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PHYLOGENETIC CLASSIFICATION OF SOME TAXA OF *HYPERICUM* L. FROM TURKEY, INFERRED FROM NRDNA ITS AND CPDNA TRNL 3ˈ–TRNF SEQUENCES

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Abstract

This study revealed the phylogenetic relationships within 26 taxa of *Hypericum* L., a genus well represented in the Turkish flora, utilizing nrDNA ITS and cpDNA *trnL* 3ˈ–*trnF* regions. Among the studied species, seven of them are endemic to Turkey, including *H. confertum* subsp. *stenobotrys* (Boiss.) Holmboe, *H. ekerii* Yüce, Aytaç & Pınar, *H. elongatum* var. *antasiaticum* (Grossh.) N. Robson , *H. pseudolaeve* N. Robson , *H. scabroides* N. Robson & Poulter, *H. thymbrifolium* Boiss. & Noë and *H. uniglandulosum* Hausskn. ex Bornm. Molecular phylogenetic analyses were performed using the Maximum Likelihood (ML) methods. The ITS dataset resolved three distinct clades. Clade I comprised species from *Hypericum* sect. *Hirtella* and sect. *Taeniocarpium*. Clade II encompassed taxa from sect. *Drosocarpium*, sect. *Hypericum*, and sect. *Crossophyllum*. Clade III was represented by sect. *Androsaemum* (only *H. hircinum*). The *trnL*-3ˈ *trnF* phylogenetic tree largely aligns with the ITS phylogeny, exhibiting similar major clades. However, *H. hircinum* L. did not form a distinct clade as observed in the ITS tree, and discrepancies were also noted at lower taxonomic levels. A strongly monophyletic and recently diverged lineage was affirmed for *H. lysimachioides* var. *spathulatum* N. Robson and *H. lysimachioides* Boiss. & Noë var. *lysimachioides*, and no molecular differentiation was observed between these two varieties. Significantly, *H. ekerii*, an endemic and critically endangered species, was included for the first time in a molecular phylogenetic study, clustering within Clade I.

Key words: Phylogeny, *Hypericum*; ITS; *trnL* 3ˈ–*trnF*

Introduction

The family Hypericaceae comprises 8 genera: *Vismia* Vand., *Triadenum* Rafinesque, *Harungana* Lamarck, *Thornea* Breedlove & McClintock, *Hypericum* L., *Cratoxylum* Blume, *Lianthus* N. Robson , and *Eliea* Cambess (Robson, 2012). The family comprises taxa characterized by translucent, essential oil-containing glands, occasionally alongside red or black secretory glands that may contain hypericin (Davis, 1967). The most prominent taxonomic characteristic of the Hypericaceae family is the morphological manifestation of schizogenous secretory canals as translucent glands in the leaves of all its species. These secretory structures have been reported to occur in the phloem, primary cortex, pericycle, central cylinder, petiole, and leaf veins (Metcalfe & Chalk, 1950).

The genus *Hypericum*, belonging to the Hypericaceae family, is a taxon widely distributed almost globally, with the exception of Antarctica, and is particularly concentrated in Europe, Asia, and North Africa. It has been reported that this genus is represented by 469 taxa worldwide (Walker *et al.,* 2001; Crockett & Robson, 2011). This genus comprises glabrous or simple uniseriate-hairy, often annual or perennial herbs, shrubs, and trees with green, yellowish-brown, or reddish stems. They are glandular or possess glands ranging from pale to dark in color, or are gland-bearing (glandiferous), exhibiting a cosmopolitan distribution in diverse temperate habitats and high mountains in tropical regions (Davis, 1967; Crockett & Robson, 2011).

Hypericum taxa are widely used in folk medicine, have been reported to be extensively utilized for various purposes, particularly in the treatment of depression. This is attributed to their rich content of naphthodianthrones such as hypericin and pseudohypericin, as well as phloroglucinol, flavonoids, biflavonoids, catechins, condensed tannins, phenolic acids, xanthones, vitamins A and C, and essential oils (Bilia *et al*., 2002; Pellati *et al*., 2005; Hostettmann & Wolfender, 2005; Varel, 2011; Çırak & Kurt, 2014).

