

Morphology and Molecular Phylogenetic Analysis of Taxa Belonging to The Genus *Picris* L. (*Asteraceae*) in Türkiye

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Received: 20/08/2024, **Revised:** 16/03/2025, **Accepted:** 14/05/2016, **Published:** 30/03/2026

Abstract

Picris L. belongs to the subfamily Cichorioideae of the family Asteraceae and is located in the subtribe Hypochaeridinae of the tribe Lactuceae. The genus *Picris* in the Flora of Turkey is represented by 9 species. In this study, morphological and phylogenetic data were combined to analyze *Picris* species distributed in Turkey. DNA isolation was performed using the phenol-chloroform-isoamylalcohol method, and the ITS (Internal Transcript Region) of nuclear ribosomal DNA (nrDNA) and the trnL-F (Transfer RNA coding) region of chloroplast DNA sequences were used as molecular markers. Phylogenetic analyses were performed using PAUP 4.0b10 software. In the analysis based on the maximum parsimony criterion, 95 parsimonious trees were evaluated. According to the maximum parsimony criteria, Branch & Bound data set analysis and linkage algorithm were used. Bootstrap analysis performed with the majority rule consensus algorithm produced a consensus tree supporting some branches. The findings confirm that *Picris* belongs in the subtribe Hypochaeridinae, along with the genera *Leontodon*, *Helminthotheca*, and *Hypochaeris*. The phylogenetic position of the genus *Picris* has long been controversial, with molecular data suggesting that some species are more closely related to different genera and deviate from traditional classifications. Therefore, *Picris* remains a taxonomically problematic group, requiring comprehensive genetic research.

Keywords: *Asteraceae*, *ITS*, *trnL-F*, morphology, phylogenetic analysis

Türkiye'deki *Picris* L. (*Asteraceae*) Cinsine Ait Taksonların Morfolojisi ve Moleküler Filogenetik Analizi

Öz

Picris L., Asteraceae familyasının Cichorioideae alt familyasına ait olup, Lactuceae tribusunun Hypochaeridinae alt tribusunda yer almaktadır. Türkiye Florası *Picris* cinsi 9 türle temsil edilmektedir. Bu çalışmada, Türkiye'de yayılış gösteren *Picris* türleri morfolojik ve filogenetik veriler birleştirilerek analiz edilmiştir. DNA izolasyonu, fenol-kloroform-izoamilalkol yöntemi kullanılarak gerçekleştirilmiş; moleküler belirteç olarak ise nükleer ribozomal DNA'nın (nrDNA) ITS (İç Transkript Bölgesi) ve kloroplast DNA dizilerinin trnL-F (Transfer RNA kodlama) bölgesi kullanılmıştır. Filogenetik analizler PAUP 4.0b10 yazılımı aracılığıyla yapılmış, maksimum parsimoni kriterine dayalı analizde 95 parsimoni ağaç değerlendirilmiş maksimum parsimony kriterlerine göre, Branch & Bound veri seti analizi ile bağlantı algoritması kullanılmıştır. Çoğunluk kuralı konsensüs algoritmasıyla gerçekleştirilen Bootstrap analizi, bazı dalları destekleyen bir konsensüs ağacı oluşturmuştur. Elde edilen bulgular, *Picris*'in *Leontodon*, *Helminthotheca* ve *Hypochaeris* cinsleriyle birlikte Hypochaeridinae alt tribusunda yer aldığını doğrulamaktadır. *Picris* cinsinin filogenetik konumu uzun süredir tartışmalı olup, moleküler veriler bazı türlerin farklı cinslerle daha yakın akraba olduğunu ve geleneksel sınıflandırmalardan sapmalar gösterdiğini ortaya koymaktadır. Bu nedenle, *Picris* hâlen taksonomik açıdan sorunlu bir grup olarak değerlendirilmekte ve kapsamlı genetik araştırmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Asteraceae, ITS, trnL-F, morfoloji, filogenetik analiz

1. Introduction

The *Asteraceae* family is a large family with more than 1100 genera and more than 23000 species. Anatomically, *Asteraceae* are characterized by the presence of resin ducts or laticiferous systems. It is also characterized by the presence of inulin in the underground organs and oil in the seeds [1]. The family is divided into three subfamilies as *Barnadesioidae*, *Cichorioideae* and *Asteroideae*. The genus *Picris* L. belongs to the *Hypochaeridinae* subtribe of the *Lactuceae* tribe of the *Cichorioideae* subfamily of the *Asteraceae* family [2,3]. The genus is represented by nearly 40 species distributed in Western Asia, South Africa, Australia, North America and Europe [4-6]. The genus *Picris* is represented in the Flora of Türkiye the East Aegea Islands (Davis & Tan 1988) by the species *P. hieracioides* L., *P. olympica* Boiss., *P. strigosa* Bieb., *P. pauciflora* Willd., *P. cyprica* Lack, *P. campylocarpa* Boiss., *P. altissima* Delile., *P. kotschy* Boiss., *P. amalecitana* (Boiss.) Eig. [7,8] (Figure 1).

