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Systematic status and phylogenetic relationships of some *Euphorbia* L. taxa from Turkey based on nrDNA internal transcribed spacer (ITS) sequences

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Euphorbia L. genus is represented in Turkey by 120 taxa which are distributed throughout the country and 18 of these taxa are endemic to Turkey. Phylogenetic relationships were determined by nrDNA Internal Transcribed Spacer (ITS) sequence analysis of 27 taxa belonging to this genus. Among the examined taxa, *E. grisophylla, E. rhytidosperma* ve *E. sanasunitensis* are endemic species. The phylogenetic relationships of the examined taxa in subgen. *Chamaesyce*, subgen. *Cystidospermum* and subgen. *Esula* of the *Euphorbia* genus were shown in dendrogram. According to the molecular phylogenetic relationship data obtained as a result of the study, it was revealed that the subgenera distinction of the *Euphorbia* genus was made correctly, but the sections and groups distinction was not supported completely. It was revealed in the Maximum Likelihood tree based upon the Tamura-Nei model of nrDNA ITS tree that *E. petiolata* and *E. chamaesyce* species belonging to subgen. *Cystidospermum* and subgen. *Chamaesyce* respectively, differed sharply from taxa belonging to subgen. *Esula*. Within the subgen. *Esula*, the sect. *Helioscopia* showed the most distant relationship to the sect. *Esula*. According to the results we obtained, it was revealed that *E. heteradena* species should be evaluated in a different Group, not in Group A. The systematic status of *E. gailladotii, E. aleppica, E. szovitsii* var. *kharputensis* and *E. falcata* subsp. *falcata* needs to be reassessed. It was also supported by molecular data that *E. sanasunitensis* is in subgen. *Esula* for the first time at the molecular level.

Keywords: *Euphorbia*, Phylogeny, nrDNA, ITS

INTRODUCTION

It has been reported that there are approximately 270,650 species of vascular plants (Tracheophyta) in the world, together with 258,650 species of seed plants (Spermatophyta) and 1200 species of ferns (Pteridophyta) (Thorne, 2002). Considering the climatic zone (between latitudes of 24^0C-35^0C, warm temperate zone) in which it is located in terms of seed plants, Turkey is very rich in terms of vascular plant species (Anonymous, 2007). One of the countries with the richest flora on earth is Turkey. Turkey has a very rich biodiversity since it is located at the intersection of 3 different phyto-geographical regions, namely Europe-Siberia, Iran-Turanian and Mediterranean (Erik and Tarıkahya, 2004; Avcı, 2005).

The Euphorbiaceae family is the fourth largest of the flowering plant families; it has a cosmopolitan distribution in all regions except Antarctica and is generally subtropical and hot climate plants. It has been reported that the genus *Euphorbia*, which gives its name to the family, has over 2000 species ranging from annual procumbent life forms to succulent tree forms (Shi et al. 2008). Euphorbiaceae, represented by about 340 genera and 7500 species on earth, shows the greatest distribution in the tropics, Indonesia and Africa (Webster, 1994; Bolaji et al. 2014; Küçüker, 2015). The genus with the highest number of species in this family is *Euphorbia*. The Euphorbiaceae family is represented by 5 genera (*Euphorbia*, *Mercurialis*, *Andrachne*, *Chrozophora* and *Ricinus*) in the Flora of Turkey (Davis et al. 1982). This number increased from 5 to 7 with the addition of 2 monotypic genera (*Acalypha australis* L. (Duman and Terzioğlu, 2009) and *Flueggea anatolica* Gemici (Gemici, 1993) by Turkish researchers in recent years. There are 109 taxa of *Euphorbia* genus in the Flora of Turkey (Davis et al. 1982). With the taxa added in the following years, this number reached approximately 120 taxa, 18 of which are endemic for Turkey (Erdoğan et al. 2012).

