

## MOLECULAR PHYLOGENY OF SECTION *DROSANTHE* (SPACH) ENDL. (*HYPERICUM* L.) INFERRED FROM CHLOROPLAST GENOME

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### Abstract

The phylogenetic relationships of *Drosanthe* section of *Hypericum* genus (Hypericaceae) were analyzed by using non-coding chloroplast DNA region (*trnL* 3'-*trnF*) for 58 individuals. The section is represented by 23 taxa and nine of which are endemic to Turkey. The chloroplast phylogeny suggested that the members of this section belonged to a polyphyletic group, which imply at least two independent origins. The individuals of this section clearly formed two main clades. One clade included all members of this section except *H. amblysepalum*, *H. spectabile*, *H. lysimachoides* var. *spathulatum* and *H. sorgerae*. Our current phylogenetic results supported the morphological grouping in the *Drosanthe* section.

**Key words:** *Hypericum*, *Drosanthe*, Chloroplast DNA, *trnL* 3'-*trnF* region, Polyphyletic.

### Introduction

*Hypericum* L. (Hypericaceae) consists of about 500 species of shrubs, herbs and a few trees. Members of *Hypericum* are distributed worldwide, with a main center of species richness in the temperate regions of the Northern Hemisphere. In cold temperate regions *Hypericum* grows mainly in lowland and upland areas, while in the tropics and warm temperate areas it is almost always confined to high elevation mountain habitats, such as the Andes. *Hypericum* occurs in almost all kind of temperate habitats, but is rarely found in water other than in very shallow depths. Hypericaceae family belongs to the clusioid clade of the Malpighiales (Wurdack & Davis, 2009). The clusioid clade includes five families (Bonnetiaceae, Calophyllaceae, Clusiaceae, Podostemaceae, and Hypericaceae) represented by 94 genera and c. 1900 species (Ruhfel *et al.*, 2011). The eudicot order Malpighiales contains c. 16,000 species and is among the most diverse rosid clades (Korotkova *et al.*, 2009; Wurdack & Davis, 2009). Malpighiales constitute a large percentage of species in the shrub and small tree layer in tropical rain forests (Davis *et al.*, 2005). Most lineages within the Malpighiales remained restricted to tropical climates. Only a few lineages made it out of the tropics and have been successful in the northern temperate zone, including Violaceae (violets), Salicaceae (willows), and *Hypericum* (Donoghue, 2008).

Nine genera belong to Hypericaceae: *Cratoxylum* Blume, *Eliea* Cambess., *Harungana* Lamarck, *Hypericum* L., *Lianthus* N. Robson, *Santomasia* N. Robson, *Thornea* Breedlove & McClintock, *Triadenum* Rafinesque and *Vismia* Vand. *Hypericum* is one of nine genera forming the family Hypericaceae. Approximately 80% of the diversity of the family is within *Hypericum* (Crockett & Robson, 2011).

The first generic description of *Hypericum* is that of Tournefort (1700). However, it was validly published by Linnaeus (1753, 1754). The first treatment of the whole genus was done by Choisy (1821), whose synoptic monograph of the "Hypericineae" contained seven genera, of which three (*Androsaemum*, *Ascyrum* and

*Hypericum*) together represent *Hypericum* in its current sense, except that Choisy included the species placed by Robson (1977) in *Triadenum*. Robson (1977, 1981, 1985, 1987, 1990, 1996, 2001, 2002, 2010) has published in eight parts the most comprehensive monograph of *Hypericum* currently available. The monograph includes a revised infrageneric classification and a review of previously published classifications of the genus (Spach, 1836a-1836b; Jaubert & Spach, 1842-1843; Keller, 1895-1925; Kimura, 1951). Currently, 486 species have been recognized based on morphology, distribution and to a certain extent cytology and classified into 36 sections.

