of cpDNA *trnL-F* and combined nrDNA ITS and cpDNA *trnL-F* sequences.

Parameter	*trnL-F*	*trnL-F*+ITS
Total number of sites	537	1420
No. of parsinomy-informative sites	175	439
No. of sigleton sites	49	266
No. of variable sites	229	733
No. of conserved sites	274	640
G + C content	33.4	49.6

Combined trnL-F+ITS data set: The total length of combined data region varied from 920bp (*E. grisophylla*) to 1235bp (*E. altissima* var. *altissima*). Of the total 1420 sites, 733 sites were variable and 640 were constant. Of the variable sites, 266 sites were singleton sites, and 439 sites were parsimoniously informative (Table 2). The maximum pair-wise distance measured between individual sequences of combined dataset was 0.3699 between *E. altissima* var. *altissima* and *E. chamaesyce*. The overall mean distance was calculated as 0.166. The transition/transversion bias (*R*) was recorded as 1.06. The estimated value of the shape parameter for the discrete Gamma Distribution is 0.5661. Substitution pattern and rates were estimated under the Tamura & Nei (1993) model (+G). Mean evolutionary rates in these categories were 0.03, 0.19, 0.52, 1.14, 3.12 substitutions per site. The nucleotide frequencies are A = 27.56%, T/U = 22.85%, C = 24.86%, and G = 24.72%.

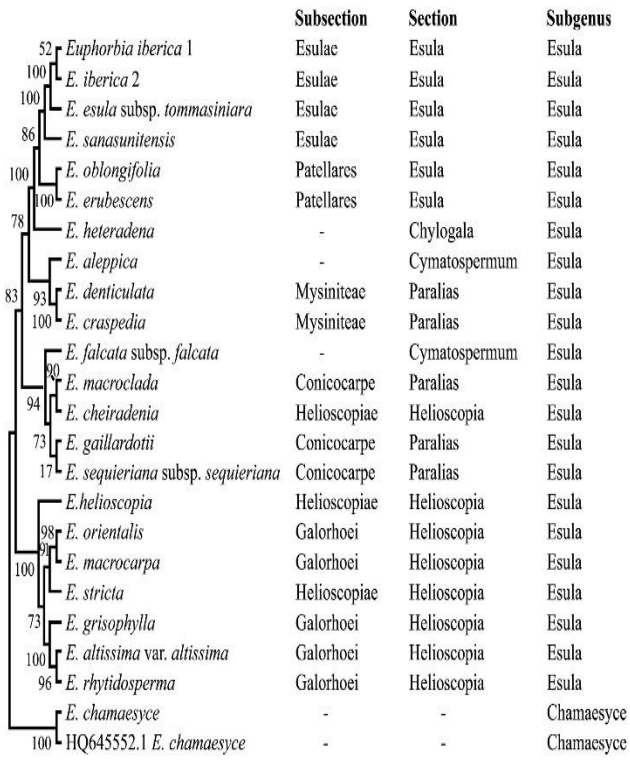

Fig. 2. Maximum Likelihood tree based upon the Tamura-Nei model of combined data set of nrDNA ITS + cpDNA *trnL-F* regions with 1000 bootstrap replicates.

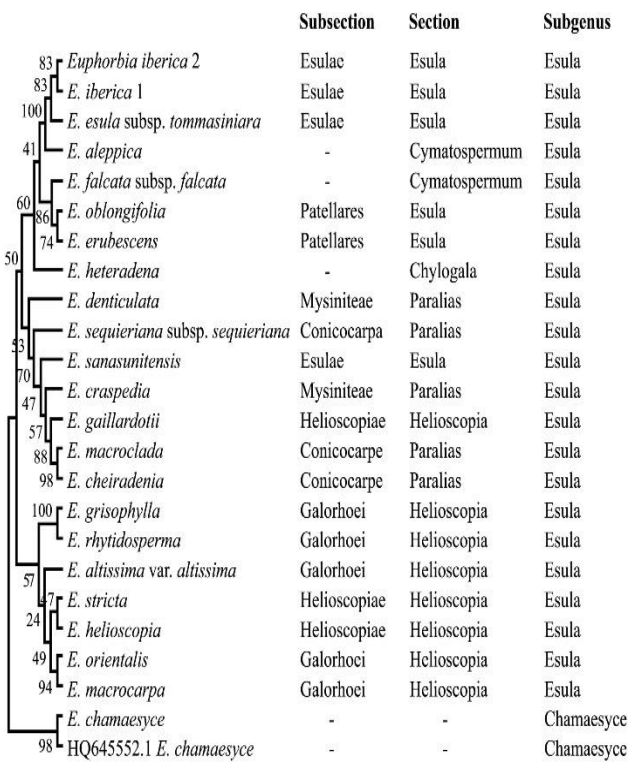

Fig. 3. Maximum Likelihood tree based upon the Tamura-Nei model of cpDNA *trnL-F* region with 1000 bootstrap replicates.