The most comprehensive monograph of *Hypericum* genus currently available was authored by Robson, published in eight parts across multiple years (1967, 1977, 1981, 1985, 1987, 1990, 1996, 2001, 2002, 2010). The genus *Hypericum* has been divided into 36 sections by recent monographs. In the Flora of Turkey, this genus is represented by 111 taxa comprising fewer than 20 sections, with an endemism rate determined as 48% (Güner *et al*., 2000; Güner *et al*., 2012; Fırat & Eroğlu, 2023). Given its intercontinental geographical position, its location at the intersection of three distinct phytogeographical regions (Euro-Siberian, Mediterranean, and Irano-Turanian), its diverse topographical features, and the prevalence of various climates, Turkey possesses an exceptionally rich flora. For these reasons, it also serves as a significant gene center for *Hypericum* taxa (Avcı, 2014; Fırat & Eroğlu, 2023).

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The Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA) is a highly favored genetic marker in phylogenetic studies of *Hypericum* genus due to its ideal combination of practical utility and evolutionary characteristics, making it excellent for resolving relationships at lower taxonomic levels. Its high copy number within the genome ensures easy and reliable amplification from even small or degraded plant samples, which is crucial for comprehensive sampling across a large and diverse genus such as *Hypericum*. Furthermore, ITS sequences evolve rapidly, providing sufficient genetic variation to discern phylogenetic relationships among closely related species and within subgenera, something slower-evolving genes often cannot achieve. This rapid evolution, combined with the availability of universal PCR primers (White *et al*., 1990; Gardes & Bruns, 1993) that work across a broad range of plant taxa, simplifies laboratory procedures for researchers studying *Hypericum* taxa worldwide. These attributes collectively make ITS an invaluable tool for clarifying species boundaries, refining infrageneric classifications, and reconstructing the evolutionary history of *Hypericum* (Nürk *et al*., 2013). Similarly, the cpDNA *trnL* 3'–*trnF* region is a highly informative marker for phylogenetic studies. This non-coding region's relatively rapid evolutionary rate allows for resolving relationships among closely related species (Gielly & Taberlet, 1994). It exhibits sufficient sequence variation for species distinction while remaining conserved enough for broad taxonomic alignment (Taberlet *et al.,* 1991). As part of the maternally inherited chloroplast genome, *trnL* 3'–*trnF* simplifies analyses by avoiding recombination complexities. Studies utilizing *trnL* 3'–*trnF* have provided critical insights into evolutionary history of *Hypericum*, helping to resolve taxonomic ambiguities and identify monophyletic groups (Robson, 2012). Both ITS and *trnL* 3'–*trnF* remain cornerstones in unraveling the evolutionary intricacies of this fascinating plant genus.

It has been reported that the distribution and configuration of glands are important in the taxonomy of the genus *Hypericum* (Robson, 1967). These morphological characters have been strongly emphasized in the descriptions of species found in the Flora of Turkey. For this reason, researchers engaged in the species identification of the genus *Hypericum* face difficulties and sometimes arrive at incorrect conclusions. This study aims to overcome the difficulties encountered during species identification and to re-evaluate the taxonomy of the genus *Hypericum* using modern molecular methods. This approach elucidates its phylogeny and provides a comprehensive perspective on the phylogenetic relationships within the genus, building upon previous research and notably incorporating certain taxa for the first time in molecular analyses.

Materials and Methods

Plant materials: Plant materials were collected from natural habitats and identified by Prof. Dr. M. Kurşat based on the Flora of Turkey and East Aegean Islands (Davis, 1965-1985). Voucher specimens were deposited in Biology Laboratory of Bitlis Eren University. Leaves were stored in silica gel until DNA extraction. The names of the taxa and collection locations are listed in Table 1.

DNA extraction, amplification and sequencing: Total genomic DNA was extracted from dried leaves of collected specimens in the wild using the protocol of the Hibrigen® plant genomic DNA isolation kit. According to the procedure, 100 mg of plant sample was homogenized with liquid nitrogen. The buffer solutions provided in the kit were used in accordance with the procedure. The obtained DNA samples were stored at -20°C until further used. Amplification of the whole region of nrDNA ITS was performed using the ITS AB101 and ITS AB102 (Douzery *et al*., 1999) and ITS5 and ITS4 universal primers in some cases (White *et al.,* 1990) and amplification of *trnL* 3'–*trnF* region was performed with primers B49317 and A49855 protocol by Taberlet *et al*., (1991). For PCR product purification, the MAGBIO 'HighPrep™ PCR Clean-up System' (AC-60005) purification kit was used for single-band samples, following the manufacturer's procedures. Sanger sequencing sample analyses were performed using ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) at BMLabosis (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).