Species of the genus can be typically recognized by their leaves (opposite, simple and entire, lacking stipules), yellow flowers with free petals and several stamens in 3 or 5 fascicles, styles free, and the presence of pale and sometimes reddish to black glandular secretions (glands). The fruit is, in general, a dehiscent capsule, containing small cylindrical light brown to black seeds. For further and more comprehensive information about characters and descriptions of the genus, the reader is particularly referred to Robson (1981), Stevens (2007), Ernst (2003) and Judd *et al.* (2008). The typical habit in *Hypericum* is a shrub or a herb, each accounting for roughly 47% of the species of the genus. The yellow (flavonoid) colored petals are characteristic for *Hypericum* and are used to define borders of the genus (Robson, 1977). Within *Hypericum*, however, such modifications also occur. Pure white forms are very rare but have been recorded in *H. geminiflorum* from Taiwan and the Philippines (Luzon) and in *H. albiflorum* from Turkey. The red tinges (anthocyanin) usually occurring in dorsal parts of the petal are either confined to veins (*H. trichocoulon* from Crete), or are more or less diffused (Robson, 1981), resulting in a 'red-spotted' flower, in *H. revolutum*, or in a 'crimson-flowered' *Hypericum*, as recorded in *H. capitatum* var. *capitatum* from Turkey and Syria.

*Hypericum* has been accepted as a medicinal plant since it has antibacterial, antimicrobial, antidepressant and antioxidant activities due to the presence of hypericin and pseudohypericin. In addition, it has been an important raw

material in the flavour and fragrance industry as it contains essential oils. We also studied most of the essential oils belongs to this section (Bagci & Yuce, 2010; 2011).

Summaries of chromosome basic numbers in *Hypericum* indicate to form a descending series from 12-7 and counts of  $n=6$  have been made for *H. setosum* and *H. cumulicola*. Counts of  $n=9$  and 10 are most frequently reported for species with a shrubby habit, while  $n=7$  and 8 is most frequent for herbs. The ploidy level is generally diploid, but tetraploids (on base numbers  $n=8, 9, 10$ ) have been reported from several sections and hexaploids have been reported from several sections (Robson, 1981).

A dataset was assembled for all 591 taxa of *Hypericum* including 457 species, 70 subspecies, 13 varieties, 11 formae and 40 hybrids (Robson, 1977). In 1977, Robson divided the genus into 30 sections in his monograph, and then added different characters to species through the years, and overall the number of sections are increased to 36 (Carine & Christenhusz, 2010).

The genus *Hypericum* L. is represented in flora of Turkey by approximately 113 species of which 41.9% are endemic.

*Drosanthe* section members in Flora of Turkey except *H. sorgerae* and *H. davisii*, are placed in *Hirtella* section according to grouping based on the morphological treatments Robson (1977). Section in *Hirtella* species comprised of 30 species with the last revision (Robson, 2010). Taxa belonging to *Hirtella* section are widely distributed in Mediterranean, Turkey, the Crimea, the Caucasus, the Altai Mountains in Iraq. However, *H. hyssopifolium*, *H. asperulum*, *H. hirtellum*, *H. libanoticum* and *H. vermicular* mentioned in this section are not distributed in Turkey (Robson, 1977).

In the monographic study of *Hirtella* section carried by Robson (2010); *Hypericum elongatum* has been divided into 3 varieties (var. *antasiaticum*, var. *elongatum*, var. *lythrifolium*). *H. elongatum* subsp. *microcalycinum* and subsp. *apiculaticum*, *H. microcalycinum*. *H. apiculatum* was treated as separate species that was previously mentioned as subspecies of *H. elongatum*.

Currently, additional research on taxonomy of the genus *Hypericum* is being performed by several groups in Europe and North America applying molecular tools to further elucidate phylogenetic relationships within the genus (Crockett & Robson, 2011).

Recently, molecular data has given excellent insight and reliable phylogenetic trees in morphologically difficult groups (Shinwari *et al.*, 1994; 1994a& b). In the present study, we try to determine non-coding region (*trn* L3'-*trn*F) in cpDNA and to determine the phylogenetic relationships within the section and compare them with the generic patterns and outgroup *Hibiscus meyeri*.

## Materials and Methods

**Plant materials:** All the members of *Drosanthe* section were collected in flowering time (May-July) from natural habitats, and deposited in Firat University Herbarium (FUH). Three different populations or three different plant samples of each member of the section were used for this study to assess the clearest result. Leaves were stored in silica gel until DNA extraction. The name of the taxa, collection locations and GenBank accession numbers are listed in Table 1.

