TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2023) 47: 152-168
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doi:10.55730/1300-008X.2753

Improvements in the phylogeny of *Epilobium* and *Chamaenerion* inferred from nrDNA and cpDNA data focusing on Türkiye

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Received: 13.09.2022 • **Accepted/Published Online:** 13.01.2023 • **Final Version:** 23.03.2023

Abstract: *Epilobium* and *Chamaenerion* included 165 and 8 species over the world, respectively. The members of the genera are distributed particularly in moist habitats from subpolar to tropical regions. This paper aims to provide improvements in the phylogeny of the genera *Epilobium* and *Chamaenerion* using Internal Transcribed Spacer (ITS) of nuclear DNA and *mat*K gene sequence data evaluation with maximum parsimony, maximum likelihood, and Bayesian inference criterations. A total of 188 individual accessions belonging to 28 taxa were newly generated and 110 sequences imported from GenBank (NCBI) were analysed. The nrDNA and plastid DNA gene trees supported to treat *Epilobium* and *Chamaenerion* as separate genera in two well-supported clades. The *mat*K analyses revealed a better resolution than nrDNA ITS within the *Chamaenerion* clade and supported the inclusion of *C. angustifolium* in the sect. *Chamaenerion*. However, most of the examined species mostly fell into the polytomy in both trees excluding *E. roseum* subsp. *consimile* which should be considered a distinct species in the *Epilobium* contrary to morphological treatment in many national flora books. Both phylogenetic trees also support distinguishing *E. prionophyllum* from *E. anatolicum* previously treated as a subspecies of *E. anatolicum*. In conclusion, these preliminary phylogenetic analyses have contributed significantly to the determination of the limits of members belonging to *Epilobium* and *Chamaenerion* distributed in Türkiye.

Key words: Anatolia, ITS, *Mat*K, Onagracaea, taxonomy

1. Introduction

Epilobium L. (Onagraceae), with approximately 165 species mostly distributed in America, Asia, Europe, France, Germany, and New Zealand, is known as a taxonomically complex genus due to its uniform external appearance and frequent intraspecific hybridization (Bleeker and Hurka, 2001; Krajšek et al., 2006, Lorimer, 2007; Granica et al., 2014). In addition, the morphological variation caused by vegetative plasticity complicates the interspecies and intraspecies identifications of *Epilobium* (Raven and Raven, 1976). In the last decades, several noteworthy manuscripts have been published to provide a treatment for *Epilobium* in Europe (Raven, 1968), Russia (Shteinberg, 1949), and Türkiye (Chamberlain and Raven, 1972); however, there has not been a consensus in the taxonomy of the genus. Haussknecht (1884) divided *Epilobium* into two sections as *E.* sect. *Chamaenerion* Tausch and *E.* sect. *Lysimachion* Tausch in his monographic study, which is principally based on the morphological (seed and stigma shape) characters. Raven (1976) divided the genus into six sections (*E.* sect. *Chamaenerion*, *E.* sect. *Cordylophorum* (Nuttall ex Torrey & A. Gray) P. H. Raven, *E.* sect. *Crossostigma* (Spach) P. H. Raven, *E.* sect. *Epilobium*, *E.* sect. *Xerolobium* P. H. Raven, *E.* sect. *Zauschneria* P. H. Raven) based on morphological, palynological, anatomical, and cytological data. Subsequently, Hoch and Raven (1992) transferred two sections (*B.* sect. *Boisduvalia* Spach and *B.* sect. *Currani* Munz) from the genus *Boisduvalia* Spach to the *Epilobium* and making totally eight sections in the genus according to absence/presence of seed comas. Salisbury (1807), Raimann (1893), and Shteinberg (1949) have been stated that sections *Epilobium* and *Chamaenerion* should be considered two separated genera in accordance with flower symmetry, the number of stamen whorls, dilated of the stamen, stamen shape and style trichome.

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Wagner et al. (2007) separated the Chamerion from the Epilobium based on morphological features, and therefore Chamerion was considered a separate genus. .

In order to contribute to the solution of taxonomic problems, some morphological (Haussknecht, 1884; Solomon, 1982; Wagner et al., 2007; Sheidai et al., 2018; Rahimi et al., 2022), micromorphological (Krajšek et al., 2006; Coşkunçelebi et al., 2017), palynological (Skvarla et al., 2008; Rahimi et al., 2018), anatomical (Keating, 1982), and caryological (Seavey and Raven, 1977; Baum et al., 1994; Husband and Schemske, 1998; Kumar and Singhal, 2011; Kumar et al., 2018) investigations have been carried out on the *Epilobium* including *Chamaenerion*. However, molecular-based studies comprising the *Epilobium* and *Chamaenerion* Ség. are limited. Molecular data (nrDNA and cpDNA sequences) have been used for exploring the relationships among members of families and genera level by several authors (Conti et al., 1993; Baum et al., 1994; Levin et al., 2003, 2004; Lorimer, 2007; Sheidai et al., 2018; Çilden and Özüdoğru, 2022; Mishra et al., 2022). It has been noted that Onagraceae is monophyletic and the relationships among its genera are poorly resolved (Levin et al., 2003). Linnaeus (1753) described the section *Chamaenerion* under the genus *Epilobium* s.l. while some researchers considered the *Chamaenerion* members as a different genus from the *Epilobium* (Raimann, 1893; Shteinberg, 1949; Sennikov, 2011), others argued that it should be evaluated under the genus *Epilobium* (Haussknecht, 1884; Rechinger, 1964; Chamberlain and Raven, 1972; Levin et al., 2003). *Chamaenerion* was first suggested as a separate genus by Salisbury (1807). However, Candolle (1828) treated members of *Chamaenerion* as section under the genus *Epilobium*. Similarly, Raimann (1893) and Holub (1972) separated the *Chamaenerion* and *Epilobium* as different genera based on morphological examination, though Raven (1962a-b) claimed that there are no distinctive characters separating these two genera; therefore, *Chamaenerion* should be considered a section within the genus *Epilobium*. *Chamaenerion* includes of 9 taxa belonging to 8 species, mostly distributed in Eurasia and northern hemisphere. The idea is also supported by the finding of Coşkunçelebi et al. (2017) and Güven et al. (2021) based on micromorphological and anatomical data respectively. On the other hand, in the previous studies, it was reported that the sect. *Chamaenerion* is monophyletic while the sect. *Epilobium* is nonmonophyletic according to nrDNA and cpDNA sequence data (Baum et al., 1994; Levin et al., 2004; Lorimer, 2007).

According to Chamberlain and Raven (1972), the genus *Epilobium* is represented with a total of 26 taxa under *E.* sect. *Chamaenerion* and *E.* sect. *Epilobium* in Türkiye. Four of them belonging to *Chamaenerion* and twenty-two of them belonging to *Epilobium*. Türkiye representatives of *Epilobium* and *Chamaenerion* taxa have been studied by several authors in terms of morphology, palynology, general anatomy (Makbul et al., 2008; Okur, 2019; Okur et al., 2021; Başer et al., 2021; Güven et al., 2021), floral variation (Okur et al., 2018), trichome morphology-micromorphology (Makbul et al., 2015; Kundakçı et al., 2016) and seed micromorphology (Coşkunçelebi et al., 2017). The previous studies have improved our taxonomical knowledge of both genera in Türkiye. However, the *Epilobium* and *Chamaenerion* taxa distributed in Türkiye have not yet been evaluated by phylogenetically up to now. In the present study, the phylogenetic relationships among the *Epilobium* and *Chamaenerion* members from Türkiye were evaluated based on nrDNA ITS and cpDNA *mat*K sequences data in combination with previously published accessions. Thus, our paper aims to: 1) contribute phylogeny of *Epilobium* and *Chamaenerion* in Türkiye, 2) describe the relationships and boundaries among some closely related species and subspecies in Türkiye.

2. Material and methods

2.1. Plant material and sampling

A total of 58 individuals belonging to 28 taxa (47 individuals belonging to *Epilobium* and 11 individuals belonging to *Chamaenerion*) collected from natural habitats of Türkiye in the year of 2010 and 2014 (Appendix) and preserved at the herbarium of Biology Department at Recep Tayyip Erdoğan University (RUB). Whenever possible, at least one individual from different populations were sampled for each taxon to account for intraspecific DNA sequence variation. *Circaea alpina* L., *Circaea lutetiana* L., *Clarkia amoena* (Lehm.) A. Nelson & J. F. Macbr., *Clarkia concinna* (Fisch. & C. A. Mey.) Greene, *Fuchsia boliviana* Carriere, *Fuchsia procumbens* R. Cunn., *Ludwigia peploides* (Kunth) P. H. Raven, *Oenothera nuttallii* Sweet., *Oenothera suffrutescens* (Ser.) W. L. Wagner & Hoch which are belonging to different tribes of the family Onagraceae are selected outgroups based on Wagner et al. (2007) for the phylogenetic analysis.

2.2. DNA isolation, amplification, and sequencing

Forty leaf samples removed from herbarium specimens and eighteen silica-dried leaves were ground without any pretreatment by using tungsten beads in TissueLyser LT-Qiagen homogenizer. Total genomic DNA was extracted following the modified CTAB extraction procedure of Doyle and Doyle (1987). Amplification of the studied markers followed the protocols described by Baum et al. (1994) and Wagner et al. (2007). Sequences of one nuclear (nrDNA ITS) and one chloroplast DNA (*mat*K) marker were used in the phylogenetic analysis. The nrDNA ITS

was amplified with primers ITS4 and ITS5 (White et al., 1990). All PCR reactions were made, according to Gültepe et al. (2010). The *mat*K cpDNA coding region was amplified using primers MG1 and MG15 (Liang and Hilu, 1996) according to the following PCR conditions. PCR cycle requirements; preliminary denaturation (95 °C; 2 min), DNA denaturation (94 °C; 1 min), annealing (56 °C for ITS, 55 °C for *mat*K; 45 s), extension (72 °C; 45 s for ITS s 1.5 min for *mat*K), number of cycles (36), final extension (72 °C; 5 min) are down below to increase these two regions. The amplification process of the *mat*K was performed in 50 µL of PCR reaction volume containing; 10 µL of reaction buffer (10 mM), 2.5 µL of $MgCl_2$ (25 mM), 20 µL of dNTP mix (200 mM), 1 µL of MG1 and µL of for MG15 primers (0.01 µM each), 0.25 µL of Taq DNA polymerase (5U/ µL), 1 µL of total template DNA (2–6 ng μL^{-1}) and 14 µL of ddH_2O. After checking the presence of PCR products with agarose gel electrophoresis, they are stored at +4 °C. PCR products were sequenced with the aid of Macrogen Inc. (Seoul, Korea) by use of the same primers. The list of samples and sequences included, with INSDC (International Nucleotide Sequence Database Collaboration) accession numbers, were given in Appendix.