Euphorbiaceae taxa have a formation that rarely grows under lianas, dioic or monoic, annual, biennial or perennial herbs, shrubs or trees (Radcliffe-Smith, 1982;

Pandey, 2006). Leaves are usually alternating or decussate, and in some species, they contain a white milky secretion called latex in mostly branched secretion tubes (Rebman and Simpson, 2006).

Molecular plant systematics has been rapidly developing in recent years (Wen et al. 1997). Phylogenetic analyzes using DNA and amino acid sequence analyzes provide great contributions to molecular plant systematics (Ro et al. 2007). Sequence analyzes have started to be used in cases where morphological characters are insufficient for phylogenetic analyzes (Yokoyama et al. 2000). Sequence analysis methods are used in many fields, from determining the geographic origins of living things to proving their phylogenies molecularly (Allan et al. 2004; Cohen and Weydmann, 2005). In studies on the molecular phylogeny of angiosperms, multi-repetitive nuclear ribosomal DNA (nrDNA) genes, chloroplast (plastid) and mitochondrial genes have been used (Qui et al. 1999; Graham and Olmstead, 2000). It has been reported that only a few of the plants can be accurately identified using classical systematic methods (Hollingsworth et al. 2011; Li et al. 2015). DNA sequence analysis can also clearly reveal the existing phylogenetic relationship between living groups that are relatively difficult to identify. DNA barcoding is a reliable diagnostic technique that allows the detection of interspecific or infraspecific differentiation of taxa based on generally short DNA sequences (600-1500 bp) found in previously determined or identified regions on the genome (Lahaye et al. 2008). In plant phylogenetic studies, the ITS (Internal Transcribed Spacer), which consists of ITS1, 5.8S and ITS2 subregions in nuclear DNA, is one of the most preferred regions. This region is primarily preferred by molecular taxonomists as it shows high genetic variation. ITS1 and ITS2 intron regions are among the genes responsible for the production of 18S, 5.8S and 26S ribosomal RNA.

There is no evaluation, phylogenetic study and literature information about the members of the *Euphorbia* species naturally distributed in Turkey. In this study the classical systematic and molecular systematic classifications were compared and the place of endemic species in the phylogeny and the position of *E. sanasunitensis*, which had not been included in any research publication before, were determined.

MATERIALS AND METHODS

Plant material:

Plant material was obtained from silica-gel dried leaved of collected specimens in the wild. The plant materials were identified by Assoc. 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 was shown in Table 1.

Table 1: The collection data 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. Kürşat 6114
E. petiolata Banks & Sol.	Malatya: Pütürge, Çevrimtaş Village, meadow lands, 11.08.2021.	M.Kürşat 6119
E. rhytidosperma Boiss. & Balansa	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M. Kürşat 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. Kürşat 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. Kürşat 6101
E. altissima Boiss. var. *altissima*	Elazığ: Baskil, Nazaruşağı neighborhood surroundings, meadow lands, 28.07.2020.	M. Kürşat 6107
E. altissima var. *glabrescens* Boiss. ex M.S.Khan	Elazığ: Baskil, Nazaruşağı neighborhood surroundings, meadow lands, 08.08.2021.	M. Kürşat 6122
E. stricta L.	Artvin: Konaklı/Ardanuç- Lahşet plateau, 1900m, 30.06.2021.	M. Kürşat 6124
E. microsphaera Boiss.	Elazığ: Sindipik Village, 1800 m, 12.08.2021.	M.Kürşat 6120
E. gaillardotii Boiss. & Blanche	Elazığ: Freeway, Meryem Mountain, in the field, 08.08.2019.	M. Kürşat 6110
E. rhabdotosperma Radcl.-	Elazığ: Keban, Keban Dam, 1430-1450 m, 2021.	M. Kürşat 6118