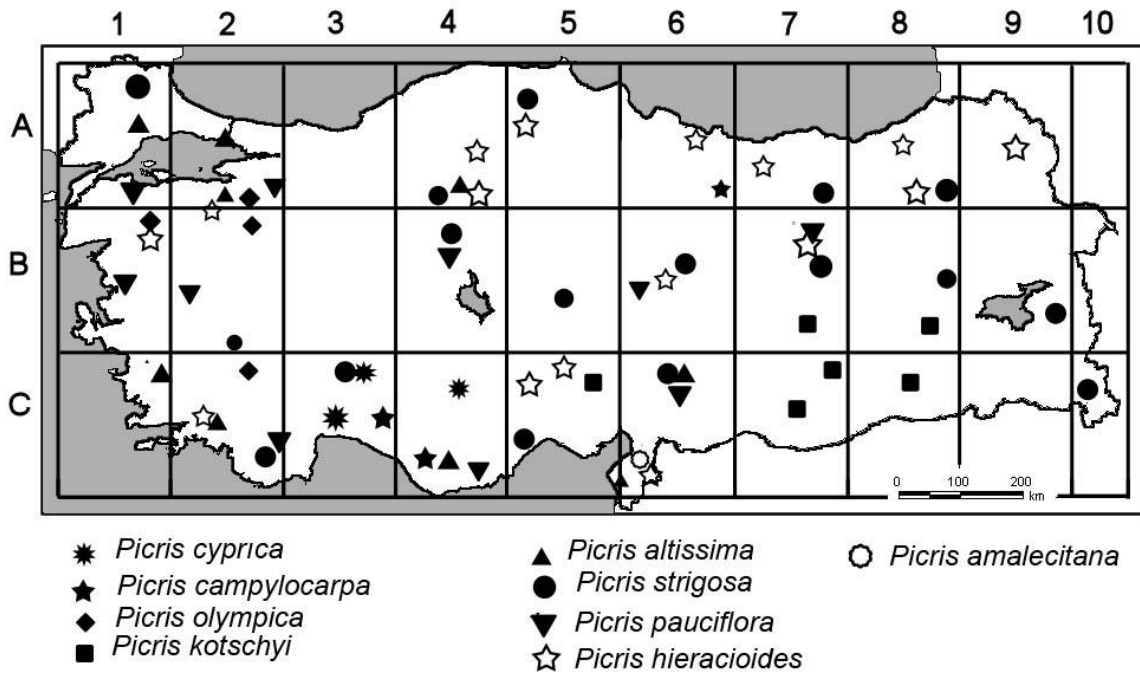


Figure 1 Distribution map of *Picris* species in Türkiye

Picris genus, which was chosen as the study material, was used in some overseas morphological and molecular studies [3,9-11] and phytochemical studies [6], no such morphological and molecular phylogenetic study has been found on its species found in Türkiye.

ITS primers are used to distinguish closely related species because they are a rapidly evolving region, while *trnL-F* primers are effective in determining large-scale phylogenetic relationships

because they are a slower evolving region. Using these two primers together allows the analysis of both rapidly and slowly changing gene regions and provides more comprehensive phylogenetic results. Therefore, *ITS* and *trnL-F* primers are often used together in phylogenetic studies in plants [12,13-15].

In this study, using morphological differences, *Picris* taxa in Türkiye were identified as the ingroup, and *Leontodon*, *Helminthotecha* and *Hypochoeris* taxa were identified as the outgroup. After determining the differences in external morphological characters, a species key was created. By combining the morphological data with the results of molecular analysis, it was aimed to determine the relatedness and phylogeny of the taxa of the genus *Picris* growing in Türkiye. With this study, we aimed to contribute to the separation boundaries of the genus *Picris* by contributing to the comparison of the compatibility of the genus distributed in Türkiye with the results of studies abroad.