2.3. Sequence alignment and coding of length mutational events

The boundaries of the nrDNA ITS (ITS1, 5.8S rDNA, ITS2) and the *mat*K (MG1/ MG15) were defined according to White et al. (1990) and Liang and Hilu (1996), respectively. The nrDNA ITS and *mat*K sequences were aligned using Muscle v.3.8.31 (Edgar, 2004), than edited in PhyDE v.0.9971 (Müller et al., 2010). The *mat*K sequences were adjusted manually to a motif-based alignment in PhyDE following the criteria outlined by Kelchner (2000), Borsch et al. (2003) and Löhne and Borsch (2005). Two separate datasets were built for the nrDNA ITS and *mat*K. Indels were coded as binary characters according to the simple indel coding (SIC) method (Simmons and Ochoterena, 2000) implemented in the program SeqState v.1.40 (Müller, 2005a).

2.4. Phylogenetic reconstruction

Data matrix was analysed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). MP analyses were carried out using the Parsimony Ratchet (Nixon, 1999) implemented in PRAP (Müller, 2004), which was run with 200 ratchet iterations with 25% of the positions randomly upweighted (weight = 2) during each replicate and 10 random addition cycles. The generated command files also including the nexus data matrix were run in PAUP* version 4.0b10 (Swofford, 2003) using heuristic search with the following parameters: all characters have equal weight, gaps are treated as "missing", simple addition of sequences, and tree bisection-reconnection branch swapping TBR branching swapping, maxtrees setting to 100 and auto-increased by 100, one nonbinary starting tree arbitrarily dichotomized before branch swapping, only one tree saved. A majority rule consensus tree was calculated from the most parsimonious trees received. Jackknife (JK) support values for the nodes found by the MP analysis were calculated in PAUP* version 4.0b10 with the recommended settings (Müller, 2005b) of 10,000 jackknife replicates with the TBR branch swapping algorithm, 36.788% of characters deleted and one tree held during each replicate.

The ML analyses were performed with RAxML-HPC2 (Stamatakis, 2006) on the Cipres Gateway (Müller et al., 2010). The analyses were done after removal of identical sequences by RAxML. Rapid bootstrapping (with the maximum set of 1000 replicates) integrated with a thorough ML search for the optimal tree was carried out using the resource-efficient CAT approximation (Stamatakis, 2006) of the general time reversible (GTR) model of nucleotide substitution under the gamma model of rate heterogeneity as the predefined substitution model in RAxML for all DNA partitions and BINCAT for the binary partition.

Prior to the BI analyses, the nucleotide substitution model that best fit for the dataset was determined for the nrDNA ITS and *mat*K datasets with MrModeltest 2.3 (Nylander, 2004). The optimal model revealed GTR+G under the Akaike Information Criterion (AIC) for both datasets. A binary (restriction site) model was implemented for the coded indels. BI analyses were performed in MrBayes 3.2 (Ronquist et al., 2012) with four simultaneous runs of Metropolis-coupled Markov Chains Monte Carlo (MCMCMC), each with four parallel Markov chains. Each chain was run for 10 million generations, saving one tree every 1000th generation. To ensure convergence of the run, a conservative burn-in of 0.2 (i.e. discarding the first 20% of the trees) was applied, during which the average standard deviation of the split frequencies between the runs dropped below 0.01 and after which the effective sampling size (ESS) for all parameters was well above 200 in either run. The postburn-in trees were used to generate a maximum clade credibility tree, of which the nodes with less than 0.5 posterior probability supports were collapsed. TreeGraph v.2 (Stöver and Müller, 2010) was used to assess the tree topologies and to visualize the trees with node supports.

Incongruence length difference (ILD) test (Farris et al., 1994) was performed to assess the congruence between the nrDNA ITS and *mat*K datasets in PAUP* version 4.0b10 (Swofford, 2003). For this test, the following parameters were used: heuristic search of 1000 replicates, each with 100 random addition searches, maxtrees set to

1 and one tree held each step. As significance threshold for congruence or homogeneity of the partitions a p-value of > 0.05 or 0.01 is considered appropriate by Sullivan et al. (1996) and Cunningham (1997), respectively.

Single nucleotide polymorphisms (SNPs) of seventy-three accessions belong to twenty-eight investigated taxa were detected with PhyDE ver. 0.9971 (Müller et al., 2010) on the original aligned sequences of nrDNA ITS and cpDNA regions (some identical accessions belonging to the same taxa not listed).

3. Results

The ILD test resulted in incongruence with high significance (p = 0.001) level between nrDNA ITS and *mat*K datasets. Therefore, the two datasets were analysed separately. The optimal model revealed GTR+G for both datasets.

3.1. nrDNA ITS dataset

In the present study, *Epilobium* and *Chamaenerion* taxa from 50 individuals were examined for with respect to nrDNA ITS region. A total of 66 nrDNA ITS sequences (for GenBank accession numbers see Appendix) were generated from *Epilobium* and *Chamaenerion* taxa. The entire length of the nrDNA ITS (ITS1, 5.8S and ITS2) varied between 616 and 621 bp in the 125 (116 in group +9 outgroup) sequences. The aligned nrDNA ITS sequences were 656 nucleotides in length. Simple Indel Coding added 59 binary characters as indels and inversions to the nrDNA ITS matrix. The final aligned nrDNA ITS dataset comprised 715 characters, of which 213 and 124 were parsimony informative and variable characters, respectively. Consistency index (CI) and retention index (RI) of the ITS region are 0.6647 and 0.8847, respectively.

The BI trees strongly support separation (JK = 100, BS = 95, PP = 1.00) of *Epilobium cylindricum* D. Don and *Chamerion latifolium* (L.) Holub. The authors believe that these two specimens obtained from NCBI were misidentified; however, taxonomic names are retained in the present paper. Correspondingly, the tree is divided into two main groups as *Epilobium* and *Chamaenerion*. While the taxa in the *Epilobium* are divided into eleven clades, and further species in polytomy, the taxa in the *Chamaenerion* are divided into two clades (Figure 1; Clade C1, C2). Among the *Epilobium* clades, the E1 clade consists of all xerophytic taxa (*E.* sect. *Xerolobium*, *E.* sect. *Crossostigma*, *E.* sect. *Cordylophorum*, *E.* sect. *Epilobiopsis* (Spegazzini) Lievens, Hoch & P. H. Raven, *E.* sect. *Boisduvalia*, *E.* sect. *Zauschneria*) with about full support values (JK = 99, BS = 99, PP = 1.00; Figure 1). Likewise, the taxa with quadrangular and thin ribbed body structure *E. tetragonum* L. grouped together with *E. obscurum* Schreb. in the same clade (JK = 99, BS = 40, PP = 0.95; Figure 1; Clade E3). However, in several cases

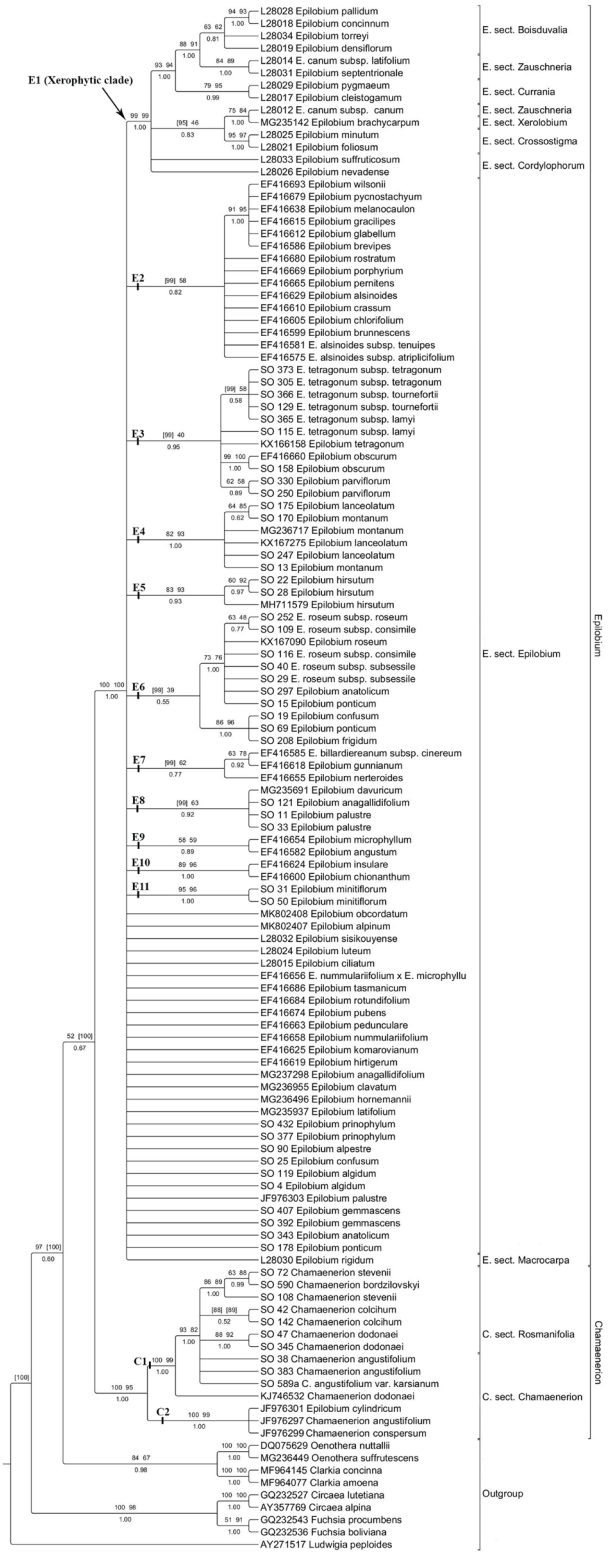

Figure 1. Bayesian phylogram (majority rule consensus tree) of *Chamaenerion* and *Epilobium* taxa based on ITS dataset. Support values: % MP jackknife (JK), and % ML bootstrap (BS) above branches, Bayesian posterior probability (PP) below branches. Section names are taken from Wagner et al. (2007).

the nrDNA ITS data do not provide sufficient resolution among the studied *Epilobium* taxa and correspondingly the tree resulted in hard polytomy (Figure 1). Among the studied *Chamaenerion* taxa, *C. colchicum* Albov, *C. dodonaei* Vill., *C. stevenii* Boiss. and three accessions of *C. angustifolium* L. (SO 38, SO 383 and SO 589a) clustered together in the *C.* sect. *Rosmarinifolium* clade with strongly supported values (JK = 93, BS = 82, PP = 1.00; Figure 1; Clad C1), and *C. angustifolium* (JF976297), *C. conspersum* (Hausskn.) Kitam. (JF976299) and *E. cylindiricum* (JF976301) in the *C.* sect. *Chamaenerion* (JK = 100, BS = 95, PP = 1.00; Figure 1; Clade C2). However, nrDNA ITS data revealed that the individuals of *C. angustifolium* formed a polytomy and the relationships between taxa could not be fully resolved (Figure 1; Clad C1).