Alignment and phylogenetic analyses: Alignment was undertaken using a data set with ClustalW (Thompson, 1994) software and subsequently checked visually. Indels were excluded from the final datasets. Variable sites, number of parsimony-informative sites, transition, transversion, genetic distance, nucleotide diversity, and divergence within species were computed as molecular diversity statistics for each dataset using Molecular Evolutionary Genetics Analysis software (MEGA 12.0) (Kumar *et al*., 2024). Ultimately, phylogenetic trees were constructed using the Maximum Likelihood method.

Results and Discussion

In this study, the nrDNA ITS and cpDNA *trnL* 3'–*trnF* regions were analyzed for 26 *Hypericum* taxa. *H. karjaginii* could not be evaluated because PCR studies with primers for *trnL* 3'–*trnF* region didn't work.

The total size of the ITS region was 639 bp composed of 152 variable sites, 63 parsimony informative sites, and 57.6% GC content. The total size of the *trnL* 3'–*trnF* region was 255 bp with gaps was composed of 129 variable sites, 99 parsimony informative sites, and 31.9% GC content (Table 2).

The phylogenetic tree constructed using ITS data and the Maximum Likelihood (ML) method from *Hypericum* taxa revealed three main clades (Fig. 1). Clade I comprised 15 taxa belonging to *Hypericum* sect. *Hirtella* (*H. lysimachioides* Boiss. & Noë var. *lysimachioides*, *H. lysimachioides* var. *spathulatum* N. Robson *,* *H. scabroides* N. Robson & Poulter, *H. helianthemoides* (Spach) Boiss., *H. scabrum* L., *H. microcalycinum* Boiss .& Heldr., *H. pseudolaeve* N. Robson , *H. thymbrifolium* Boiss. & Noë , *H. davisii* N. Robson , *H. elongatum* (Grossh.) N. Robson var. *elongatum*, *H. elongatum* var. *antasiaticum* (Grossh.) N. Robson , *H. ekerii* Yüce, Aytaç & Pınar,, *H. karjaginii* Rzazade, *H. lydium* Boiss. and *H. uniglandulosum* Hausskn. ex Bornm.) and 4 taxa belonging to sect. *Taeniocarpium* (*H. confertum* subsp. *Stenobotrys* (Boiss.) Holmboe, *H. armenum* Jaub. & Spach subsp. *armenum, H. linarioides*

Bosse subsp. *linarioides* and *H. venustum* Fenzl). Clade II included 2 taxa from sect. *Drosocarpium* (*H. bithynicum* Boiss. and *H. montbretii* Spach), 3 taxa from sect. *Hypericum* (*H. triquetrifolium* Turra, *H. perforatum* L. subsp. *perforatum and H. tetrapterum* Fr. var. *tetrapterum*), and one species from sect. *Crossophyllum* (*H. orientale* L.). Clade III contained a single species, *H. hircinum*, L. belonging to the sect. *Androsaemum*.

In the phylogenetic tree based on *trnL* 3'–*trnF* data, 2 clades were identified (Fig. 2). These 2 clades comprised the same taxa as those found in the first 2 clades of the ITS data-derived phylogenetic tree. However, the third clade identified in the ITS phylogenetic tree containing *H. hircinum* from sect. *Androsaemum*, was not observed in the *trnL* 3'–*trnF* tree. Instead, *H. hircinum* was placed within clade II, which was observed to further divide into two subclades. The first of these subclades contained taxa belonging to sections *Drosocarpium*, *Hypericum*, and *Crossophyllum*, while the second subclade exclusively included *H. hircinum* from sect. *Androsaemum*.

Seven of the 26 taxa used in this study were endemic (*H. confertum* subsp. *stenobotry*s, *H. ekerii*, *H. elongatum* var. *antasiaticum*, *H. pseudolaeve*, *H. scabroides*, *H. thymbrifolium* and *H. uniglandulosum*) (Davis *et al.,* 1967; Ekim *et al.,* 2000; Güner *et al*., 2000; Babacan *et al.,* 2017).