**Table 1. Plant species with their location, altitude information and GenBank accession numbers.**

Specimens	Location (altitude)-voucher	GenBank Acc. No.
<i>H. spectabile</i>	Kahramanmaraş (900 m)-GD 2202	KU324564
<i>H. amblysepalum</i>	Gaziantep (700-800 m)-EY 1037	KU324576
<i>H. lysmachioides</i> var. <i>lysismachioides</i>	Elazığ (1550 m)-EY 1025	KU324591
<i>H. lysmachioides</i> var. <i>spathulatum</i>	Elazığ (1000 m)-GD 2277	KU324597
<i>H. elongatum</i> var. <i>elongatum</i>	Kahramanmaraş (1000 m)-GD 2203	KU324561
<i>H. microcalycinum</i>	Elazığ (1400 m)-EY 1023	KU324603
<i>H. sorgerae</i>	Sivas (1350 m)-GD 2330	KU324606
<i>H. lydium</i>	Malatya (1100 m)-GD 2213	KU324555
<i>H. retusum</i>	Şanlıurfa (675 m)-GD 2271	KU324585
<i>H. pseudolaeve</i>	Malatya (1200 m)-EY 1061	KU324570
<i>H. helianthemoides</i>	Van (1990 m)-EB 2029	KU324579
<i>H. thymbrifolium</i>	Malatya (1500 m)-GD 2248	KU324549
<i>H. uniglandulosum</i>	Elazığ (1000 m)-GD 1334	KU324582
<i>H. salsolifolium</i>	Şanlıurfa (694 m)-EY 1047	KU324573
<i>H. capitatum</i> var. <i>capitatum</i>	Adıyaman (1200 m)-GD 2284	KU324588
<i>H. capitatum</i> var. <i>luteum</i>	Gaziantep (900 m)-GD 2212	KU324600
<i>H. scabroides</i>	Elazığ (1315 m)-GD 1413	KU324594
<i>H. scabrum</i>	Kahramanmaraş (950 m)-GD 2209	KU324558
<i>H. thymopsis</i>	Malatya (1500 m)-EY 1087	KU324567
<i>H. olivieri</i>	Malatya (1330 m)-GD 2272	KU324552
<b>Accession from the NCBI database (outgroup)</b>		
<i>Hibiscus meyeri</i>		KR738407.1

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

Region name	Primer name	DNA sequence
<i>trnL 3'-trnF</i> IGS	e (forward)	5' GGT TCA AGT CCC TCT ATC CC 3'
	f (reverse)	5' ATT TGA ACT GGT GAC ACG AG 3'

**DNA extraction, amplification of *trnL-F* region and sequencing:**

Total genomic DNA was extracted from silica gel-dried leaves using a modified version of the CTAB method (Doyle & Doyle 1987). Isolated genomic DNA was quantified by spectrophotometry. Then, each sample was diluted to 10 ng/uL for amplification of the desired region. The *trnL3'-trnF* region was amplified with one primer pair depicted in Table 2 (Taberlet *et al.*, 1991). PCR amplifications were performed in 50  $\mu$ L reaction volume with 5  $\mu$ L of 10x PCR Mg free buffer (Promega, Madison, WI, USA), 4  $\mu$ L of MgCl<sub>2</sub> (25 mM), 0.5  $\mu$ L of dNTP mixture (10 mM), 0.5  $\mu$ L of each of the primers (100  $\mu$ M), 0.18  $\mu$ L Taq DNA Polymerase, and approximately 50 ng template DNA. The PCR conditions were carried out as: 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 15 s, annealing 58°C for 30 s and elongation at 72°C for 45 s and a final extension at 72°C for 5 min. Purified PCR products were sequenced with ABI 310 Genetic Analyzer (PE Applied Biosystem) Automatic Sequencer in the RefGen Biotechnology Co., METU Teknokent (Ankara). The chromatogram data were opened and edited with the Finch TV (Version 1.4.0) manufactured by the Geopiza Research Team (Patterson *et al.*, 2004, 2006).

**Sequence alignment and phylogenetic analysis:** The nucleotide sequences were aligned with ClustalW multiple sequence alignment program. Molecular diversity parameters (total nucleotide length (bp), GC content (%), variable sites and parsimony informative sites) were calculated with the MEGA 6.0 software (Tamura *et al.*, 2013). Maximum Likelihood (ML) method with Tamura-Nei model (Tamura & Nei, 1993) were used to construct phylogenetic tree with 1000 bootstrap replicates. *Hibiscus meyeri* (with the accession number: KR738407) was selected as a potential out-group for the construction of the phylogenetic tree. The accession numbers of the samples uploaded in GenBank (NCBI) for present study are also given in Table 1.