The SNP sites based on the ITS region of the species are presented in Table 1. According to ITS data, eighty-one polymorphic sites have been detected. The species belonging to *Chamaenerion* have SNPs significantly differed from the *Epilobium* taxa. However, the *Chamaenerion* species are quite like each other. In the sect. *Epilobium*, the accessions of *E. roseum* subsp. *roseum* Schreb. (SO 252) has SNPs ('T') at position 180 and 439, and ("A") at position 555 are different from rest of two subspecies. The specimen belonging to *E. tetragonum* subsp. *lamyi* (F. W. Schultz) Nyman (SO 115) has two SNPs at the positions 72 and 560 ('T' and 'A') that are different from all subspecies of *E. tetragonum*. *E. anatolicum* Hausskn. accessions (SO 297) have SNPs at positions of 180, 190, 439, 555, and 569 which is different from the accessions of *E. prinophyllum* Hausskn. (SO 377).

3.2. *matK* dataset

In the present study, *Epilobium* and *Chamaenerion* taxa from 28 individuals were examined for with respect to *mat*K gene. A total of 35 *mat*K sequences (for GenBank accession numbers see Appendix) were generated from *Epilobium* and *Chamaenerion* taxa. The partial length of the *mat*K varied between 1558 and 1550 bp in the 63 (54 in group +9 outgroup) sequences. The aligned *mat*K sequences were 1570 nucleotides in length. Simple Indel Coding added 8 binary characters as indels and inversions to the *mat*K matrix. The final aligned *mat*K dataset comprised 1578 characters of which of which 145 and 125 were parsimony informative and variable characters, respectively. Consistency index (CI) and retention index (RI) of the *mat*K are 0.8552 and 0.9165, respectively.

The BI trees strongly support the differentiation of *Epilobium* and *Chamaenerion* taxa (JK = 99, BS = 96, PP = 1.00). The trees are divided into two main groups as *Epilobium* and *Chamaenerion*. *Epilobium* are divided into two subgroups (Xerophytic Clade and *E.* sect. *Epilobium*) and then *E.* sect. *Epilobium* is divided into eight clades, and several species in polytomy; however, the taxa in the *Chamaenerion* consist of a clade (Figure 2, note: the clades name were designated in accordance with the ITS tree and two further clades (E12 and E13) which are not resolved in the ITS tree newly assigned for *mat*K tree). Similarly, the differentiation of taxa with divided stigma deeply 4-lobed, *E. hirsutum* L. is strongly (JK = 93, BS = 97, PP = 1.00), *E. lanceolatum* Sebast. & Mauri, and *E. montanum* L. (JK = 70, BS = 59, PP = 0.56) is moderately supported (Figure 2; Clade E4, E5). Despite that deeply 4-lobed stigmated *E. parvifolium* Schreb. is clustered with *Epilobium* species which have divided stigma entire (Figure 2; Clade E3). Moreover, it was determined that *E. roseum* subspecies are not clustered together (Figure 2; Clade E3, E6). In addition, according to *mat*K data, it was determined that hard polytomy is common among *Epilobium* taxa and taxonomic relationships were not fully resolved. In the C1 Clade, the *Chamaenerion* taxa strongly clustered (JK = 99, BS = 99, PP = 1.00) together. However, *C. colchicum*, which is in *Rosmarinifolium* section of *Chamaenerion* taxa grouped separately from *C. dodonaei* and *C. stevenii* at the same section (JK = 100, BS = 97, PP = 1.00; Figure 2; Clade C1).

The SNP sites based on the *mat*K region of the species are presented in Table 2. According to *mat*K data fifty-nine polymorphic sites has been detected. The species belonging to *Chamaenerion* have SNPs significantly differed from the *Epilobium* taxa. However, the *C. angustifolium* is quite different from the rest of the *Chamaenerion* taxa. In the sect. *Epilobium*, the *E. roseum* subsp. *consimile* (Hausskn.) P. H. Raven accessions (SO 342, SO 109) have SNPs ('C', 'A', 'T', 'A', 'C', 'A', 'C', 'G') at position 608, 757, 876, 890, 1121, 1145, 1452, 1453, respectively (Table 2), those are different from the rest of the subspecies of the *E. roseum*. There are no nucleotide variations among the subspecies of *E. tetragonum*. There are nine different SNPs between *E. prinophyllum* and *E. anatolicum*.

4. Discussion

The generic name and taxonomical level of *Chamaenerion* have become a source of disagreement among the different authors. However, Sennikov (2011) reported that the correct generic name is *Chamaenerion*, and *Chamerion* is a heterotypic synonym. Thus, we prefer to use the generic name as *Chamaenerion* in the present paper. The genus *Chamaenerion* is treated under the genus *Epilobium* as a section in the Flora of Türkiye (Chamberlain and Raven, 1972). However, molecular studies on the Epilobieae tribe revealed that Chamaenerion and Epilobium along with the other seven sections are clustered at

Table 1. Single nucleotide polymorphisms based on the ITS in the accession belonging to *Epilobium* species. Point indicates nuclotide similarity with the first row.

																				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1	2	4	4	5	6	6	7	7	7	8	8	8	8	8	8	9	9	9	0	1	1	1	2	2	2	2	3	3	3	5	6	8	8	8	8	8
	4	7	1	7	7	4	9	2	3	5	0	2	4	5	6	7	4	5	6	9	7	8	9	0	2	3	9	0	2	3	3	7	0	3	5	7	8
C. angustifolium (SO 38)	C	T	T	A	T	A	C	C	T	T	C	G	A	T	T	C	T	C	T	T	A	A	G	T	T	T	A	A	G	T	T	C	T	A	T	C	T
C. colchium (SO 42)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. dodonaei (SO 345)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. stevenii (SO 108)	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. algidum (SO 119)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. alpestre (SO 90)	?	?	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	G	A	C	G	C	T	C	C	.	C	.	.	T	C
E. anagallidifolium (SO 121)	?	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	T	C	.	.	T	C
E. anatolicum (SO 297)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	.	.	.	T	C
E. confusum (SO 25)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	G	A	C	G	C	T	C	C	.	C	.	.	T	C
E. frigidum (SO 208)	T	C	C	G	C	.	.	T	G	C	A	A	G	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. gemmascens (SO 407)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. hirsutum (SO 28)	T	C	C	G	C	.	.	T	.	C	A	A	G	C	C	.	C	T	G	C	.	C	A	G	A	C	G	C	T	C	C	.	C	G	.	.	C
E. lanceolatum (SO 247)	T	C	C	G	C	G	.	T	.	C	A	A	.	C	C	.	.	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. montanum (SO 13)	T	C	C	G	C	.	T	T	.	C	A	A	.	C	C	.	.	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. minitiflorum (SO 31)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. obscurum (SO 158)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	G	C	T	C
E. palustre (SO 11)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	T	C	.	.	T	C
E. parviflorum (SO 250)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	G	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. ponticum (SO 15)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	.	.	.	T	C
E. prinophylum (SO 377)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	G	A	C	G	C	T	C	C	.	C	.	.	T	C
E. roseum subsp. *consimile* (SO 116)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. roseum subsp. *roseum* (SO 252)	T	.	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	.	.	.	T	C
E. roseum subsp. *subsessile* (SO 40)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. tetraganum subsp. *lamyi* (SO 115)	T	C	C	G	C	.	.	T	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. tetragonum subsp. *tetragonum* (SO 373)	T	C	C	G	C	.	.	.	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C
E. tetragonum subsp. *tournefortii* (SO 366)	T	C	C	G	C	.	.	.	.	C	A	A	.	C	C	.	C	T	G	C	.	C	A	A	A	C	G	C	T	C	C	.	C	.	.	T	C

Table 1. (Continued).

	1	1	1	1	2	2	2	2	2	2	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6
	9	9	9	9	1	1	1	2	4	4	7	8	9	3	3	4	4	4	5	5	5	5	6	6	8	8	9	9	1	1	2	2	3	5	5	5	6	6	7	7	8	8	8	9	9	9	9	1
	0	1	2	3	1	5	9	7	7	8	9	3	3	3	9	0	2	3	2	3	4	9	0	4	4	5	4	9	0	1	2	3	2	3	5	8	0	9	6	7	3	4	7	0	2	3	5	4
C. angustifolium (SO 38)	C	T	A	C	C	G	C	C	G	A	C	T	C	G	C	A	T	T	T	C	G	T	T	T	G	G	C	C	T	C	T	T	C	A	T	T	A	T	C	C	C	A	T	A	A	T	G	A
C. colcihum (SO 42)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. dodonaei (SO 345)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C. stevenii (SO 108)	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E. algidum (SO 119)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. alpestre (SO 90)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. anagallidifolium (SO 121)	T	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	G	C	A	T
E. anatolicum (SO 297)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	T	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	A	C	.	.	A	A	G	T	C	G	.	C	A	T
E. confusum (SO 25)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	.	A	A	G	T	C	G	.	C	A	T
E. frigidum (SO 208)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	T	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. gemmascens (SO 407)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. hirsutum (SO 28)	G	C	.	G	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	.	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. lanceolatum (SO 247)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	T	G	A	A	A	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	.	.	.	A	A	G	T	C	G	.	C	A	T
E. montanum (SO 13)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	A	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	.	.	.	A	A	G	T	C	G	.	C	A	?
E. minitiflorum (SO 31)	G	C	.	.	G	C	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	C	T	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. obscurum (SO 158)	G	C	G	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	C	C	A	A	G	T	C	G	.	C	.	T
E. palustre (SO 11)	T	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	G	C	A	T
E. parviflorum (SO 250)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	.	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	C	C	A	A	G	T	C	G	.	C	A	T
E. ponticum (SO 15)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	T	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	A	C	.	.	A	A	G	T	C	G	.	C	A	T
E. prinophylum (SO 377)	T	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. ros. subsp. *consimile* (SO 116)	T	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	.	A	A	G	T	C	G	.	C	A	T
E. ros .subsp. *roseum* (SO 252)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	T	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	A	C	.	.	A	A	G	T	C	G	.	C	A	T
E. ros. subsp. subsessile (SO 40)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. tet. subsp. *lamyi* (SO 115)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	.	C	A	A	G	T	C	G	.	C	A	T
E. tet. subsp. *tetragonum* (SO 373)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	C	C	A	A	G	T	C	G	.	C	A	T
E. tet. subsp. *tournefortii* (SO 366)	G	C	.	.	G	T	.	A	A	T	T	C	T	.	.	G	A	A	.	T	T	C	C	.	.	C	.	.	C	T	C	.	.	C	.	C	C	C	A	A	G	T	C	G	.	C	A	T