The IUCN conservation status of the included *Hypericum* species, as determined by the Turkish Plant Red Data Book, showed varying levels of risk. *H. davisii, H. microcalycinum*, and *H. lysimachioides* var. *lysimachioides* are classified as Near Threatened (*NT*). Meanwhile, *H. lydium* and *H. scabrum* are designated as Least Concern (*LC*), suggesting stable populations. *H. pseudolaeve* is categorized as Vulnerable (*VU*), indicating a higher degree of concern. Most critically, *H. helianthemoides*, *H. thymbrifolium*, *H. scabroides*, and *H. uniglandulosum* are all listed as Endangered (*EN*), highlighting their significant threat of extinction in Turkey (Ekim *et al*., 2000). The current study included several taxa whose ITS and *trnL* 3'–*trnF* sequences namely *H. armenum* subsp. *armenum*, *H. ekerii*, *H. karjaginii*, *H. lydium, H. lysimachioides* var. *spathulatum*, *H. microcalycinum*, and *H. uniglandulosum* had not been included in previous comprehensive studies (Meseguer *et al*., 2013; Nürk *et al*., 2013).

Table 1. The collection data of investigated *Hypericum* L. specimens.

Taxa	Locality, voucher and specimen code
H. hircinum L.	Gaziantep province, İslahiye district, Koccağız village, 1020 m, 19.06.2006, S. Çakır 902
H. lysimachioides Boiss. & Noë var. *lysimachioides*	Van province, Kuzgun Kıran pass, 1800 m, 14.06.2022, M. Kurşat 7068
H. lysimachioides var. *spathulatum* N. Robson	Bitlis province, Kambos mountain, 1750 m, 16.07.2022. M. Kurşat 7063
H. elongatum (Grossh.) N. Robson var. *elongatum*	Elazığ province, Baskil district, Doğançık village, 1800 m, 03.06.2021, M. Kurşat 7076
H. elongatum var. *antasiaticum* (Grossh.) N. Robson	Van province, Güzeldere pass, Western slope, 2600m, 04.07.20022, M. Kurşat 7082
H. karjaginii Rzazade	Sivas province, Between Hekimhan and Kangal district, 38.km, 1500-1600 m, 30.06.2008, E. Yüce 1096
H. microcalycinum Boiss. & Heldr.	Elazığ province, Baskil district, Haroğlu Village, 1450 m. 03.06.2021, M. Kurşat 7072
H. davisii N. Robson	Van province, Güzeldere pass, Eastern slope, 2700 m, 04.07.20022, M. Kurşat 7083
H. lydium Boiss.	Van province, Peli mountain, 2700 m, 14.06.2021, M. Kurşat 7070
H. pseudolaeve N. Robson	Malatya province, Between Malatya and Gölbaşı district, 50.km, 860 m, 02.06.2008, E.Yüce 1052
H. helianthemoides (Spach) Boiss.	Van province, Van-Gürpınar district fork in the road, Koyunyatağı village, 1750 m, 04.07.2022, M. Kurşat 7085
H. thymbrifolium Boiss. & Noë	Malatya province, Between Malatya and Darende district, 50. km, 1500 m, 29.06.2008, E.Yüce 1084
H. uniglandulosum Hausskn. ex Bornm.	Elazığ province, Hasan mountain, 2100 m, 28.07.2022, M. Kurşat 7073
H. ekerii Yüce, Aytaç & Pınar	Tunceli province, Mazgirt district, c. 21 km E of Tunceli, c. 3 km SE of Çevrecik, Munzur Mountain ranges, 1800–2060 m, 11.06.2015, E. Yüce 2246
H. scabroides N. Robson & Poulter	Elazığ province, Baskil district, Doğançık Village, around Bolucuk town, 1480 m, 03.06.2021, M. Kurşat 7074
H. scabrum L.	Elazığ province, Baskil district, Doğançık Village, around Bolucuk town, 1530 m, 03.06.2021, M. Kurşat 7075
H. confertum subsp. *stenobotrys* (Boiss.) Holmboe	Gaziantep province, İslahiye district, Köklü village, 840 m, 22.04.2006, S. Çakır 460
H. venustum Fenzl	Bitlis; Tatvan district and Van, 15. km, 1900 m, 09.07.2021, M. Kurşat 7066
H. linarioides Bosse subsp. *Linarioides*	Erzurum province, Şenkaya district, Gülvere village, 2500 m, 20.08.1980, Y. Altan 2913
H. armenum Jaub. & Spach subsp. *Armenum*	Muş province, Kurtik mountain, 2450 m, 27.04.2018, M. Kurşat 7056
H. montbretii Spach	Osmaniye province, Zorkun plateau, in the Forest, 1650 m, 22.06.2021, M. Kurşat 7059
H. bithynicum Boiss.	Rize province, Çamlıhemşin district, Between Ortayayla village and Başköy village, Wetland,, 2100 m, 01.07.2021, M. Kurşat 7067
H. orientale L.	Rize province, Çamlıhemşin district, Between Ortayayla village and Başköy village, Wetlands, 2160 m, 01.07.2021, M. Kurşat 7060
H. tetrapterum Fr. var. *tetrapterum*	Malatya province, Kubbe mountain, stream, 2000 m, 29.07.1981, Y. Altan 1962
H. perforatum L. subsp. *perforatum*	Elazığ province, Baskil district, Doğançık Village, around Bolucuk town, 1530 m, 03.06.2021, M. Kurşat 7077
H. triquetrifolium Turra	Elazığ province, Baskil district, 1300 m, 28.07.2021, M. Kurşat 7065