Sm.		
E. helioscopia L.	Siirt: Tillo, Around Ismail Fakirullah Tomb, 1100 m, 09.04.2021.	M. Kürşat 6121
E. aleppica L.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6105
E. szovitsii var. *kharputensis* Azn. ex M.S.Khan	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6115
E. falcata L. subsp. *falcata*	Elazı: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 04.08.2019.	M. Kürşat 6111
E. denticulata Lam.	Elazığ: Baskil, Doğançık Village, around Bolucuk town, 1530 m, 01.08.2019.	M. Kürşat 6102
E. craspedia Boiss.	Mardin: Savur, Pınardere neighborhood, Stony land,899 m, 08.04.2020.	M. Ayaz 070
E. macroclada Boiss.	Van,:Gevaş, Roadside, Slopes, 1750 m, 28.07.2019.	M. Kürşat 6103
E. cheiradenia Boiss. & Hohen.	Van: Kuzgun Kıran Pass, 2240 m, 22.07.2019.	M. Kürşat 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.	Hakkari: Cilo plateau, Avaspi glaciers, 2540 m, 28.06.2021.	M. Kurşat 6117
E.oblongifolia (K.Koch) K.Koch	Artvin: Murgul-Damar, Kabaca plateau, Öküzyatağı location,2200 m, 30.06.2021.	M. Kurşat 6123
E. erubescens Boiss.	Osmaniye: Zorkun plateau, in the Forest, 1650 m, 22.06.2021.	M. Kurşat 6126

DNA extraction, amplification and sequencing:

Total genomic DNA was extracted by protocol of the the Hibrigen® plant genomic DNA isolation kit. Amplification of the whole region of nrDNA ITS were performed using the ITS AB101 and ITS AB102 primers (Douzery et al. 1999).

Alignment and phylogenetic analyses:

Phylogenetic analyses were undertaken using data set of samples aligned usin ClustalW (Thompson et al. 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.

RESULTS AND DISCUSSION

The characteristics of sequences:

The aligned data set of entire ITS included a total of 27 taxa. ITS sequences length, GC% content, conserved sites, parsinomy informative and variable sites statistics are showed in Table 2.

Table 2: Numeric information of ITS

	ITS
Length of the aligned sequence	890
GC% content	59.4
Conserved sites	536
Parsinomy informative sites	234
Variable sites	343

The evolutionary characteristics:

Euphorbia genus is divided into 3 subgenus (*Chamaesyce Cystidospermum* and *Esula*), 5 sections of subgen. *Esula* (*Helioscopia*, *Cymatospermum*, *Paralias*, *Chylogala* and *Esula*) and 9 groups (A, B, C, D, E, F, G and I) in the Flora of Turkey. The status of the studied taxa according to the Flora of Turkey is shown in Table 3.

Table 3: The status of the investigated *Euphorbia* taxa according to the flora of Turkey

Taxa	Taxon number	Group	Subsection	Section	Subgenus
E. chamaesyce	4	A	-	-	*Chamaesyce*
E. petiolata	6	A	-	-	*Cystidospermum*
E. rhytidosperma	11	B	*Galarhoei*	*Helioscopia*	*Esula*
E. grisophylla	14	B	*Galarhoei*	*Helioscopia*	
E. macrocarpa	15	B	*Galarhoei*	*Helioscopia*	
E. orientalis	23	B	*Galarhoei*	*Helioscopia*	
E. altissima var. *altissima*	24	B	*Galarhoei*	*Helioscopia*	
E. altissima var. *glabrescens*	24	B	*Galarhoei*	*Helioscopia*	
E. stricta	31	C	*Helioscopiae*	*Helioscopia*	
E. microsphaera	32	C	*Helioscopiae*	*Helioscopia*	
E. gaillardotii	34	C	*Helioscopiae*	*Helioscopia*	
E. rhabdotosperma	38	C	*Helioscopiae*	*Helioscopia*	
E. helioscopia	39	C	*Helioscopiae*	*Helioscopia*	
E. aleppica	47	D	-	*Cymatospermum*	
E. szovitsii var. *kharputensis*	49	D	-	*Cymatospermum*	
E. falcata subsp. *falcata*	55	D	-	*Cymatospermum*	
E. denticulate	58	F	*Myrsiniteae*	*Paralias*	
E. craspedia	59	F	*Myrsiniteae*	*Paralias*	
E. macroclada	66	G	*Conicocarpae*	*Paralias*	
E. cheiradenia	73	G	*Conicocarpae*	*Paralias*	
E. seguieriana subsp. *seguieriana*	74	G	*Conicocarpae*	*Paralias*	
E. heteradena	76	A	-	*Chylogala*	
E. esula subsp. *tommasiniana*	78	H	*Esulae*	*Esula*	
E. sanasunitensis	79	H	*Esulae*	*Esula*	
E. iberica	81	H	*Esulae*	*Esula*	
E.oblongifolia	83	I	*Patellares*	*Esula*	
E. erubescens	87	I	*Patellares*	*Esula*	