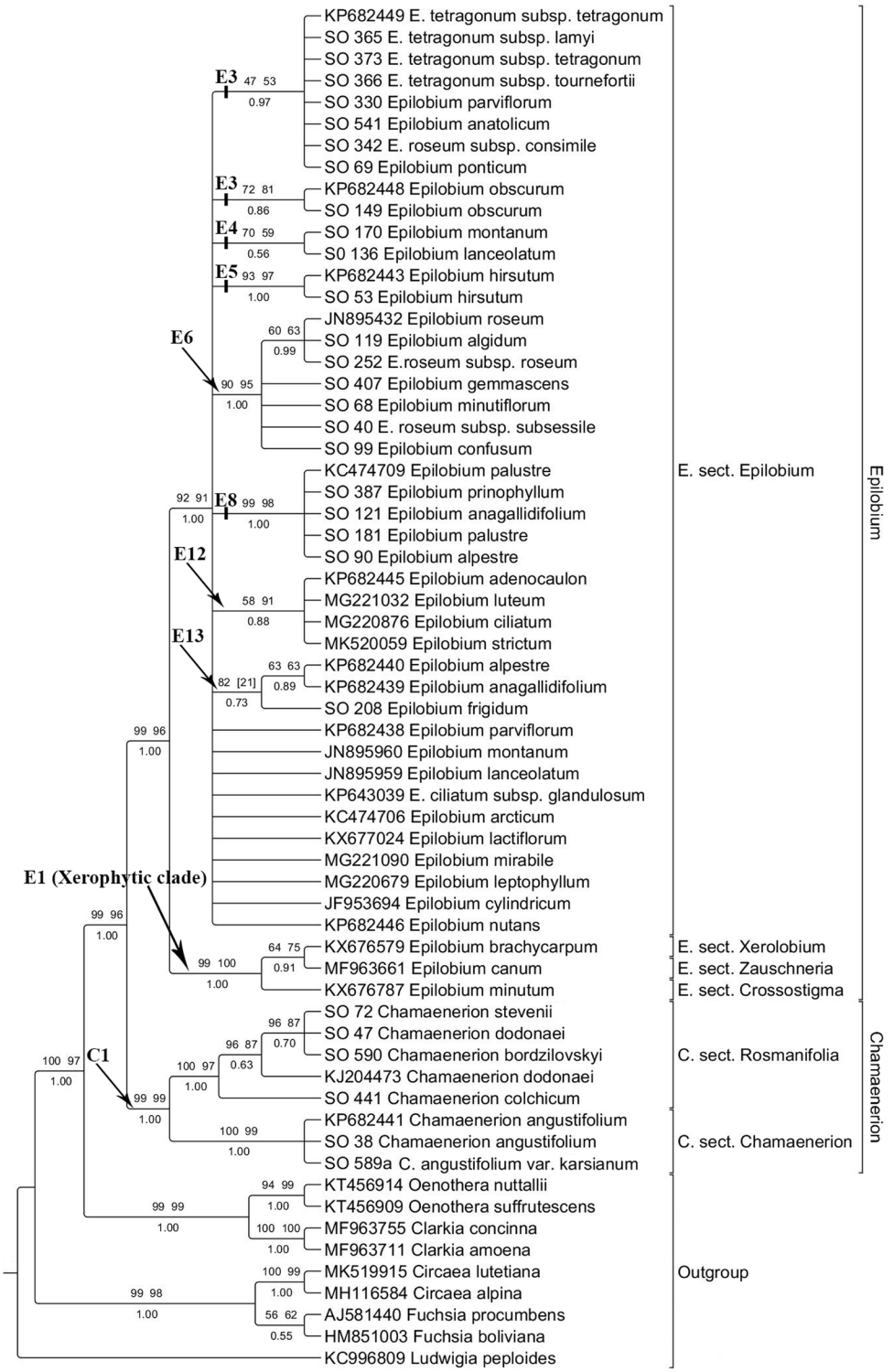

Figure 2. Bayesian phylogram (majority rule consensus tree) of *Chamaenerion* and *Epilobium* taxa based on *mat*K dataset. Support values: % MP jackknife (JK), and % ML bootstrap (BS) above branches, Bayesian posterior probability (PP) below branches. Section names are taken from Wagner et al. (2007) (Designations of the clades as in Figure 1).

Table 2. Single nucleotide polymorphisms based on the *mat*K in the accession belonging to *Epilobium* species. Point indicates nucleotide similarity with the first row.

						1	1	1	2	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	6	6	6	7	7	7	7	7	7
	1	1	3	6	7	1	3	4	1	2	3	7	8	1	3	5	9	1	3	3	4	1	1	6	0	2	7	0	2	5	6	9	9
	2	8	5	6	4	4	7	3	9	9	5	0	2	3	6	1	0	1	8	9	1	0	3	2	8	0	8	2	0	7	9	3	6
C. angustifolium (SO38)	T	A	G	G	G	T	A	C	A	G	C	C	G	G	G	T	T	A	T	A	A	T	C	C	C	G	C	T	C	G	A	G	T
C. colchicum (SO 441)	C	G	A	.	A	G	.	A	T	.	.	.	T	.	.	.	.	.	.	T	C	C	.	.	.	.	.	.	.	A	.	.	.
C. dodonaei (SO 47)	C	G	A	.	A	G	.	A	T	.	.	.	T	.	.	.	.	C	A	G	C	C	.	.	.	.	.	.	.	A	.	.	.
C. stevenii (SO 72)	C	G	A	.	A	G	.	A	T	.	.	.	T	.	.	.	.	C	A	G	C	C	.	.	.	.	.	.	.	A	.	.	.
E. algidum (SO 119)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. alpestre (SO 90)	.	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	A	G
E. anagallidifoium (SO 121)	.	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	A	G
E. anatolicum (SO 541)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. confusum (SO 99)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. frigidum (SO 208)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. gemmascens (SO 407)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. hirsutum (SO 53)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. lanceolatum (S0 136)	C	.	.	T	.	.	C	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. minutiflorum (SO 68)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. montanum (SO 170)	C	.	.	T	.	.	C	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. obscurum (SO 149)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	A	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. palustre (SO 181)	.	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	A	G
E. parviflorum (SO 330)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. ponticum (SO 69)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. prinophyllum (SO 387)	.	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	A	G
E. roseum subsp. *consimile* (SO 342)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. roseum subsp. *roseum* (SO 252)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. roseum subsp. *subsessile* (SO 40)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	G	T	T	C	G	T	C	.	G
E. tetragonum subsp. *lamyi* (SO 365)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. tetragonum subsp. *tetragonum* (SO 373)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
E. tetragonum subsp. *tournefortii* (SO 366)	C	.	.	T	.	.	.	.	G	C	G	G	.	A	C	G	C	.	.	T	.	.	A	T	.	T	T	C	G	A	C	.	G
C. angustifolium (SO38)	C	G	A	C	G	C	A	C	A	C	A	G	G	C	C	T	A	A	G	G	G	T	C	G	C	C							
C. colchicum (SO 441)	T	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.							
C. dodonaei (SO 47)	T	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.							
C. stevenii (SO 590)	T	.	.	.	A	.	.	A	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	A	.	.							
E. algidum (SO 119)	.	T	.	.	N	T	C	.	T	T	C	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. alpestre (SO 90)	.	T	.	.	C	T	C	.	T	.	.	C	A	T	T	.	.	G	A	.	.	.	T	.	T	T							
E. anagallidifolium (SO 121)	.	T	.	.	C	T	C	.	T	.	.	C	A	T	T	.	.	G	A	.	.	.	T	.	T	T							
E. anatolicum (SO 541)	.	T	T	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. confusum (SO 99)	.	T	.	.	A	T	C	.	T	T	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. frigidum (SO 208)	.	T	.	.	A	T	C	.	T	.	.	C	.	.	T	C	.	G	A	.	C	G	T	.	T	T							
E. gemmascens (SO 407)	.	T	.	.	A	T	C	.	T	T	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. hirsutum (SO 53)	.	T	.	.	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	.	.	G	.	T	T							
E. lanceolatum (S0 136)	.	T	.	.	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							

Table 2. (Continued).