2. Material and Methods

2.1. Morphological findings

Picris samples selected as study material were collected during the flowering periods between 2013 and 2015 and stored as herbarium materials. During the field study, fresh leaf samples were taken from plant material into silica gel for molecular analysis. All of these samples were given to Balıkesir University herbarium after the study was completed. (Table 1).

Table 1 Coollection data of studied *Picris* taxa

Taxa	Collection data and of collector's number
<i>Picris hierocioides</i> L.	A2 Bursa; Bursa, Uludağ National Park road, 400m, 21.06.2013, BS -BY 1051
<i>Picris olympica</i> Boiss.	B1 Bursa; Uludağ- Sarıkız around, 1700m, 19.06.2013, BS-BY 1043
<i>Picris strigosa</i> Bieb.	B4 Ankara; Sivrihisar- Oğlakçı between, 06.07.2014, BS-BY 1076 B5 Nevşehir; Zelve- Ürgüp road 2km, 1000m, 07.07.2014, BS-BY 1079
<i>Picris pauciflora</i> Willd.	C3 Antalya; Antalya Korkuteli between, 33km, forest clearing, 10.06.2013, BS-BY 1011 B6 Malatya; Darende-Gürpınar crossroad, 1150m, marl area, 23.05.2014, BY-VU 17347

<i>Picris cyprica</i> Lack	C3 Antalya; Manavgat- Akseki between, 15km, 11.06.2013, BS-BY 1019
<i>Picris campylocarpa</i> Boiss.	C3 Antalya; Manavgat- Akseki between, 15km, 11.06.2013, BS-BY 1023 C3Antalya; Serik- Manavgat between, 10km, 10m, 11.06.0213, BS-BY 1028
<i>Picris altissima</i> Delile	C3 Antalya; Akseki-Seydişehir between, 10-12km, 11.06.2013, BS-BY 1017
<i>Picris kotschy</i> Boiss.	C6 Osmaniye; Mount Nur Pass 500m, forest clearing, 19.05.2014, BY-VU 17308 B7 Malatya; Kale Kömürhan around the bridge, northern slope, 700m, 23.05.2014, BY-VU 17338
<i>Helminthotecha echioides</i> (L.) Holub	B1 İzmir; Narlıdere-Balıkesir between, SL, 50m, 15.06.2013, BS-BY 1032
<i>Leontodon asperimus</i> (L.)	C3 Burdur; Söğüt-Çavdar between, 3km, steppe, 1500m, 15.06.2013, BS-BY 1005
<i>Hypochoeris radicata</i> (L.)	B1 Bursa; Uludağ- hotel area, 1900m, 21.06.2013, BS-BY 1067

Along with the field studies, domestic herbariums (ANK, GAZİ, HUB, EGE, AEF) were visited and the existing specimens were examined. In addition, Geneva (G) and Edinburgh (E) herbariums were visited by Prof. Dr. Bayram Yıldız and the specimens collected from Türkiye and neighboring countries in these herbariums were examined.

After the studied specimens were identified according to their morphological characters, relevant resources were scanned to determine the ancestral and derived character states in the morphological characters [16-21].

Distinctive character states were collected in a table for phylogenetic analysis (Table 3). In Table 4, character states are organized in numerical coding. 29 morphological characters were used in the analysis using parsimony criteria.

2.2. Molecular findings

Genomic DNA isolations of the samples belonging to the study material and the taxa *Leontodon*, *Helminthotecha* and *Hypochoeris* selected as outgroups were made with the Phenol-Chloroform-Isoamylalcohol protocol developed by Dellaporta [18].

For molecular analyses, *ITS* (Internal Transcribed Spacer) nuclear ribosomal DNA (nrDNA) region sequences and *trnL-F* (L-F region encoding tRNAs of Transfer Chloroplast) regions, which have been mentioned many times in the literature and have been proven to be reliable, were used [3,12, 22-29].

Table 2 *ITS* and *trnL-F* primers used in this study with their designers

	Primer	Nucleotide Sequence (5'- 3')	T _m Value	Primer Designer
Forward	<i>ITS</i> 5A	TCCTCCGCTTATTGATATGC	49.9 °C	[12]
Reverse	<i>ITS</i> 4	CCTTATCATTAGAGGAAGGAG	52.1 °C	[12,13]
Forward	<i>trnL-e</i>	GGTTCAAGTCCCTCTATCCC	51	[14]
Reverse	<i>trnF-f</i>	ATTTGAACTGGTGACACGAG	51	[14]

PCR (Techno Thermal Cycler -Techno, Cambridge, UK) program steps for amplification of the target regions of the samples using the identified primers (Table 2): 5 min first denaturation at 94 °C, denaturation for 30 s at 94 °C, 45 s 50°C annealing and 1s 68°C extension. These steps were repeated 35 times. In the last step, final extension was completed at 68°C for 5s and the samples were stored at 4°C.