Table 2. Molecular diversity parameters of ITS and *trnL* 3ˈ–*trnF* sequences.

Molecular diversity parameters	ITS region	*trnL* 3ˈ–*trnF*
Total number of sites	639	255
Parsinomy-informative sites (PI)	63	99
Sigleton sites (S)	86	30
Variable sites (V)	152	129
Conserved sites (C)	476	100
GC content (%)	57.6	31.9

A molecular phylogenetic analysis conducted by Nürk *et al*., (2013), employing the complete ITS region data, showed that members of sect. *Androsaemum* were grouped under the *Hypericum* Mediterranean II group, while members of sect. *Hypericum*, sect. *Crossophyllum*, sect. *Hirtella*, sect. *Drosocarpium* and sect. *Taeniocarpium* formed part of the core *Hypericum* group (Nürk *et al*. 2013). Meseguer *et al*., (2013) delineated clades A-E within *Hypericum* based on phylogenetic analysis using ITS and various cpDNA markers across diverse sections. Their study placed *Hirtella* group (encompassing species from sections *Coridium, Monanthema, Inodora, Triadenioides, Taeniocarpium,* and *Hirtella*) and *Hypericum*-group (accomodating species from sections *Crossophyllum, Drosocarpium,* and *Hypericum*) within clade E. The sections containing *H. hircinum* (sect. *Androsaemum*) were positioned in clade C (Crockett *et al.,* 2004; Meseguer *et al*., 2013). Our phylogenetic analysis based on ITS distinctly resolved three significant clades, which clearly delineated the evolutionary relationships among the taxa examined. As a result, these findings are in concordance with previous studies. The *trnL* 3ˈ–*trnF* phylogenetic tree largely aligned with the ITS phylogeny, showing similar main clades and inter-sectional connections. The only difference was *H. hircinum*, which appeared as a distinct subclade within clade II. Despite this overall agreement, we observed some inconsistencies between the ITS and *trnL* 3ˈ–*trnF* phylogeny at low taxonomic levels. Several reasons could account for these differences: hybridization, incomplete lineage sorting positive selection, and paralogy. Additionally, the ITS marker itself can be prone to homoplasy because of significant sequence variation, compensatory base changes, and the buildup of indels (Álvarez & Wendel, 2003; Meseguer *et al*., 2013).

Members of the sect. *Drosanthe* in the Flora of Turkey except for *H. sorgerae* and *H. davisii* are categorized under the sect. *Hirtella* according to Robson (1977) based on the established morphological classification. In both our phylogenetic trees, *H. davisii* formed a sister group with taxa belonging to the *Hirtella* sections as reported by Nürk *et al*., (2013).