**Results**

In this study of noncoding *trn L3'-trn F* region of cpDNA belongs to the 20 taxa of *Drosanthe* section (Fig. 1). Two main clades are differentiated (Fig. 1). At the beginning of the current study, it was decided and collected 23 (all) taxa of *Drosanthe* section. However, we could not amplify three of them during PCR studies due to their lack of DNA quality. These taxa were *H. davisii*, *H. apricum* and *H. apiculatum*.

The size of the *trn L3'-trn F* region ranged from 160-202bp. A total of 288bp final data set with gaps and missing data including the outgroup (*Hibiscus meyeri*) were composed of 106 variable site, 22 parsimony informative site (PI) and 30.8% GC content (Table 3). 22 PI was indicated in Table 4.

**Table 3. Molecular diversity parameters of individuals.**

Molecular diversity parameters	<i>trnL3'-trnF</i> Region
Number of taxa	20
Number of sequences	59
Total length (bp)	288
GC content (%)	30.8
Variable sites (V)	106
Parsimony informative sites (PI)	22

The phylogenetic tree constructed by using the ML method, displayed the polyphyly of the section *Drosanthe* (Fig. 1). Two main clades were formed in the tree. First clade consisted of the all taxa of the *Drosanthe* section except *H. spectabile*, *H. amblysepalum*, *H. lysimachoides* var. *spathulatum* and *H. sorgerae* taxa. A large deletion was observed between the region of 97th and 141th bp in the taxa of second clade. This 44 bp differences indicated the formation of this two main clades among the section of *Drosanthe*.

**Discussion**

The *trnL3'-trnF* sequence in the section *Drosanthe* give us an important information to clarify phylogenetic relationships among the morphologically different members of this section. According to the phylogenetic tree, there were two main clades. Fig. 1 illustrated that two clades were formed to show phylogenetic relationships within the *Drosanthe* section of the genus *Hypericum*. The first clade was composed of 16 taxa (*H. thymbrifolium*, *H. olivieri*, *H. lydium*, *H. scabrum*, *H. elongatum* var. *elongatum*, *H. thymopsis*, *H. pseudolaeva*, *H. salsolifolium*, *H. helianthemoides*, *H. uniglandulosum*, *H. retusum*, *H. capitatum* var. *capitatum*, *H. lysimachoides* var. *lysimachoides*, *H. capitatum* var. *luteum*, *H. scabroides* and *H. microcalycinum*) in *Drosanthe*, on the other hand, the second clade included the 4 taxa (*H. spectabile*, *H. amblysepalum*, *H. lysimachoides* var. *spathulatum* and *H. sorgerae*). Except *H. sorgerae*, other members of this clade were declared that similar by morphological characters as shown in some studies (Davis *et al.*, 1967; Robson, 1977; Yuce, 2009).

It is shown that the varieties of *H. lysimachoides*: *H. lysimachoides* var. *lysimachoides* and *H. lysimachoides* var. *spathulatum* were branched in two different clades (Fig. 1). The reason of this may derived from the clear morphological differences (sepal type, margin of the sepal and leaf type) among this two varieties.

Two subspecies of *Hypericum elongatum* (*H. elongatum* subsp. *microcalycinum* and *H. elongatum* subsp. *elongatum*) were grouped into two different branches. It was depicted by Robson that one was elevated to the rank of species namely, *H. microcalycinum* while the other was treated as variety *H. elongatum* var. *elongatum* (Robson, 2010). This situation supported that two varieties were grouped into two distant clades.