As can be seen from the dendrogram of the studied 27 *Euphorbia* taxa (Figure 1), the taxa belonging to subgen. *Chamaesyce*, subgen. *Cystidospermum* subgen. *Esula* are sharply separated from each other (Figure 1). It was observed that molecular nrDNA ITS sequence analyzes did not support the groups and sections separation made by morphological characters.

Our analysis results showed that subgen. *Chamaesyce, Cystidospermum* and *Esula* were clearly separated from each other. When the nrDNA ITS results are evaluated based on Flora of Turkey, subgen. *Esula* is divided into two main clades; the first includes sect. *Helioscopia*, *Paralias* and *Cymatospermum*, the second includes sect. *Paralias*, *Cymatospermum*, *Chylogala* and *Esula* (Figure 1). According to this evaluation, clearly there is no complete separation in sections. In 2013 (Riiana et al.) and 2015 (Pahlevani et al.) publications, the ITS and *ndhF* regions of some *Euphorbia* species collected from Iranian regions were studied and their phylogenetic relatedness degrees were discussed. While our study results comply with the classical systematic in Flora of Turkey classification, they differ in some respects and are compatible with molecular data results obtained with previous studies.

According to classical systematic data, *E. rhytidosperma*, *E. grisophylla*, *E. macrocarpa*, *E. orientalis*, *E. altissima* var. *altissima*, *E. altissima* var. *glabrencens*, *E. stricta*, *E. microsphaera*, *E. gaillardotii*, *E. rhabdotosperma* and *E. helioscopia* subsp. *helioscopia* are in sect. *Helioscopia* (Table 3). In this study, on the other hand, a discrepancy was determined in the systematic status of *E. gailladotii*. Our studies revealed that, *E. gailladotii* is not in clade *Helioscopia*, but in clade *Paralias*, and this result conflicts with the classical systematic. In the sequence studies of *E. gailladotii* nrDNA ITS and plastid *trn*T–*trn*F Frajman and Geltman (2021) it was determined that *E. gailladotii* was included in sect. *Pithyusa*. To illuminate this complexity, the systematic status of *E. gailladotii* should be re-evaluated.