E. minutiflorum (SO 68)	.	T	.	.	A	T	C	.	T	T	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. montanum (SO 170)	.	T	.	.	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. obscurum (SO 149)	.	T	.	.	A	T	C	.	T	.	.	C	.	.	T	C	.	G	A	.	C	G	T	.	T	T							
E. palustre (SO 181)	.	T	.	.	C	T	C	.	T	.	.	C	A	T	T	.	.	G	A	.	.	.	T	.	T	T							
E. parviflorum (SO 330)	.	T	.	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. ponticum (SO 69)	.	T	.	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. prinophyllum (SO 387)	.	T	.	.	C	T	C	.	T	.	.	C	A	T	T	.	.	G	A	.	.	.	T	.	T	T							
E. roseum subsp. *consimile* (SO 342)	.	T	T	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. roseum subsp. *roseum* (SO 252)	.	T	.	.	A	T	C	.	T	T	C	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. roseum subsp. *subsessile* (SO 40)	.	T	.	.	A	T	C	.	T	T	.	C	.	.	T	.	.	G	A	.	.	.	T	.	T	T							
E. tetragonum subsp. *lamyi* (SO 365)	.	T	.	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. tetragonum subsp. *tetragonum* (SO 373)	.	T	.	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							
E. tetragonum subsp. *tournefortii* (SO 366)	.	T	.	A	A	T	C	.	T	.	.	C	.	.	T	.	.	G	A	.	C	G	T	.	T	T							

two monophyletic clades and should be considered separate genera. (Conti et al., 1993; Levin et al., 2003, 2004; Wagner et al., 2007; Luo et al., 2021; Rahimi et al., 2022). The ITS and *mat*K tree topologies obtained from the current study strongly supported to treat *Chamaenerion* and *Epilobium* as separate genera as well. Similarly, Baum et al. (1994) and Wagner et al. (2007) disputed that members of *Chamaenerion* formed a sister group with members of the genus *Epilobium*; therefore, *Chamaenerion* should be a separate genus. Likewise, seed morphology, e.g., seed shape, presence/absence of papillae and beak, papillae shape and ornamentation, and periclinal wall features (Coşkunçelebi et al., 2017), anatomical features, e.g., midrib and idioblast shape, leaf and pericarp properties (Güven et al., 2021), pollen shape, e.g., monad and tetrad (Rahimi et al., 2018) and chromosomes numbers (x = 9, 18, 36, 72) detected that *Epilobium* and *Chamaenerion* should be separate genera (Keating, 1982; Wagner et al., 2007). It is seen that the molecular data obtained in this study are compatible with the morphological, palynological, cytological and anatomical data, and support the idea that they are the two distinct genera as previously cited.

4.1. *Chamaenerion*

Taxa belonging to genus *Chamaenerion* form strongly supported group according to molecular data (Figures 1 and 2; Clade C1, C2). The *Chamaenerion* species (Figures 1 and 2, Tables 1 and 2) are distinguished from *Epilobium* members by having less fibrous roots (not densely fibrous root), alternate leaf arrangement on the lower parts of the stem (not opposite or whorled leaf arrangement), free sepals (not free sepals), flowers with zygomorphic symmetry (not actinomorphic symmetry), and obtuse petals tip (not emarginate petal tip) (Figure 3). In addition to abovementioned identifying morphological characters, *Chamaenerion* differs from *Epilobium* by having equifacial leaves with cylindrical idioblasts in mesophyll and circular or cordate midrib as well monad pollen shedding (Coşkunçelebi et al., 2017; Rahimi et al., 2018; Güven et al., 2021).

Wagner et al. (2007) treated the genus *Chamaenerion* as two sections (sect. *Chamaenerion* and sect. *Rosmanifolia*) in his revisional study, which is principally based on the morphological (inflorescence, style pubescent or glabrous, appearance of buds, fruit and seed surface) characters and molecular data. As seen in the Figure 1, members of the genus *Chamaenerion* received strong support (JK = 100, BS = 95, PP = 1.00) in analyses based on ITS; however, accessions of the *C. angustifolium* treated under the sect. *Chamaenerion* appears in polytomy with the members of *C.* sect. *Rosmarinifolium*. On the other hand, sect. *Chamaenerion* and sect. *Rosmanifolia* received strong support (JK = 99, BS = 99, PP = 1.00) and better resolved in the analyses of the *mat*K in contrast to ITS (Figures 1 and 2).

C. dodonaei and *C. stevenii* shared similar morphological features (Chamberlain and Raven, 1972), the *mat*K tree and *mat*K SNPs also supported the similarity between these two taxa (Figure 2, Table 2). However, they are separated in the ITS tree. Recent studies regarding to seed micromorphology and general anatomy showed that no significant differences between *C. dodonaei* and *C. stevenii* (Coşkunçelebi et al., 2017, Güven et al., 2021). Pollen surface ornamentation of these two taxa displayed differences (Rahimi et al., 2018) in

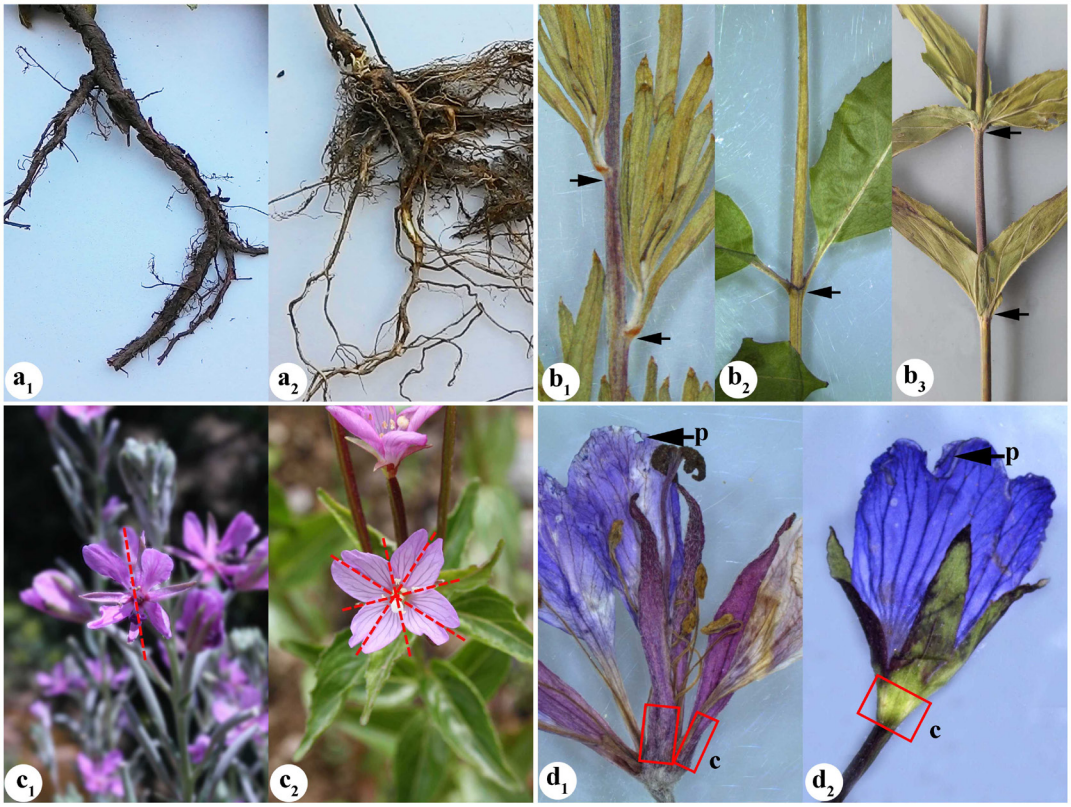

Figure 3. Morphological comparison of *Chamaenerion* and *Epilobium*; a_1. ± Fibrillous root, a_2. Fibrillous root; b_1. Alternate leaf arrangement, b_2. Opposite leaf arrangement, b_3. Whorled leaf arrangement; c_1. Bilateral symmetry, c_2. Radially symmetry: d_1. Calyx lobe free and petal tip obtuse, d_2. Calyx lobe not free and petal tip emarginate; Calyx (c), Petal tip (p), (a_1, b_1, c_1, d_1: *Chamaenerion*; a_2, $b_{2,3}$, c_2, d_2: *Epilobium*).

contrast to abovementioned studies as shown in the ITS tree (Figure 1). On the other hand, accessions of the *C. dodonaei* (KJ746532) retrieved from NCBI are clustered away from newly generated sequences of *C. dodonaei* (Figures 1 and 2; Clade C1). Therefore, diagnosis of the specimen belonging to *C. dodonaei* (KJ746532) needs a confirmation. However, *mat*K provides better resolution at this point as stated by Liang and Hilu (1996). Similarly, *C. bordzilovskyi* Tzvelev described as a new species by Tzvelev (2014) clustered together with *C. stevenii* at the same Clade in both trees with moderately support values (Figures 1 and 2; Clade C1). Okur (2019) reported that there are strong similarities between *C. bordzilovskyi* and *C. stevenii* according to general morphology, seed and palynological features and suggested that *C. bordzilovskyi* is conspecific with *C. stevenii* which shows variation in flower color even in the same sample throughout the flowering time. Moreover, the present results obtained from both nrITS and *matK* support that they are conspecific (= *Chamaenerion bordzilovskyi* Tzvelev, Novosti Sist. Vyssh. Rast. 45: 47. 2014. **syn. nov.)**.

C. colchicum (Albov) Holub is evaluated in the *C.* sect. *Rosmarinifolium* (Rahimi et al., 2018) and distributed in the Caucasus, north-eastern part of Türkiye and northwestern part of Iran (Wagner et al., 2007). *C. colchicum* is externally linked with *C. dodonaei* and *C. stevenii* in both trees (Figures 1 and 2). Although palynological features of these three species are similar (Okur, 2019), in fact they are distinct in their characteristics of the seed micromorphology (Coşkunçelebi et al., 2017) and anatomical features (Güven et al., 2021).

C. angustifolium differs from the other species of *Chamaenerion* by having anatomical features such as circular shape of midrib and hypostomatic leaves, one-layered palisade parenchyma beneath the lower epidermis and periclinal walls of the seed epidermis are without papillae (Coşkunçelebi et al., 2017; Güven et al., 2021). *C. angustifolium* is derived species in the genus *Chamaenerion* (Baum et al., 1994), has a derived mode of herkogamy (Guo et al., 2016) with polyploidy (Chen et al., 1988) and may have facilitated the dispersal of the species to new habitats (Guo et al., 2016).