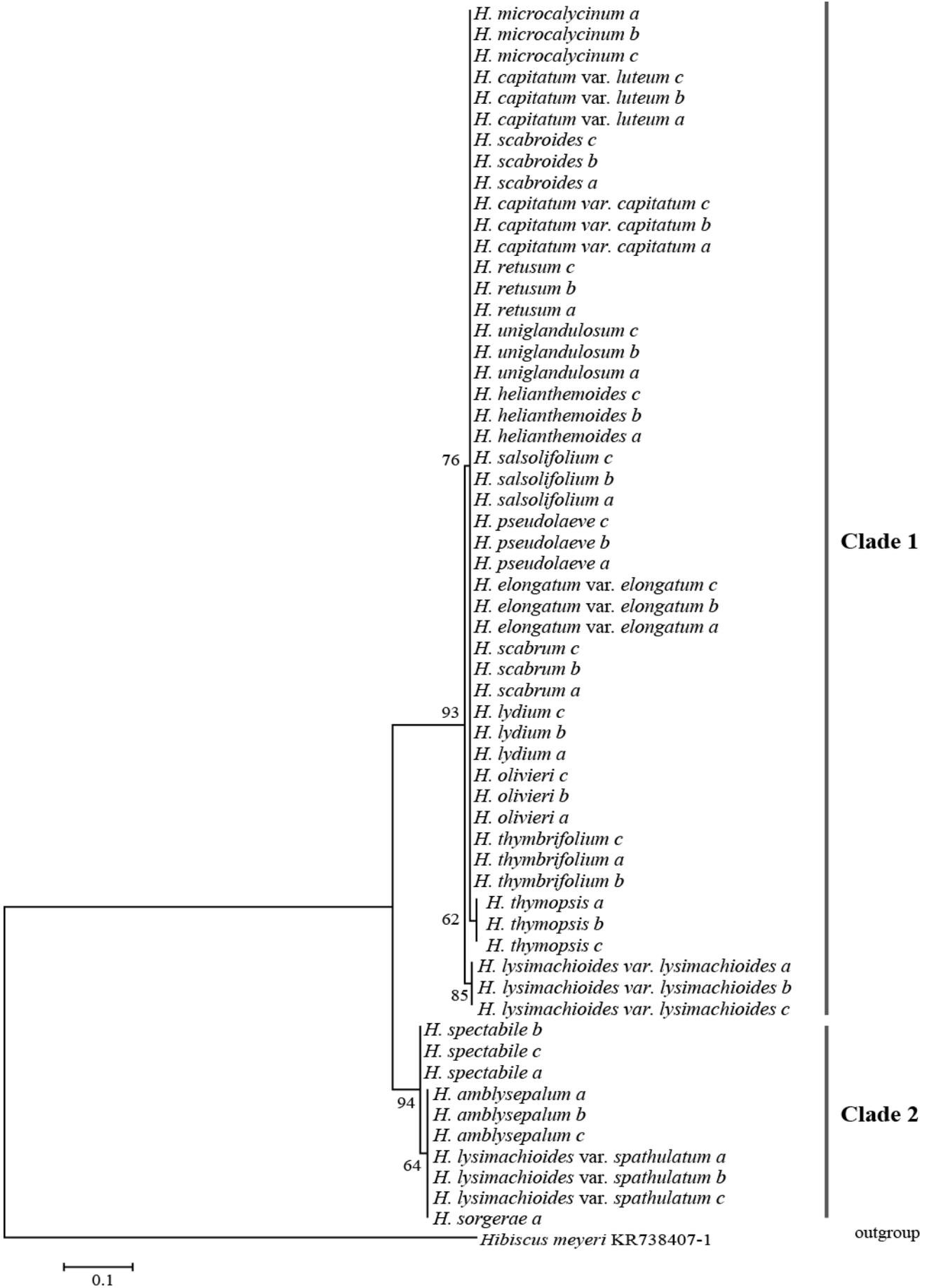


Fig. 1. The phylogenetic tree constructed using maximum likelihood method.

Table 4. The Parsimony-informative sites in the non-coding *trnL* 3'-*trnF* sequence of *Drosanthe* section.

Taxa	52	115	128	141	154	158	160	161	164	165	166	168	169	170	171	173	174	175	176	189	208	210	
<i>H. thymbrifolium a</i>	C	C	C	C	T	C	T	C	T	C	C	A	G	T	C	T	T	T	T	T	A	C	
<i>H. thymbrifolium b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. thymbrifolium c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. olivieri a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. olivieri b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. olivieri c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. lydiium a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. lydiium b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. lydiium c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. scabrum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. scabrum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. scabrum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. elongatum var. elongatum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. elongatum var. elongatum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. elongatum var. elongatum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. spectabile a</i>	.	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	
<i>H. spectabile b</i>	.	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	
<i>H. spectabile c</i>	.	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	
<i>H. thymopsis a</i>	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. thymopsis b</i>	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. thymopsis c</i>	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>H. pseudolaeva a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. pseudolaeva b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. pseudolaeva c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. salsolifolium a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. salsolifolium b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. salsolifolium c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.
<i>H. amblysepalum a</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	
<i>H. amblysepalum b</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	
<i>H. amblysepalum c</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	C	-	-	

Table 4. (Cont'd.).