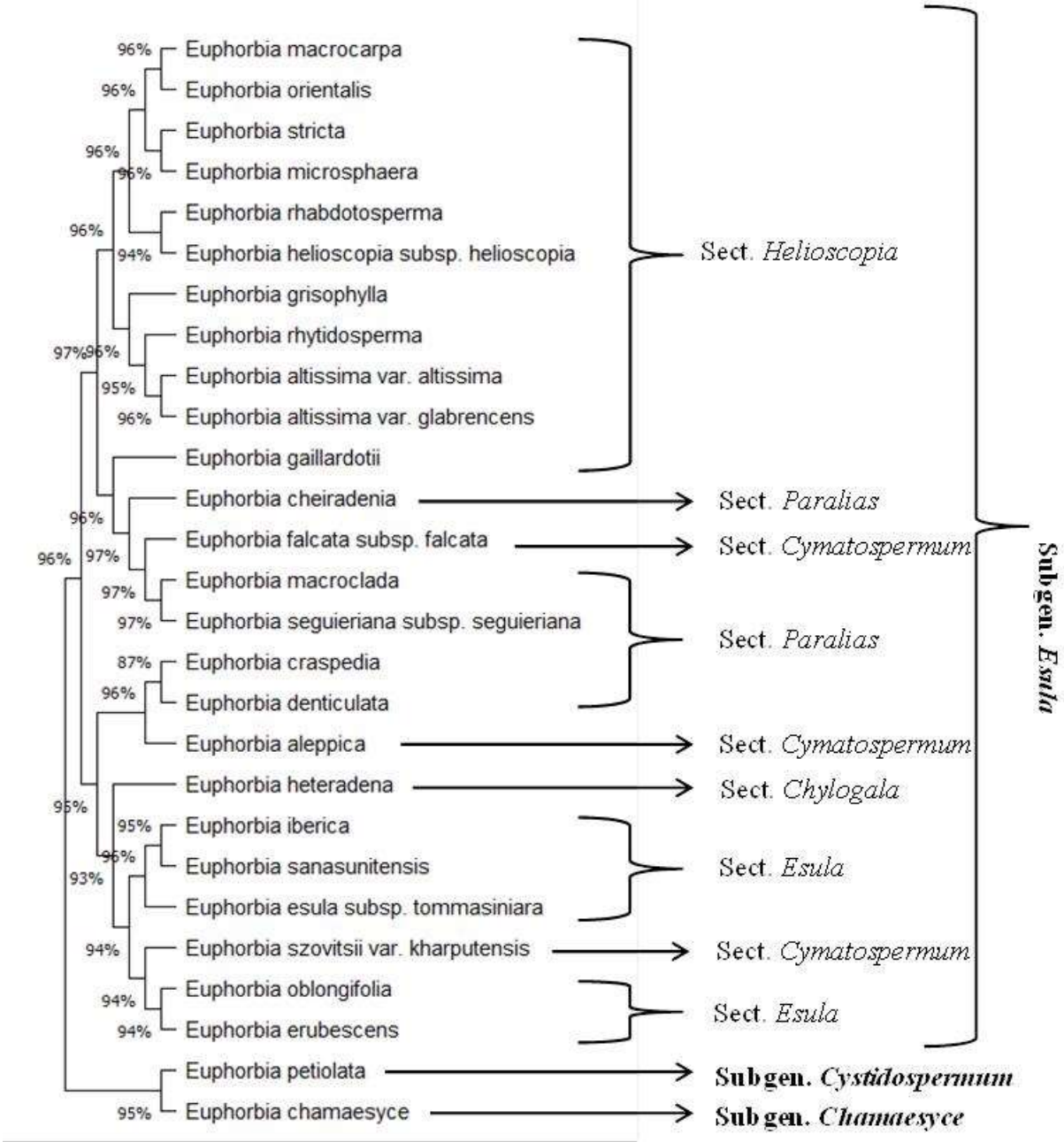

Figure 1: Maximum Likelihood tree based upon the Tamura-Nei model of nrDNA ITS region with 1000 bootstrap replicates

Sect. Cymatospermum includes E. aleppica, E. szovitsii var. kharputensis and E. falcata subsp. falcata in Flora of Turkey. The molecular phylogenetic analysis confirmed the placement E. aleppica is in sect. Myrsinitae, E. szovitsii is in sect. Szovitsiae and E. falcata is in sect. Pithyusa (Riiana et al. 2013; Pahlevani et al. 2015; Frajman and Geltman, 2021). Figure 1 clearly illustrates these three taxa are located in different clades.