A molecular investigation of *Hypericum* sect. *Drosanthe* according to the Flora of Turkey, employing the *trnL* 3ˈ–*trnF* region, demonstrated that the *H. lysimachioides* varieties, *H. lysimachioides* var. *lysimachioides* and *H. lysimachioides* var. *spathulatum*, exhibited distinct branching into two separate clades. This phylogenetic separation was previously attributed to notable morphological disparities between these two varieties (Doğan *et al*., 2017). However, in the present study, such differentiation was not observed within these varieties in either ITS and *trnL* 3ˈ–*trnF* trees. The high bootstrap support between *H. lysimachioides* var. *spathulatum* and *H. lysimachioides* var. *lysimachioides* indicates that these varieties form a strongly monophyletic group and likely diverged recently. This finding suggests that taxonomic distinctions at the varietal level are clearly supported at the genetic level, reflecting the intraspecific diversity within the genus *Hypericum*. Similar findings in the earlier studies demonstrated that varieties and subspecies of the genus *Hypericum* exhibited genetic differentiation alongside morphological and ecological adaptations (Greiner *et al*., 2011; Renner, 1934).

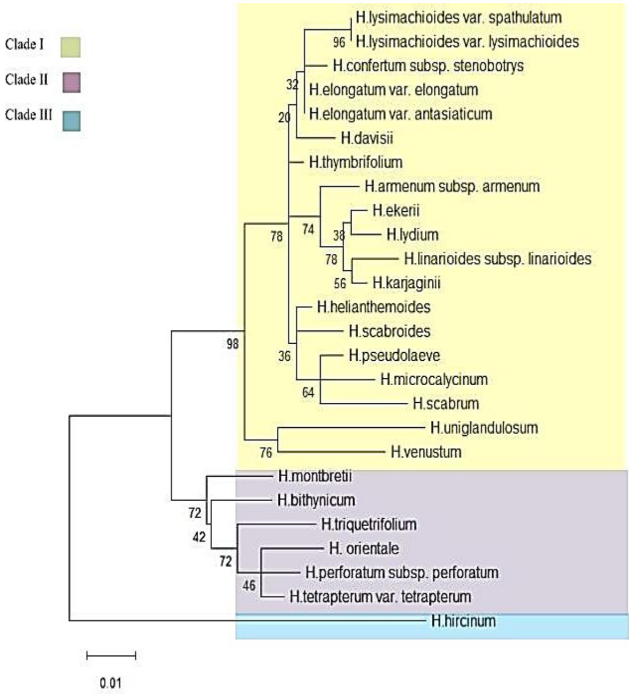

Fig. 1. The phylogenetic tree of ITS constructed using Maximum Likelihood method.

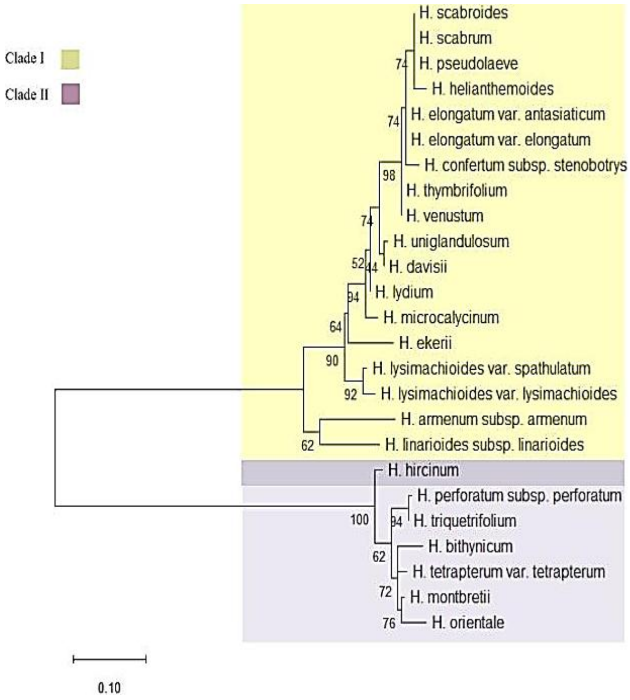

Fig. 2. The phylogenetic tree of *trnL* 3ˈ–*trnF* constructed using Maximum Likelihood method.