Taxa	52	115	128	141	154	158	160	161	164	165	166	168	169	170	171	173	174	175	176	189	208	210	
<i>H. helianthemoides a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
<i>H. helianthemoides b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
<i>H. helianthemoides c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
<i>H. uniglandulosum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. uniglandulosum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. uniglandulosum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. retusum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. retusum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. retusum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. capitatum var. capitatum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. capitatum var. capitatum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. capitatum var. capitatum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
<i>H. lysimachiooides var. lysimachiooides a</i>	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. lysimachiooides var. lysimachiooides b</i>	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. lysimachiooides var. lysimachiooides c</i>	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. scabroides a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. scabroides b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. scabroides c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. lysimachiooides var. spathulatum a</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	-	-	-	-
<i>H. lysimachiooides var. spathulatum b</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	-	-	-	-
<i>H. lysimachiooides var. spathulatum c</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	-	-	-	-
<i>H. capitatum var. luteum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
<i>H. capitatum var. luteum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
<i>H. capitatum var. luteum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
<i>H. microcalycinum a</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. microcalycinum b</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. microcalycinum c</i>	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
<i>H. sorgerae a</i>	A	.	.	T	A	A	G	T	G	A	A	G	A	C	G	C	C	C	C	-	-	-	-

Nine taxa out of twenty three were endemic and these were *H. scabroides*, *H. spectabile*, *H. sorgerae*, *H. thymopsis*, *H. pseudolaeve*, *H. salsolifolium*, *H. thymbrifolium*, *H. uniglandulosum* and *H. capitatum*. In respect to the morphological features, *H. spectabile* was so close to *H. amblysepalum*, *H. lysimachioides* var. *spathulatum* and same result was found in present study. Taxon *H. pseudolaeve* had close relationship with *H. thymbrifolium*, *H. helianthemoides* and *H. olivieri* additionally, *H. uniglandulosum* was closely related with *H. salsolifolium*. With regard to our current phylogenetic tree, all these taxa were grouped in the same clade and this indicated that both morphological and present molecular results has congruency by each other.

In the study of genus *Hypericum*, nuclear ribosomal DNA internal transcribed spacer sequences were analyzed among 36 species of *Hypericum* as ingroup and two species *Thornea* as outgroup. This sampling included most of the previously described species from Korea and Japan. The ITS phylogeny suggested that the surveyed *Hypericum* species belong to a monophyletic section, *Trigynobrathys*, and a polyphyletic section, *Hypericum*. In addition, two monotypic sections, *Sampsonia* and *Roscyna*, were identified. Members of section *Hypericum* occur in four different lineages worldwide, which imply at least four independent origins (Park & Kim, 2004). With respect to the study on the phylogenetic analysis of the genus *Hypericum*, the members of the section *Hirtella* (*Drosanthe*), namely, *H. scabrum*, *H. scabroides*, *H. thymbrifolium*, *H. pseudolaeve*, *H. davisii* ve *H. elongatum* subsp. *lythrifolium* were grouped in the same clade as such in our study (Nurk *et al.*, 2013). Moreover, both morphological and molecular results obtained from the study conducted by Nurk *et al.* the section *Hirtella* covering the members of section *Drosanthe* supports current results (2013). It is possible to say that phylogenetic tree and morphological data (Yuce, 2009) and the literature review have supported that *Drosanthe* section may be divided at least into two groups in the *Drosanthe* or *Hirtella* sections within the genus *Hypericum*. The results are concordant to earlier reports (Shinwari, 1995; Mahmood *et al.*, 2010).

### Acknowledgement

This study has been funded by the FUBAP (project number FF.11.09).