The evolutionary characteristics: In the Flora of Turkey *Euphorbia* genus is divided into 4 subgenera (*Chamaesyce*, *Cytidospermum*, *Poinsettia* and *Esula*), among this, *Esula* is divided into 8 sections (*Balsamis*, *Helioscopia*, *Cymatospermum*, *Herpetorrhiza, Paralias*, *Chylogala*, *Esula* and *Lathyris*). From these sections, sect. *Helioscopia* has two subsections namely, *Galarhoei* and *Helioscopiae*, sect. *Paralias*; three subsect. namely *Myrsiniteae*, *Paralioideae* and *Conicocarpae*, and sect. *Esula*; two subsects. namely *Esulae* and *Patellares* (Davis, 1982).

Reconstructing the evolutionary aspects of interspecies relationships is currently one of the most important issues in molecular evolution. If reliable phylogenies can be created, they may help in tracing phylogentic sequence in terms of the evolutionary events that provide today's diversity. In the previous study taxonomy, phylogeny, and systematics of *Euphorbia* species collected from Turkey were investigated using DNA sequences from complete nrDNA ITS regions (ITS1 and ITS2) (Koçak *et al.*, 2023). In the present study, combined sequences of ITS1+2 and *trnL-F* and *trnL-F* loci from cpDNA (Figs. 2 and 3) were used to compare phylogenetic relationship of *Euphorbia* species with previous ITS data (Koçak *et al.*, 2023) and compatibility with traditional and molecular systematics. When all three phylogenetic trees of both studies are compared it is seen that the distinction between subgenus, section and subsection is compatible with the classification in Flora of Turkey. However, there are discrepancies observed in the distribution of species especially in phylogenetic tree derived from *trnL-F* sequences. In this study, inferences have been tried to be made by taking into account the studies published in recent years in order to resolve these inconsistencies that arise with the classical systematics. *E. chamaesyce* (subgen. *Chamaesyce*) was completely distinguished from the species found in subgen. *Esula* in the cladistics trees created based on nrDNA data, cpDNA data and combined data set. In all three data set results, species belonging to sect. *Helioscopia* were gathered under the same cluster, and similarly, species belonging to sect. *Esula* were also included under one cluster.

Combined data set: In Flora of Turkey, *E. helioscopia*, *E. orientalis*, *E. macrocarpa*, *E. stricta*, *E. grisophylla*, *E. altissima* var. *altissima* and *E. rhytidosperma* are classified under sect. *Helioscopia*. All these species were grouped under the same cluster in all three data set consensus trees. *E. gaillardotii* species classified in sect. *Helioscopia* in the Flora of Tukey is not included in the cluster of sect. *Helioscopia* species in the combined data set tree and thus systematic status of *E gaillardotii* contradicts with the classical systematic classification. In studies conducted by Riina *et al.*, (2013) and Frajman & Geltman (2021), it was included in sect. *Pithyusa E. gaillardotii* based on nrDNA and cpDNA data not in sect. *Helioscopia*. Sect. *Pithyusa* was previously described as a subsect. of sect. *Paralias* (Prokhanov, 1949; Boisser, 1862; Pahlevani *et al.*, 2011). According to the Prokhanov system, species found in sect. *Pithyusa* are grouped under sect. *Paralias* subsect. *Conicocarpae.* The species in the same group of which *E. gaillardotii* is included in the combined data tree are *E. falcata* subsp. *falcata*, *E. macroclada*, *E. cheiradenia* and *E. seguieriana* subsp. *seguieriana*. In Flora of Turkey, *E. falcata* subsp. *falcata* is placed in sect. *Cymatospermum* and *E. macroclada*, *E. cheiradenia* and *E. seguieriana* subsp. *seguieriana* are classified as members of sect. *Paralias* subsect. *Conicocarpe*. In Riina *et al.*, (2013), *E. falcata*, *E.*

macroclada and *E. cheiradenia* are treated under the sect. *Pithyusa*. Classification of *E. seguieriana* subsp. *seguieriana* has not been included in any previous study however it seems that in the systematic classification made according to our molecular data results, it is closely related to sect. *Pithyusa* species and is clustered under the same group.

Myrsiniteae was previously treated as a subsect. of sect. *Paralias* and in recent studies, it is accepted as a section and include 14 species of which are; *E. aleppica*, *E. anacampseros*, *E. corsica*, *E. craspedia*, *E. denticulata*, *E. fontqueriana*, *E. marschalliana*, *E. monostyla*, *E. myrsinites*, *E. oxyphylla*, *E. rechingeri*, *E. rigida*, *E. spinidens* and *E. veneris* (Prokhanov, 1949; Boisser, 1862; Pahlevani *et al.*, 2011; Riina *et al.*, 2013). In Riina *et al.*, (2013) the *Myrsiniteae-Pithyusa* clade is noted, and the existence of this clade agrees with Frajman & Schönswetter (2011) and Horn *el al*., (2012) and also supported based on some morphological characters. The placement of widespread Mediterranean species *E. aleppica* was formerly uncertain because of its some morphological characters. Nonetheless, *E. aleppica* shares many morphological and ecological similarities with the species included in sect. *Myrsiniteae* (Riina *et al.*, 2013; Frajman & Geltman, 2021). In Flora of Turkey *E. aleppica* is placed in sect. *Cymatospermum*. Our phylogenetic tree based on combined data set showed that *E. aleppica* was clearly positioned within sect. *Myrsiniteae* and sister taxa to *E. denticulata* and *E. craspedia*. *E. denticulata* and *E. craspedia* are included in sect. *Paralias* subsect. *Myrsiniteae* in Flora of Turkey but in sect. *Myrsiniteae* according to studies based on molecular data (Riina *et al.*, 2013; Frajman & Geltman, 2021).