In the monographic studies of the sect. *Hirtella* by Robson (2010), *H. elongatum* was split into 3 varieties: *var. antasiaticum*, *var. elongatum*, and *var. lythrifolium*. Furthermore, *H. elongatum* subsp. *microcalycinum* and subsp. *apiculaticum*, which were previously treated as subspecies, were elevated to species rank as *H. microcalycinum* and *H. apiculatum*. This taxonomic revision supports the placement of *H. microcalycinum* in a distinct subclade, separate from the varieties of *H. elongatum*. The relationship between *H. elongatum* var. *elongatum* and *H. elongatum* var. *antasiaticum*, while supported by relatively low bootstrap values, indicated that these 2 taxa belonged to the same species. The low support values suggest that more genetic data or different molecular markers are needed to increase the resolution of relationships within this group. Studies, including that by Nürk *et al*., (2013), have shown that the phylogenetic resolution of certain species complexes and varieties within the genus *Hypericum* can vary depending on the markers used.

A new plant species endemic to Turkey, *H. ekerii* Yüce, Aytaç & Pınar, was discovered on Düzgün Baba Mountain, Tunceli and was included for the first time in a molecular phylogenetic investigation in our study. This species is known only from its type locality, where it forms a pure population with a restricted habitat spanning less than 10 km^2. Its total population size is estimated to be no more than 70-80 individuals. (Babacan *et al*., 2017). In our study, *H. ekerii* was shown to be a member of clade I, and formed a sister group with taxa from sect. *Hirtella*.

The findings presented in this study generally support previous classifications of *Hypericum* taxa. Importantly, new relationships are also unraveled that will guide future research within this genus. Furthermore, these results clarified the classification of certain taxa that were not previously included in molecular analyses. Future research could elucidate the evolutionary relationships of *Hypericum* taxa by integrating additional genetic markers (e.g., nuclear genes, single-copy genes) and employing more comprehensive sampling to build upon this phylogenetic tree.

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Authors Contribution: M.K. and O.Ö; collected and identified the plants materials, A.K; conducted the experiment and collected the data, G.K; designed the study, performed the experiments and wrote the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Alvarez, I. and J.F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Mol. Phylogenet. Evol*., 29: 417-434.

Avcı, M. 2014. *Türkiye'nin Bitki Çeşitliliği ve Coğrafi Açıdan Değerlendirmesi*. Ü. Akkemik (Ed.), Türkiye'nin Bitki Çeşitliliği ve Coğrafi Açıdan Değerlendirmesi içinde (pp. 28-53). Ankara: Orman Genel Müdürlüğü Yayını.

Babacan, Y.E., Z. Aytaç and B.M. Pınar. 2017. *Hypericum ekerii* (Hypericaceae), a new species from Turkey. *Pak. J. Bot.,* 49(5): 1763-1768.

Bilia, A.R., Gallori, S. and Vincieri, F.F. 2002. St. John's wort and depression: efficacy, safety and tolerability an update. *Life Sci.*, 70(26): 3077-3096.

Crockett, S.L. and N.K.B. Robson. 2011. Taxonomy and chemotaxonomy of the genus *Hypericum*. *Medicinal Aromatic Plant Sci. Biotech*., 5(1): 1-13.

Crockett, S.L., A.W. Douglas, B.E. Scheffler, and I.A. Khan. 2004. Genetic profiling of *Hypericum* (St. John's Wort) species by nuclear ribosomal ITS sequence analysis. *Planta Med.,* 70: 929-935.

Çırak, C. and D. Kurt. 2014. Önemli tıbbi bitkiler olarak *Hypericum* türleri ve kullanım alanları. *Anadolu.,* 24(1): 38-52.

Davis, P.H. 1967. *Flora of Turkey and the East Aegean Islands*. Vol. 2. Edinburgh University Press, Edinburgh.

Davis, P.H. (Ed.), 1965–1985. *Flora of Turkey and the East Aegean Islands*, vol. 1–9. Edinburgh University Press, Edinburgh.

Doğan, G., A. Yılmaz, E. Bağcı, E and Z. Kaya. 2017. Molecular phylogeny of section *Drosanthe* (Spach) Endl. (*Hypericum* L.) Inferred from chloroplast genome. *Pak. J. Bot.,* 49(6): 2235-2242.

Douzery, E.J.P., A.M. Pridgeon, P. Kores, H.P. Linder, H. Kurzweil and M.W. Chase. 1999. Molecular phylogenetics of Diseae (Orchidaceae): A contribution from nuclear ribosomal ITS sequences. *Amer. J. Bot.,* 86: 887-899.