### References

- Bagci, E. and E. Yuce. 2010. The essential oils of the aerial parts of two *Hypericum* (*H. pseudolaeve* Robson and *H. thymbrifolium* Boiss. & Noë) species from east anatolian region of Turkey. *JEOBP*, 18(3).
- Bagci, E. and E. Yuce. 2011. Composition of the essential oil of *Hypericum salsolifolium* Hand. Mazz. and *Hypericum retusum* Aucher from Turkey. *Acta Bot. Gall.*, 158(2): 169-173.
- Carine, M.A. and M.C.M. Christenhusz. 2010. About this volume: the monograph of *Hypericum* by Norman Robson, Department of Botany, Natural History Museum. *Phytotaxa*, 4: 1-4.
- Choisy, J.D. 1821. *Prodromus d'une Monographie de la Famille des Hypericinées*. Genève, Paris: J.J. Paschoud, Imprimeur-Libraire.
- Crockett, S.L. and K.B.N. Robson. 2011. Taxonomy and chemotaxonomy of the genus *Hypericum*. *Med. and Aro. Plant Sci. and Biotech.*, 5: 1-13.
- Davis, C.C., C.O. Webb, K.J. Wurdack, C.A. Jaramillo and M.J. Donoghue. 2005. Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests. *American Naturalist*, 165: 36-65.
- Davis, P.H., R.R. Mill and K. Tan. 1967. *Flora of Turkey and The East Aegean Island*. (2<sup>nd</sup> Ed) Edinburgh University Press, London.
- Donoghue, M.J. 2008. A phylogenetic perspective on the distribution of plant diversity. *Procee. of the Nat. Aca. of Sci.*, 105: 11549-11555.
- Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, 19: 11-15.
- Ernst, E. 2003. *Hypericum. The genus Hypericum*. London, New York: Taylor and Francis.
- Jaubert, C. and E. Spach. 1842-1843. *Illustrationes Plantarum Orientalium 1. Roret Bibliopolam*, Paris.
- Judd, W.S., C.S. Campbell, E.A. Kellogg, P.F. Stevens and M.J. Donoghue. 2008. *Plant systematics. A Phylogenetic Approach*. (3<sup>rd</sup> Ed) Sunderland: Sinauer Associates.
- Keller, R. 1895. *Hypericum*. In: Engler A. and Prantl, K. *Die natürlichen Pflanzenfamilien*. Vol. 3. Leipzig: Engelmann. pp. 208-215.
- Keller, R. 1925. *Hypericum*. In: Engler, A. and Prantl, K. *Die natürlich Pflanzenfamilien*. 2nd ed. 21. pp. 175-183.
- Kimura, Y. 1951. *Hypericaceae*. In: T. Nakai and M. Honda [eds]. *Nova Flora Japonica*. Vol. 10. Tokyo: Sanseido.
- Korotkova, N., J.V. Schneider, D. Ouadant, A. Worberg, G. Zizka and T. Borsch. 2009. Phylogeny of the eudicot order Malpighiales: analysis of arecalcitrant clade with sequences of the petD group II intron. *Plant Syst. and Evo.*, 282: 201-228.
- Linnaeus, C. 1753. *Species Plantarum*, 2:783. Laurentius Salvius, Stockholm.
- Linnaeus C. 1754. *Genera Plantarum*, 5: 362, 368-370. Stockholm.
- Mahmood, T., S. Muhammad and Z.K. Shinwari. 2010. Molecular and morphological characterization of *Caralluma* species. *Pak. J. Bot.*, 42(2): 1163-1171.
- Nurk, N.M., S. Madrinan, M.A. Carine, M.W. Chase and F.R. Blattner. 2013. Molecular phylogenetics and morphological evolution of St. Jhon's wort (*Hypericum*; Hypericaceae). *Molecular Phyl. and Evol.*, 66: 1-16.
- Park, S.J. and K.J. Kim. 2004. Molecular phylogeny of the genus *Hypericum* (Hypericaceae) from Korea and Japan: Evidence from nuclear rDNA ITS sequence data. *J. of Plant Bio.*, 47(4): 366-374.
- Patterson, J., B. Chamberlain and D. Thayer. 2004-2006. Finch TV Version 1.4.0
- Robson, N.K.B. 1977. Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. *Bull. of the Brit. Mus. (Nat. Hist.) Bot.*, 5: 291-355.
- Robson, N.K.B. 1981. Studies in the genus *Hypericum* L. (Guttiferae): 2. Characters of the genus. *Bull. of the Brit. Mus. (Nat. Hist.) Bot.*, 8(2): 55-226.
- Robson, N.K.B. 1985. Studies in the genus *Hypericum* L. (Guttiferae): 3. Sections: 1. *Campylosporus* to: 6a. *Umbraculoides*. *Bull. of the Brit. Mus. (Nat. Hist.) Bot.*, 12(4): 1-325.
- Robson, N.K.B. 1987. Studies in the genus *Hypericum* L. (Guttiferae): 7. Section 29. *Brathys* (part 1). *Bull. of the Brit. Mus. (Nat. Hist.) Bot.*, 16(1): 1-106.