When we make a subgeneric comparison with the classification created according to the results of studies based on molecular sequences in recent years, we see that sect. *Esula* members (*E. iberica, E. esula* subsp. *tommasiniara, E. sanasunitensis, E. oblongifolia* and *E. erubescens*); sect. *Myrsiniteae* members (*E. aleppica, E. denticulata* and *E. craspedia*), sect. *Pithyusa* members (*E. falcata* subsp. *falcata, E. seguieriana* subsp. *seguieriana, E. gaillardotii, E. macroclada* and *E. cheiradenia*), and sect. *Helioscopia* members (*E. helioscopia, E. orientalis, E. macrocarpa, E. stricta, E. altissima* var. *altissima, E. grisophylla* and *E. rhytidosperma*) are clearly separated from each other, forming distinct clusters.

***trnL-F* data:** Although phylogenetic tree based on *trnL-F* data is compatible with the combined data results in terms of the distribution of sections, the groups in which some included species are contradictory. The relevant classification of *E. falcata*. subsp. *falcata* was discussed in the combined data set results. The *trnL-F* analysis places it within sect. *Esula,* however, this placement does not coincide with both the classical classification and the results of ITS and combined data set molecular studies. Another disagreement involves the group containing *E. sanatunisensis* which is included in sect. *Esula* in classical classification, but other members of the group are species of sect. *Paralias* according to Flora of Turkey. The systematic status of *E. aleppica* is also controversial. It is a member of sect. *Cymatospermum* in Flora of Turkey; however, it is included in sect. *Myrsiniteae* in Riina *et al.*, (2013) and Frajman & Geltman (2021) and our ITS and combined data set confirms these molecular studies results. In the cpDNA sequences analysis *E. aleppica* is sister to *E. iberica* and *E. esula* subsp. *tommasiniara* which are the members of sect. *Esula*.

The *trnL-F* intergenic spacer contains high levels of variation even among very close species, and due to this feature, it provides important distinction at species and infraspecific levels in phylogenetic studies and has been used in many phylogeny studies (Taberlet *et al.*, 1991; Clegg, 1993; McDade & Moody, 1999; Vir *et al.*, 2023). However, some studies have revealed the inconsistency of the information provided by different regions of the nrDNA and cpDNA genome in terms of phylogeny at the species level (Vir *et al.*, 2023) When the results of this study are evaluated, the phylogeny inferred from cpDNA *trnL-F* sequences was inefficient to infer differentiation of groups and species relationships. The discrepancy in phylogenetic relationships presented by the trees obtained based on the results of ITS data and *trnL-F* data can be attributed to more than one reason. One of these is that different genome regions, such as the nrDNA ITS region and the cpDNA *trnL-F* regions, have different evolutionary rates. In fact, different regions of the chloroplast genome may have different evolutionary rates. This gives rise to a large number of possibilities in determining species, genus, family and even higher-level relationships of the data obtained from the chloroplast genome. In addition, the fact that the phylogenetic tree results created based on cpDNA *trnL-F* data are not compatible with traditional systematic results may be due to the fact that some of the data obtained from the genome may not reflect the phylogeny based on different characters such as morphology (Jin & Nei, 1990). However, the conservative evolution of the chloroplast is one of the disadvantages of inferring phylogeny, limiting its applicability in distinguishing between closely related species and at the population level. Another limitation of cpDNA for species-level phylogeny estimation involves the potential occurrence of chloroplast transfer, i.e., the movement of the chloroplast genome from one species to another through ingression. The implicit assumption of only a single mode of plastid transfer within genera or even species may have significant implications for phylogeny construction performed by cladistics methods. Variations in intraspecific regions and the mode of plastid transfer are of great importance (Harris & Ingram, 1991; Rieseberg & Soltis, 1991; Rieseberg & Brunsfeld, 1992).

Conclusion

As a result, it was seen that the phylogenic tree of nrDNA ITS and combined ITS+*trnL-F* data set did not fully coherent with that of cpDNA *trnL-F*. When ITS and ITS+*trnF-L* data set results are evaluated together, it is seen that they conflict with the classical systematics in terms of classification of some species, but compatible with systematics based on new molecular data. Consequently sect. *Cymatospermum* needs to be reconsidered and the systematic status of *E. aleppica*, *E. denticulata*, *E. craspedia*, *E. macroclada*, *E. cheiradenia* and *E. seguieriana* subsp. *seguieriana* and *E. gaillardotii* should be rearranged based on new information derived from these phylogenetic studies.

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