Tzvelev (2014) separated some plant specimens previously identified as *C. angustifolium* collected from near Kars (Türkiye) and published it as *C. angustifolium*

L. var. *karsianum* Tzvelev according to petal colour. The *mat*K tree showed that the specimens of *C. angustifolium* var. *karsianum* are clustered with *C. angustifolium* at the same Clade (Figure 2; C1); however, it is a part of polytomy with some members of the *C. angustifolium* and *C.* sect. *Rosmanifolium* in the ITS tree (Figure 1). General and seed morphology with palynological data are not enough to discriminate these two taxa (Okur, 2019) and *mat*K (Figure 2) data is support to close relationships between *C. angustifolium* var. *karsianum* and *C. angustifolium*. *C. angustifolium* has diploid, tetraploid, and hexaploid populations and white-flowered variants are more common among the diploid populations than tetraploid and hexaploid those (Mosquin, 1966). Throughout its vast range, *C. angustifolium* shows considerable variations in terms of the leaf size and shape, pubescence, and flower and pollen morphology. These variations have a strong genetic basis, but some populations on the species are also subject to profound environmental modifications (Mosquin, 1966). Therefore, the colour of the petals is not important to evaluate the subspecific rank in *C. angustifolium*. Conseqeuently, *C. angustifolium* var. *karsianum* should be conspecific with *C. angustifolium*. Mosquin (1966) divided *C. angustifolium* into two subspecies based on palynological properties, polyploidy level, and leaf size, and reported that *C. angustifolium* subsp. *circumvagum* Mosquin was distributed also in Türkiye. However, there is no morphological and cytological data about the accessions belonging to *C. angustifolium* collected from Türkiye (SO 383, SO 38), Austria (KP682441), and China (JF976297) used in molecular study. Thus, all these accessions are treated under the *C. angustifolium* in molecular studies. However, to reveal subspecific boundaries of *C. angustifolium*, further morphological, molecular, polynological, and cytological studies are needed from Türkiye. The most prevalent species of the genus, *C. angustifolium*, (Keating et al., 1982; Jürgenson et al., 2012) also differs from the rest of species in the sect. *Chamaenerion* by having buds arising from the root tissue (Keating et al., 1982) and in cross section of the fruit, *C. angustifolium* has type I capsule shape (characterized by circular-shaped central column and T-form lobes with dense mechanical tissue cells on the inner side of mesocarp) in contrast to members of the *C.* sect. *Rosmanifolium* which have type II capsule shape (characterized by rectangular-shaped central column and T-form lobes with sparse mechanical tissue cells on the inner side of mesocarp) (Güven et al., 2021). The current results especially obtained from the *mat*K dataset revealed that *C. angustifolium* is a unique species among the members of the *C.* sect. *Chamaenerion* (Tables 1 and 2) and, for this reason, it is taken under a different section. Therefore, the *mat*K tree provides better resolution than ITS in the current study as indicated by Levin et al. (2004) within Onagreae.

Baum et al. (1994) stated that *C. angustifolium* (*E. angustifolium*) is the most closely related species to *C. latifolium* (L.) Holub (Moreover, the present results obtaine). However, the accession (MG235937) belonging to *C. latifolium* is not in the same group with *C. angustifolium*, possibly due to misidentification (Figure 1). Additionally, accession (JF976297) of the *C. conspersum* and *E. cylindricum* at the ITS tree (Figure 1; Clade C2). Besides, the ITS tree topology has shown that relationships among the accessions of the *C. angustifolium* are not fully resolved and form a polytomy.

4.2. *Epilobium*

In the current sense, the genus *Epilobium* is evaluated under the eight sections (Wagner et al., 2007) as reviewed in the introduction part of this paper and the majority of the species belong to *E.* sect. *Epilobium* (Baum et al., 1994). Phylogenetic relationships of many species in this section are not resolved due to hard polytomy and the section is not a monophyletic based on ITS tree (Figure 1). However, it is seen that taxa with the entire stigma fell into two clades in the phylogenetic trees (Figure 1; Clade E3, Figure 2; Clade E6). Although they have different SNPs (Tables 1 and 2), *E. obscurum* and *E. tetragonum* are two morphologically related taxa with quadrangular stems and thin ribs on the surface and were clustered within the same clade (Figures 1 and 2; Clade E3). *E. tetragonum* is represented with three subspecies in the flora of Türkiye (Chamberlain and Raven, 1972). In the ITS tree, subspecies of the *E. tetragonum* formed a group in the Clade E3 (Figure 1). However, an accession (SO115) of *E. tetragonum* subsp. *lamyi* is separated from the rest accessions (SO 373, SO 305, SO 366, SO 129, SO 365) collected from far away locations in the phylogenetic tree and has different SNPs (Figure 1; Clade E3, Table 1). However, *E. tetragonum* subsp. *lamyi* is considered a distinct species by some researchers (Haussknecht, 1884; Shteinberg, 1949). A recent study carried out on the genus *Epilobium* has shown that *E. tetragonum* subsp. *lamyi* differs from other subspecies by lack of stem sclerenchyma (Güven et al., 2021). Moreover, according to Okur (2019), the subspecies of the *E. tetragonum* are different based on palynological and micromorphological properties. Although the current molecular data do not discriminate sufficiently *E. tetragonum* subsp. *lamyi*, it is treated as a separate species in this study.

The other clade that includes species with entire stigma (*E. anatolicum* Hausskn., *E. ponticum* Hausskn., *E. roseum* subsp. *consimile*) is grouped together with *E. parviflorum* Schreb. which have 4-parted stigma (Figure 2; Clade E6). *E. roseum* is represented with three subspecies in Türkiye (Chamberlain and Raven,

1972). In the Flora USSR (Shteinberg, 1949), *E. roseum* subsp. *consimile* and *E. roseum* subsp. *roseum* were evaluated as distinct taxa. Similarly, based on molecular and morphological data, these three subspecies were recognized as distinct from each other (Wagner et al., 2007). In our ITS trees, all the subspecies are grouped in the same clade, but forming a polytomy (Figure 1; Clade E6). In the *mat*K tree (Figure 2; Clade E3, E6), one accession of *E. roseum* subsp. *consimile* (SO 342) is separated from the two subsp. of *E. roseum*. Coşkunçelebi et al. (2017) reported that these subspecies are different in terms of seed micromorphological features. However, they do not reveal significant differences in terms of anatomical features (Güven et al., 2021). In accordance with seed microfeatures and different SNPs *mat*K sequences (Table 2), *E. roseum* subsp. *consimile* is separated. Therefore, *E. roseum* subsp. *consimile* can be considered a separated species. On the other hand, taking into consideration of both ITS and *mat*K tree topologies, the species of *Epilobium* and *Chamaenerion* mostly fell into the polytomy. Although ITS provides more effective results in phylogenetic studies especially at the genus level or higher, it does not provide sufficient data at the infraspecific level (Baldwin et al., 1995; Levin et al., 2004; Lorimer, 2007; Rahimi et al., 2022). Hence, subspecies of *E. roseum* except subsp. *consimile* are not separated based on ITS and *mat*K sequences.

E. lanceolatum, *E. montanum*, *E. parviflorum*, and *E. hirsutum* are morphologically similar species in terms of 4-parted stigmas (Shteinberg, 1949; Chamberlain and Raven, 1972). *E. hirsutum* is grouped in a separate clade from *E. lanceolatum*, *E. montanum*, and *E. parviflorum* taxa, which having 4-parted stigma (Figure 1 and 2; Clade E5). This result reveals that the parted stigma structure is not a stable feature among taxa and cannot be associated with molecular data. However, *E. hirsutum* differs morphologically from other *Epilobium* members with large flowers and dense hirsute stem hairs (Chamberlain and Raven, 1972). Similarly, pollen grain features (Makbul et al. 2008; Rahimi et al. 2018), seed micromorphology (Coşkunçelebi et al., 2017), and vegetative and fruit anatomy on this species have revealed that this taxon differs from the other taxa (Güven et al., 2021). This shows that *E. hirsutum*, which has different morphological, palynological, and anatomical features, has also a characteristic genome structure.

Taxonomic rank of *E. prionophyllum* is still in a disputed position in the current taxonomical studies. While *E. prionophyllum* (Hausskn.) P. H. Raven is considered a distinct species (Hauscknecht, 1884; Shteinberg, 1949), by some researchers and in the Flora of Türkiye, this species was evaluated as a subspecies of *E. anatolicum* (Raven, 1964; Chamberlain and Raven, 1972; Wagner et al., 2007). While *E. anatolicum* and *E. prinophyllum* formed polytomy based on the ITS tree, they were placed in different clades in the *mat*K tree (Figure 1; E6, Figure 2; E3, E8). Additionally, these species have different SNPs both ITS and *mat*K sequences (Tables 1 and 2). Güven et al. (2021) reported a limited general anatomical difference between *E. prionophyllum* and *E. anatolicum*, but Coşkunçelebi et al. (2017) stated that these species can be easily distinguished based on the micromorphological features of the seed surface. Similarly, Makbul et al. (2016) reported that *E. prionophyllum* differs from *E. anatolicum* due to morphological features such as hairs of the stem, leaf, flower, and fruit. Thus, the present molecular findings are in accordance with the abovementioned studies conducted on the species.

5. Taxonomic conclusion

The phylogenetic relationships of 28 taxa belonging to *Epilobium* (24 taxa) and *Chamaenerion* (4 taxa) in the tribe *Epilobiae* which are widespread in Türkiye have been investigated. The current results support the discrimination of this group which are considered a different genus in many studies (Shteinberg, 1949; Chamberlain and Raven, 1972; Wagner et al., 2007). The xerophytic clade (E1) separated and revealed monophyly in the genus *Epilobium* both in ITS and *mat*K tree (Figures 1 and 2). Current results especially obtained from *mat*K sequence analysis supported the separation of the *C. angustifolium* from the *C.* sect. *Rosmarinifolium*. *E. tetragonum* subsp. *lamyi* and *E. roseum* subsp. *consimile* are supported to be separate species based on the available data. This study also supported the discrimination between *E. anatolicum* and *E. prionophyllum*. Besides this, genetic variability in the current regions is low and not enough to solve taxonomical problems more clearly in the genus *Epilobium*. The genus can be more robustly addressed with a holistic approach using different data provided from cytological, palynological, micromorphological, morphological, and molecular sources based on multiple accessions to elucidate inter- and/or intraspecific relationships.

Acknowledgments

The authors would like to express their thanks to The Scientific and Technological Research Council of Türkiye (TÜBİTAK, Project number: 113Z782) for financial support.

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Appendix. Taxa used in the phylogenetic analysis. Authors' voucher ([1]) and GenBank ([2]) accessions (SO: Okur & S. Makbul).