- Robson, N.K.B. 1990. Studies in the genus *Hypericum* L. (Guttiferae): 8. Sections 29. Brathys (part 2) and 30. Trigynobrathys. *Bull. of the Brit. Mus. (Nat. Hist.). Bot.*, 20(1): 1-151.
- Robson, N.K.B. 1996. Studies in the genus *Hypericum* L. (Guttiferae): 6. Sections 20. Myriandra to 28. Elodes. *Bull. of the Brit. Mus. (Nat. Hist.). Bot.*, 26(2): 75-217.
- Robson, N.K.B. 2001. Studies in the genus *Hypericum* L. (Guttiferae) 4(1). Sections 7. Roscyna to 9. *Hypericum* sensu lato (part 1). *Bull. of the Brit. Mus. (Nat. Hist.). Bot.*, 31(2): 37-88.
- Robson, N.K.B. 2002. Studies in the genus *Hypericum* L. (Guttiferae) 4(2). Section 9. *Hypericum* sensu lato (part 2): subsection 1. *Hypericum* series 1. *Hypericum*. *Bull. of the Brit. Mus. (Nat. Hist.). Bot.* 32(2): 61-123.
- Robson, N.K.B. 2010. Studies in the genus *Hypericum* L. (Hypericaceae) 5(2). Sections 17. *Hirtella* to 19. *Coridium*. *Phytotaxa*, 4: 127-258.
- Ruhfel, B., V. Bittrich, C. Bove, M. Gustafsson, C. Philbrick, R. Rutishauser, Z. Xi and C. Davis. 2011. Phylogeny of the clusioid clade (Malpighiales): evidence from the plastid and mitochondrial genomes. *American J. of Bot.*, 98: 306-325.
- Shinwari, Z.K. 1995. Congruence between morphology and molecular phylogenies in *Prosartes* (Liliaceae). *Pak. J. Bot.*, 27(2): 361-369.
- Shinwari, Z.K., R. Terauchi and S. Kawano 1994b Phylogenetic relationships among genera in the Liliaceae-Asparagoideae-Polygonatae sensu lato inferred from rbcL gene sequence data. *Pl. Systematic & Evolution*, 192: 263-277.
- Shinwari, Z.K., R. Terauchi and S. Kawano. 1994. Molecular systematics of liliaceae-asparagoideae-polygonatae. 1. RFLP analysis of cpDNA in several species of asiatic disporum species. *Plant Species Bio.*, 9: 11-18.
- Shinwari, Z.K., R. Terauchi, F.H. Utech and S. Kawano. 1994a. Recognition of the New World Disporum Section *Prosartes* as *Prosartes* (Liliaceae) based on the sequence data of the rbcL gene. *Taxon*, 43(3): 353-366.
- Spach, E. 1836a. Conspectus monographiae Hypericacearum. *Annales des Sci. Naturelles (Botanique) II*, 5: 349-369.
- Spach, E. 1836b. Hypericacearum monographiae fragmenta. *Annales des Sci. Naturelles (Botanique) II*, 5: 157-176.
- Stevens, P.F. 2007. *Clusiaceae-Guttiferae* In: Kubitzki K(Ed) *The Families and Genera of Vascular Plants*. Springer, IX, 48-66, Berlin, Germany.
- Taberlet, P., L. Gielly, G. Patou and J. Bouvet. 1991. Universal Primers amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol. Int. J. Mol. Biol. Biochem. Genet. Eng.*, 17: 1105-1109.
- Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.*, 10: 512-526.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 30(12): 2725-2729.
- Tournefort, J.P. 1700. *Institutiones Rei Herbariae*. Paris: Typographia Regia J. Anisson. [www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)
- Wurdack, K.J. and C.C. Davis. 2009. Malpighiales phylogenetics: Gaining ground one of the most recalcitrant clades in the angiosperm tree of life. *American J. of Botany*, 96: 1551-1570.
- Yuce, E. 2009. *Hypericum* L. (Hypericaceae) cinsine ait *Drosanthe* (Spach) Endl. seksiyonunun biyosistematiği. *Doktora Tezi, Fırat Üniversitesi, Fen Bilimleri Enstitüsü*, Elazığ.

(Received for publication 20 August 2016)