E. aleppica was separated from *E. craspedia* and *E. denticulata*, which is sister group and included in sect. *Paralias* subsect. *Myrsiniteae*, *E. szovitsii* var.

kharputensis was separated from *E. oblongifolia* and *E. erubescens* included in sect. *Esula*. subsect. *Patellares*. The ITS phylogeny of Kryukov et al. (2010) and Salmaki et al. (2011) placed *E. szovitsii* sister to sect. *Esula*. This placement is similar to our results. However *E. szovitsii* is included in sect. *Szovitsiae* in molecular classification and is included in sect*. Cymatospermum* in traditional classification. *E. falcata* subsp. *falcata* is separated from *E. macroclada* and *E. seguieriana* subsp. *seguieriana*, both of which are in sect. *Paralias.* subsect. *Cornicocarpae*. *E. falcata* subsp. *falcata* was clearly positioned within taxa in sect. *Paralias* as sister to most taxa of the sections. In another molecular publication using (ITS, ETS) and chloroplast (*trn*L-*trn*F, *psb*A-*trn*H, *ycf*3-*trn*S, *trn*G, *atp*B-*rbc*L, *trn*K-*mat*K, *trn*T-*trn*L) markers (Barres et al. 2011) *E. falcata* was included in sect. *Cymatospermum*, but phylogeny results has placed it in sect. *Paralias*. According to the ITS phlogeny results, *E. seguieriana* subsp. *seguieriana*, *E. macroclada*, *E. falcata* subsp. *falcata*, *E. cheiradenia* and *E. gaillardotii* are in the same clade and clearly inconsistent with the traditional classification. *E. cheiradenia* was included in sect. *Pityusa* in 2013 (Riiana et al.), 2015 (Pahlevani et al.) and 2021 (Frajman and Geltman) publications. In Flora of Turkey, sect. *Paralias* is divided into two subsect; *Myrsiniteae* includes *E. denticulata* and *E. craspedia* species and subsect. *Conicocarpae* includes *E. macroclada, E. cheiradenia* and *E. seguierana* subsp. *seguierana* taxa*. Myrsiniteae* is one of the most distinctive groups of *E.* subgen. *Esula* and it is also treated as a subsections of sect*. Paralias* in some of the publications (Boissier, 1862; Prokhanov, 1949; Pahlevani et al. 2011). Prokhanov's system also included members of sect. *Pithyusa* within sect*. Paralias*, under the name subsect. *Conicocarpae*. These taxonomic placements are not supported by some recent classification, which show clearly that sect. *Paralias* and the *Myrsiniteae-Pithyusa* clade are not closely related to each other. When the results are re-evaluated in the light of this information, it is better understood why *E. denticulata* and *E. craspedia*, which are included in the subsect. *Myrsiniteae* according to traditionally classification were included in sect. *Myrsiniteae* in the recent studies. In addition, *E. macroclada, E. segueriana* subsp. *seguierana and E. cheiradana*, which are located in sect. *Paralias*, subsect *Cornicocarpae* according to classical systematic, were included in sect. *Pithyusa* in 2013 (Riiana et al. 2013) publication.

E. esula subsp*. tommasiniara*, *E. sanasunitensis* and *E. iberica* are in sect. *Esula* according to Flora of Turkey. In the ITS phylogeny, these three taxa are in the same clade and are consistent with the traditional and molecular classifications. Also, the molecular status of *E. sanasunitensis* was determined for the first time by this study. *E. heteradena* was included in sect. *Chylogala* in accordance with our phylogeny results in both traditional classification and molecular publications furthermore, this species must be excluded from group A.

CONCLUSIONS

In conclusion, the dendrogram obtained from the nrDNA ITS sequence analysis result provided tremendous information. Although the dendrogram results seem to be compatible with the traditional classification at the subgenus level, parallel results have been obtained in terms of section with the classification discussed in recent publications. The fact that *E. grisophylla*, *E. rhytidosperma* and *E. sanasunitensis* are endemic and *E. sanasunitensis* is included in a molecular publication for the first time makes this study remarkable. Our research result shows that *E. heteradena* species included in Group A should be evaluated in a different group. In addition, the classification of *E. gailladotii*, *E. aleppica*, *E. szovitsii* var. *kharputensis* and *E. falcata* subsp. *falcata* needs to be reviewed.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/10.3390/antiox12081524/s1,

Author contributions

AK and GK conceived of the presented idea. AK and GK designed and performed the experiments and also wrote the manuscript. MK collected and identified the plant samples.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Nature Conservation and National Parks.

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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