Taxa	Code in the MJ cladogram	Voucher data	ITS	*mat*K	References
C. angustifolium L.	JF976297[2]	China, A228	+		Li et al. 2011
	KP682441[2]	E173		+	Unpubl. (Genbank)
	SO 38[1]	Türkiye, Erzurum, Okur 38 & S. Makbul	+	+	Present study
	SO 383[1]	Türkiye, Trabzon, Okur 383 & S. Makbul	+		Present study
C. angustifolium (L.) Scop. var. *karsianum* Tzvelev	SO 589a[1]	Türkiye, Kars, Okur 589a & S. Makbul	+	+	Present study
C. bordzilovskyi Tzvelev	SO 590[1]	Türkiye, Kars, Okur 590 & S. Makbul	+	+	Present study
C. colchicum Albow.	SO 42[1]	Türkiye, Artvin, Okur 42 & S. Makbul	+		Present study
	SO 142[1]	Türkiye, Rize, Okur 142 & S. Makbul	+		Present study
	SO 441[1]	Türkiye, Artvin, Okur 441 & S. Makbul		+	Present study
C. conspersum (Hausskn.) Holub	JF976299[2]	China, D1705	+		Li et al. 2011
C. dodonaei Vill	KJ746532[2]	Czech, 3LF-1089	+		Heneberg and Rezac, 2014
	KJ204473[2]	Geneva, Faure, L., G00199072		+	Unpubl. (Genbank)
	SO 47[1]	Türkiye, Kastamonu, Okur 47 & S. Makbul	+	+	Present study
	SO 345[1]	Türkiye, Kastamonu, Okur 345 & S. Makbul	+		Present study
C. latifolium (L.) Holub	MG235937[2]	Canada, CCDB-18332-E4	+		Kuzmina et al. 2017
C. stevenii Boiss.	SO 72[1]	Türkiye, Gümüşhane, Okur 72 & S. Makbul	+	+	Present study
	SO 108[1]	Türkiye, Gümüşhane, Okur 108 & S. Makbul	+		Present study
E. adenocaulon Hausskn.	KP682445[2]	Austria, E34			Unpubl. (Genbank)
E. algidum Bieb.	SO 4[1]	Türkiye, Rize, Okur 4 & S. Makbul	+		Present study
	SO 119[1]	Türkiye, Kütahya, Okur 119 & S. Makbul	+	+	Present study
E. alpestre (Jacq) Krocker.	KP682440[2]	Austria, E152		+	Unpubl. (Genbank)
	SO 90[1]	Türkiye, Rize, Okur 90 & S. Makbul	+	+	Present study
E. alpinum L.	MK802407[2]	USA, H. E. Marx, ID169166	+		Marx et al. 2019
E. alsinoides A. Cunn.	EF416629[2]	New Zealand, AK298165	+		Unpubl. (Genbank)
E. alsinoides subsp. *atriplicifolium* (A. Cunn.) P. H. Raven & Engelhorn	EF416575[2]	New Zealand, Peel.HR55	+		Unpubl. (Genbank)
E. alsinoides subsp. *tenuipes* (Hook. f.) P. H. Raven & Engelhorn	EF416581[2]	New Zealand, Pukaki.HR108	+		Unpubl. (Genbank)
E. anagallidifolium Lam.	MG237298[2]	Canada, CCDB-18332-D4	+		Kuzmina et al. 2017
	KP682439[2]	Austria, E147		+	Unpubl. (Genbank)
	SO 121[1]	Türkiye, Rize, Okur 121 & S. Makbul	+	+	Present study
E. anatolicum Hausskn.	SO 297[1]	Türkiye, Amasya, Okur 297 & S. Makbul	+		Present study
	SO 343[1]	Türkiye, Amasya, Okur 343 & S. Makbul	+		Present study
	SO 541[1]	Türkiye, Artvin, Okur 541 & S. Makbul		+	Present study
E. angustum (Cheesemen) P. H. Raven & Engelhorn	EF416582[2]	New Zealand, Pukaki.HR109	+		Unpubl. (Genbank)
E. arcticum Sam	KC474706[2]	Canada, Gillespie 7276 CAN		+	Saarela et al. 2013

Appendix. (Continued).

E. billardiereanum subsp. *cinereum* (A. Rich.) P. H. Raven & Engelhorn	EF416585[2]	New Zealand, Puhipuhi.HR215	+		Unpubl. (Genbank)
E. brachycarpum Presl.	MG235142[2]	California, Davis, Sytsma s.n. (WIS).	+		Baum et. al 1994
	KX676579[2]	Canada, E.R. Manton & J. Fennemon, MF285		+	Unpubl. (Genbank)
E. brevipes Hook. f.	EF416586[2]	New Zealand, Fyffe.EP20	+		Unpubl. (Genbank)
E. brunnescens (Cockayne) P. H. Raven & Engelhorn	EF416599[2]	New Zealand, Arthur.HR74	+		Unpubl. (Genbank)
E. canum (E. Greene) P. H. Raven subsp. *canum*	L28012[2]	U.S.A., Beard and Beard in 1959 (UC).	+		Baum et. al 1994
E. canum (Greene) P. H. Raven	MF963661[2]	U.S.A., Beard s.n., JEPSI05539		+	Unpubl. (Genbank)
E. canum subsp. *latifolium* (Hook). Raven	L28014[2]	U.S.A., Butte Co., Cresta Reservoir, See in 1954. (UC).	+		Baum et al. 1994
E. chionanthum Hausskn.	EF416600[2]	New Zealand, Chatham.PdL2	+		Unpubl. (Genbank)
E. chlorifolium Hausskn.	EF416605[2]	New Zealand, Peel.HR56	+		Unpubl. (Genbank)
E. ciliatum Raf.	L28015[2]	U.S.A., Multnomah Co., Seavey 1149 (MO)	+		Baum et al. 1994
	MG220876[2]	Canada, Alexandra Bergeron, Jean-Sebastian Mignot, Marie-Helene Brice, Basil Fayet, MT0018650		+	Kuzmina et al. 2017
E. ciliatum subsp. *glandulosum* (Lehm.) Hoch & P. H. Raven	KP643039[2]	U.S.A., N. A. Bourg,		+	Unpubl. (Genbank)
E. clavatum Trel.	MG236955[2]	Canada, CCDB-18332-H6	+		Kuzmina et al. 2017
E. cleistogamum (Curran) Hoch & Raven	L28017[2]	U.S.A., Butte Co., Broyles 1089 (CHSC, MO)	+		Baum et al. 1994
E. concinnum (D. Don) Hoch & P. H. Raven	L28018[2]	Chile, Munioz 2383 (MO).	+		Baum et al. 1994
E. confusum Hausskn.	SO 19[1]	Türkiye, Rize, Okur 19 & S. Makbul	+		Present study
	SO 25[1]	Türkiye, Artvin, Okur 25 & S. Makbul	+		Present study
	SO 99[1]	Türkiye, Rize, Okur 99 & S. Makbul		+	Present study
E. crassum Hook. f.	EF416610[2]	New Zealand, Lyford.HR145	+		Unpubl. (Genbank)
E. cylindricum D. Don	JF976301[2]	China, D1100	+		Li et al. 2011
	JF953694[2]	China, D134		+	Li et al. 2011
E. davuricum Fisch. ex Hornem	MG235691[2]	Canada, CCDB-18332-A5	+		Kuzmina et al. 2017
E. densiflorum (Lindl.) Hoch & Raven	L28019[2]	U.S.A., Butte Co., Oswald 794 (CHSC)	+		Baum et. al 1994
E. foliosum Nutt. ex Torr. & A. Gray) Suksd.	L28021[2]	U.S.A., Butte Co., Schlising in 1987 (MO)	+		Baum et. al 1994
E. frigidum Hausskn.	SO 208[1]	Türkiye, Muğla, Okur 208 & S. Makbul	+	+	Present study
E. gemmancens C. A. Mayer.	SO 392[1]	Türkiye, Trabzon, Okur 392 & S. Makbul	+		Present study
	SO 407[1]	Türkiye, Rize, Okur 407 & S. Makbul	+	+	Present study
E. glabellum G. Forst.	EF416612[2]	New Zealand, Haast.HR86	+		Unpubl. (Genbank)
E. gracilipes Kirk	EF416615[2]	New Zealand, Arthur.HR69	+		Unpubl. (Genbank)
E. gunnianum Hausskn.	EF416618[2]	New Zealand, Mangarakau.HR66	+		Unpubl. (Genbank)

Appendix. (Continued).

E. hirsutum L.	MH711579[2]	China, Dong, S.-L.,Q738	+		Unpubl. (Genbank)
	KP682443[2]	Austria, E189		+	Unpubl. (Genbank)
	SO 22[1]	Türkiye, Artvin, Okur 22 & S. Makbul	+		Present study
	SO 28[1]	Türkiye, Gümüşhane, Okur 28 & S. Makbul	+		Present study
	SO 53[1]	Türkiye, Kahramanmaraş, Okur 53 & S. Makbul		+	Present study
E. hirtigerum A. Cunn.	EF416619[2]	New Zealand, Akl.A	+		Unpubl. (Genbank)
E. hornemannii Rchb.	MG236496[2]	Canada, CCDB-18332-E5	+		Kuzmina et al. 2017
E. insulare Hausskn.	EF416624[2]	New Zealand, Pukaki.HR116	+		Unpubl. (Genbank)
E. komarovianum H. Le'v.	EF416625[2]	New Zealand, Pisa.HR101	+		Unpubl. (Genbank)
E. lactiflorum Hausskn.	KX677024[2]	Canada, E. R. Manton, ERM838		+	Unpubl. (Genbank)
E. lanceolatum Sebast. & Mauri	JN895959[2]	United Kingdom, NMW5025		+	Unpubl. (Genbank)
	SO 136[1]	Türkiye, Samsun, Okur 136 & S. Makbul		+	Present study
	SO 175[1]	Türkiye, Trabzon, Okur 175 & S. Makbul	+		Present study
	SO 247[1]	Türkiye, Kırklareli, Okur 247 & S. Makbul	+		Present study
E. leptophyllum Raf.	MG220679[2]	Canada, Alexandra Bergeron, Jean-Sebastian Mignot, Marie-Helene Brice, Basil Fayet, MT00186436		+	Kuzmina et al. 2017
E. luteum Pursh	L28024[2]	U.S.A., Marion Co., Seavey 1452 (MO)	+		Baum et al. 1994
	MG221032[2]	Canada, J. Macoun, CCDB-20333-D11		+	Kuzmina et al. 2017
E. melanocaulon Hook	EF416638[2]	New Zealand, Molesworth.EP35	+		Unpubl. (Genbank)
E. microphyllum A. Rich.	EF416654[2]	New Zealand, Weld.EP02	+		Unpubl. (Genbank)
E.minutiflorum Hausskn.	SO 31[1]	Türkiye, Bayburt, Okur 31 & S. Makbul	+		Present study
	SO 50[1]	Türkiye, Hakkari, Okur 50 & S. Makbul	+		Present study
	SO 68[1]	Türkiye, Giresun, Okur 68 & S. Makbul		+	Present study
E. minutum Lind. ex Lehm.	L28025[2]	U.S.A., Curry Co., Chambers 4847 (MO, OSC)	+		Baum et al. 1994
	KX676787[2]	Canada, E.R. Manton & J. Fenneman, MF297		+	Unpubl. (Genbank)
E. mirabile Trel. ex Piper	MG221090[2]	Canada, F. Lomer, CDB-24917-B01		+	Kuzmina et al. 2017
E. montanum L.	MG236717[2]	Canada, O. Vitikainen 56699HIM	+		Kuzmina et al. 2017
	JN895960[2]	United Kingdom, NMW5025		+	Unpubl. (Genbank)
	SO 13[1]	Türkiye, Rize, Okur 13 & S. Makbul	+		Present study
	SO 170[1]	Türkiye, Artvin, Okur 170 & S. Makbul	+	+	Present study
E. nerteroides A. Cunn.	EF416655[2]	New Zealand, Nelson ck.HR78	+		Unpubl. (Genbank)
E. nevadense Munz	L28026[2]	U.S.A.,Clark Co., Hoch 3440 (MO)	+		Baum et al. 1994
E. nummuleriifolium R. Cunn. ex A. Cunn.	EF416658[2]	New Zealand, Owhiro.EP36	+		Unpubl. (Genbank)
E. nummuleriifolium x *E. microphyllum*	EF416656[2]	New Zealand, Owhiro.EP37	+		Unpubl. (Genbank)
E. nutans F. W. Schmidt	KP682446[2]	Austria, E47			Unpubl. (Genbank)
E. obcordatum A. Gray	MK802408[2]	U.S.A., P. Z. Klos & H. E. Marx ID:169319	+		Marx et al. 2019
E. obscurum (Schreb.) Schreb	EF416660[2]	New Zealand, Pukaki.HR112	+		Unpubl. (Genbank)
	KP682448[2]	Austria, E50		+	Unpubl. (Genbank)
	SO 149[1]	Türkiye, Hatay, Okur 149 & S. Makbul		+	Present study
	SO 158[1]	Türkiye, Hatay, Okur 158 & S. Makbul	+		Present study