Ekim, T., M. Koyuncu, M. Vural, H. Duman, Z. Aytaç and N. Adıgüzel. 2000. *Türkiye Bitkileri Kırmızı Kitabı (Red Data Book of Turkish Plants)*. Türkiye Tabiatını Koruma Derneği, Ankara.

Fırat, M. and H. Eroğlu. 2023. *Hypericum celikaensis* (Hypericaceae), a new species from southeastern Anatolia (Adıyaman–Turkey). *Candollea*, 78: 79-87.

Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol*., 2(2): 113-118.

Gielly, L. and P. Taberlet. 1994. The use of chloroplast DNA to resolve taxonomic relationships: An example in the genus *Saxifraga*. *Pl. Syst. Evol.*, 190(1-2): 131-143.

Greiner, S., U. Rauwolf, J. Meurer and R.G. Herrmann. 2011. The role of plastids in plant speciation. *Mol. Ecol.*, 20: 671-691.

Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer. 2000. *Flora of Turkey and the East Aegean Islands*. Vol. 11 (Supplement 2). Edinburgh University Press, Edinburgh.

Güner, A., S. Aslan, T. Ekim, M. Vural and M.T. Babaç. (Eds.) 2012. *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.

Hostettmann, K. and J.L. Wolfender. 2005. St. John's Wort and its active principles in depression and anxiety, *Phytochem.*, 5-20.

Kumar, S., G. Stecher, M. Suleski, M. Sanderford, S. Sharma and K. Tamura. 2024. MEGA12: Molecular evolutionary genetics analysis version 12.0. *Mol. Biol. Evol.*, 41(12): 1-9.

Metcalfe, C.R. and L. Chalk. 1950. *Anatomy of the Dicotyledons.* Vol. 1 and II Clarendon Press, Oxford.

Meseguer, A.S., J.J. Aldasoro and I. Sanmartin. 2013. Bayesian inference of phylogeny, morphology and range evolution reveals a complex evolutionary history in St. John's wort (Hypericum). *Mol. Phylogenet. Evol.,* 67: 379-403.

Nürk, N.M., S. Madrinan, M.A. Carine, M.W. Chase and F.R. Blattner. 2013. Molecular phylogenetics and morpological evolution of St. Jhon's wort (*Hypericum*; Hypericaceae). *Mol. Phylogenet. Evol.*, 66: 1-16.

Patterson, J., B. Chamberlain and D. Thayer. 2004-2006. *Finch TV Version 1.4.0.*

Pellati, F., S. Benvenuti and M. Melegari. 2005. Chromatographic performance of a new polar poly (ethylene glycol) bonded phase for the phytochemical analysis of *Hypericum perforatum* L. *J. Chromatogr. A*, 1088(1-2): 205-217.

Renner, O. 1934. Die pflanzlichen Plastiden als selbstandige Elemente der genetischen Konstitution. Berichte der mathemathisch-physikalischen Klasse der sachsischen Akademie der Wissenschaften zu Leibzig, 86: 241-266.

Robson, N.K.B. 1967. *Hypericum* L. In: (Ed.): Davis, P.H. Flora of Turkey and the East Aegean Islands (pp. 355-401). Edinburgh: Edinburgh University Press.

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.

Robson, N.K.B. 2012. Studies in the genus *Hypericum* L. (Hypericaceae) 9. Addenda, corrigenda, keys, lists and general discussion. *Phytotaxa*, 72: 1-111.

Taberlet, P., L. Gielly, G. Pautou and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.*, 17(5): 1105-1109.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22(22): 4673-4680.

Varel, M. 2011. *Hypericum perforatum* Binbirdelikotu, Sarı Kantaron. (Ed.): Demirezer, L.O. FFD Monografları Tedavide Kullanılan Bitkiler içinde (s. 309-319). Ankara: MN Medikal & Nobel Tıp Kitap Sarayı.

Walker, L., T. Sirvent, D.M. Gibson and N. Vance. 2001. Regional differences in hypericin and pseudohypericin concentrations and five morphological traits among *Hypericum perforatum* plants in the northwestern United States. *Can. J. Bot.*, 79(10): 1248-1251.

White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: (Eds.): Innis, M.A., D.H. Gelfand, J.J. Sninsky & T.J. White. PCR Protocols: A Guide to Methods and Applications (pp. 315-322). Academic Press, San Diego.