Appendix. (Continued).

E. pallidum (Eastw.) Hoch & P. H. Raven	L28028[2]	U.S.A., Butte Co., Oswald 554 (CHSC)	+		Baum et al. 1994
E. palustre L.	JF976303[2]	China D1388	+		Li et al. 2011
	KC474709[2]	Canada, Consaul 4319b CAN		+	Saarela et al. 2013
	SO 11[1]	Türkiye, Rize, Okur 11 & S. Makbul	+		Present study
	SO 33[1]	Türkiye, Giresun, Okur 33 & S. Makbul	+		Present study
	SO 181[1]	Türkiye, Gümüşhane, Okur 181 & S. Makbul		+	Present study
E. parviflorum Schreb.	KP682438[2]	Austria, E08		+	Unpubl. (Genbank)
	SO 250[1]	Türkiye, Kırklareli, Okur 250 & S. Makbul	+		Present study
	SO 330[1]	Türkiye, Kastamonu, Okur 330 & S. Makbul	+	+	Present study
E. pedunculare A. Cunn.	EF416663[2]	New Zealand, Matukituki.HR96	+		Unpubl. (Genbank)
E. pernitens Cockayne & Allan	EF416665[2]	New Zealand, Pukaki.HR115	+		Unpubl. (Genbank)
E. ponticum Hausskn.	SO 15[1]	Türkiye, Rize, Okur 15 & S. Makbul	+		Present study
	SO 69[1]	Türkiye, Gümüşhane, Okur 69 & S. Makbul	+	+	Present study
	SO 178[1]	Türkiye, Gümüşhane, Okur 178 & S. Makbul	+		Present study
E. porphyrium G. Simpson	EF416669[2]	New Zealand, Porters. HR38	+		Unpubl. (Genbank)
E.prionophyllum Hausskn.	SO 377[1]	Türkiye, Trabzon, Okur 377 & S. Makbul	+		Present study
	SO 387[1]	Türkiye, Trabzon, Okur 387 & S. Makbul		+	Present study
	SO 432[1]	Türkiye, Giresun, Okur 432 & S. Makbul	+		Present study
E. pubens A. Rich.	EF416674[2]	New Zealand, Tararua.HR6	+		Unpubl. (Genbank)
E. pycnostachyum Hausskn.	EF416679[2]	New Zealand, Porters. HR39	+		Unpubl. (Genbank)
E. pygmaeum (Speg.) Hoch & Raven	L28029[2]	U.S.A., Butte Co., Broyles 1090 (CHSC, MO)	+		Baum et al. 1994
E. rigidum Hausskn.	L28030[2]	U.S.A., Del Norte Co., Wiens 6797 (MO).	+		Baum et al. 1994
E. roseum (Schreb.) Schreb	KX167090[2]	United Kingdom, NMW4343	+		Unpubl. (Genbank)
	JN895432[2]	United Kingdom, NMW4343		+	Unpubl. (Genbank)
E. roseum Schreber subsp. *consimile* (Hausskn.) P.H. Raven.	SO 109[1]	Türkiye, Erzincan, Okur 109 & S. Makbul	+		Present study
	SO 116[1]	Türkiye, Trabzon, Okur 116 & S. Makbul	+		Present study
	SO 342[1]	Türkiye, Karabük, Okur 342 & S. Makbul		+	Present study
E. roseum Schreber subsp. *roseum*	SO 252[1]	Türkiye, Kırklareli, Okur 252 & S. Makbul	+	+	Present study
E. roseum Schreber subsp. *subsessile* (Boiss) P.H. Raven.	SO 29[1]	Türkiye, Bursa, Okur 29 & S. Makbul	+		Present study
	SO 40[1]	Türkiye, Erzurum, Okur 40 & S. Makbul	+	+	Present study
E. rostratum Cheeseman	EF416680[2]	New Zealand, Pukaki.HR105			Unpubl. (Genbank)
E. rotundifolium G. Forst.	EF416684[2]	New Zealand, Ruahine.HR4	+		Unpubl. (Genbank)
E. septentrionale (Keck) Raven	L28031[2]	U.S.A., Raiche 30551 (UC)	+		Baum et al. 1994
E. sisikouyense (Munz.) Hoch and Raven	L28032[2]	U.S.A., Jackson Co., Seavey 1150 (MO).	+		Baum et al. 1994
E. sitrictum Muhl. ex Spreng.	MK520059[2]	U.S.A., Kriebel, R v0256824WIS		+	Direct submission
E. suffruticosum Nutt.	L28033[2]	U.S.A., Wyoming, Teton Co., Raven s.n. (MO)	+		Baum et al. 1994
E. tasmanicum Hausskn.	EF416686[2]	New Zealand, Porters.HR42	+		Unpubl. (Genbank)
E. tetragonum L.	KX166158[2]	United Kingdom, NMW6385	+		Unpubl. (Genbank)

Appendix. (Continued).

E. tetragonum subsp. *lamyi* (F. W. Schultz) Nyman	SO 115[1]	Türkiye, Samsun, Okur 115 & S. Makbul	+		Present study
	SO 365[1]	Türkiye, Samsun, Okur 365 & S. Makbul	+	+	
E. tetragonum subsp. *tetragonum* L.	KP682449[2]	Austria, E60		+	Unpubl. (Genbank)
	SO 305[1]	Türkiye, Samsun, Okur 305 & S. Makbul	+		Present study
	SO 373[1]	Türkiye, Samsun, Okur 373 & S. Makbul	+	+	Present study
E. tetragonum subsp. *tournefortii* (Michalet) H. Lev.	SO 129[1]	Türkiye, Samsun, Okur 129 & S. Makbul	+		Present study
	SO 366[1]	Türkiye, Samsun, Okur 366 & S. Makbul	+	+	Present study
E. torreyi (S. Watson) Hoch & P. H. Raven	L28034[2]	U.S.A., Fresno Co., Seavey in 1974 (MO).	+		Baum et al. 1994
E. wilsonii Petrie	EF416693[2]	New Zealand, Woodside. HR229	+		Unpubl. (Genbank)
Circaea alpina L.	AY357769[2]	USA, Smith 1052 (WIS)	+		Berry et al. 2004
	MH116584[2]	China, MLDP022B		+	Tan et al. 2018
Circaea lutetiana L.	GQ232527[2]	China, Wen10363	+		Xie et al. 2009
	MK519915[2]	U.S.A., Kriebel, R v0139816W1S		+	Direct submission
Clarkia amoena (Lehm.) A. Nelson & J. F. Macbr.	MF964077[2]	U.S.A., Gottlieb 8909 JEPS103040 (HV)	+		Unpubl. (Genbank)
	MF963711[2]	U.S.A., Keller s. n., JEPS117964		+	Unpubl. (Genbank)
Clarkia concinna (Fisch. & C. A. Mey.) Greene	MF964145[2]	U.S.A., Keller s. n., JEPS117964 (HV)	+		Unpubl. (Genbank)
	MF963755[2]	U.S.A., Keller s. n., JEPS117964 (HV)		+	Unpubl. (Genbank)
Fuchsia procumbens R. Cunn.	GQ232543[2]	China	+		Xie et al. 2009
	AJ581440[2]	United Kingdom, Chase 2501 K		+	Hilu et al. 2003
Fuchsia boliviana Carriere	GQ232536[2]	China	+		Xie et al. 2009
	HM851003[2]	Portugal, H. Schaefer, BM 2009/4		+	Schaefer et al. 2011
Ludwigia peploides (Kunth) P. H. Raven	AY271517[2]	Alameda Co., CA, Sytsma 5010 (WIS)	+		Levin et al. 2004
	KC996809[2]	U.S.A.		+	Armitage et al. 2013
Oenothera nuttallii Sweet	DQ075629[2]	U.S.A.	+		Unpubl. (Genbank)
	KT456914[2]	U.S.A., Steele 1322		+	Unpubl. (Genbank)
Oenothera suffrutescens (Ser.) W. L. Wagner & Hoch	MG236449[2]	Canada, CCDB-18332-C3	+		Kuzmina et al. 2017
	KT456909[2]	U.S.A., Steele 1322		+	Unpubl. (Genbank)