Techno Thermal Cycler (Techno, Cambridge, UK) program steps applied for the other primer studied, *trnL-F* (Table 2): 5 min first denaturation at 94 °C, denaturation for 30 s at 94 °C, 45 s 50°C annealing and 1s 68°C extension. After these steps were repeated 30 times, the final extension was completed at 68°C for 5s and stored at 4°C. Sequencing reactions of the primers used were performed through a commercial service provider (Ligand Biotechnology, Izmir, Türkiye). To check the accuracy of the DNA sequences obtained, a professional computer program called Sequencher, which is frequently used in molecular systematic studies, was used.

Alignment of the DNA sequences to make them suitable for analysis was done with ClustalW software. Phylogenetic trees were then constructed using the appropriate parameters of PAUP* (Phylogenetic Analysis Using Parsimony) 4.0b10 phylogenetic analysis software [26].

3. Results

3.1 Morphological findings

After the identification of the studied specimens according to morphological characters, relevant sources were reviewed to determine their ancestral and derived character status [17-19,21,30]. These distinctive characters were organized into numerical coding and a table was created (Table 3). The character states coded separately for taxa are organized in Table 4 to form a matrix. In the analysis using parsimony criteria, 29 morphological characters were used. As a result of Branch-and-Bound analysis, 27 characters are informative characters. In the analysis, 95 best trees were determined. 13 of them have the most reliable tree topology. Among these trees, the most suitable tree topology was determined. (Figure 2). In tree topology, *Picris* species are seen as a monophyletic group the species selected as the outgroup has become distinctly separated from the *Picris* species. The distinction between homomorphic and heteromorphic species given in the identification key is also evident in the tree topology.

Table 3 Morphological Character States to be Used for Phylogenetic Analysis

Morphological Characters	Character Number	Character States
Habitus	1	Perennial(0) Biennial (1) Annual(2)
Herbs status	2	decumbent(0) erect (1)
Stems feather condition (Gloshit)	3	2,3,4 branched (0) 2 branched (1) 4-5 branched (2)
Stems leaf density	4	Sparse leaved (0) Dense leaved (1)
Leaf lobes of the stem	5	acuminate (0) acute (1)
stem leaf type	6	Oblanseolate (0) Linear elliptic(1) Ovate- lanseolate (2) Linear-lanseolate(3) Oblong (4)
leaf	7	skinny(0) herbs (1)
phyllaries	8	two rows (0) one rows (1) three rows (2)
Outer phyllaries	9	taller than inner phyllaries (0) shorter than the inner phyllaries (1) equal to inner phyllaries (2)
outer phyllary shape	10	lineer lanceolate(0) oblong lanceolate(1) ovate-accuminate(2)
inner phyllary hair shape	11	Simple pubescent (0) densely pubescent (1)
inner phyllary hair dimensions (mm)	12	2-4 (0) 3-7 (1) 7-10 (2)
inner phyllary shape	13	lineer lanceolate (0) oblong lanceolate (1)
inner phyllary dimensions (mm)	14	6-10,5 (0) 10-16 (1) 13-20 (2)
Basal leaf density	15	sparsely leafy (0) dense leafy (1)

Basal leaf type	16	oblanceolate (0)	lanceolate (1)	Linear- lanceolate (2)
Basal leaf apex	17	obtus (0)	acute(1)	
Basal leaf length (cm)	18	3-8(0)	3-15(1)	4-9 (2) 4-14(3)
Width of capitulum	19	equal width (0)	Wide at the base (1)	narrow at the base (2)
Capitulum shape	20	knuckled (0)	Not knuckled (1)	
Width of capitulum (mm)	21	3-6 (0)	4-8(1)	6-11(2) 8-13(3) 0,7-2.5(4)
Peduncle status	22	Thinned in fruit (0)	thickened in fruit (1)	Same with fruit (2)
Pappus length in flower	23	shorter than perianth (0)	longer than perianth (1)	Same length as perianth (2)
Pappus	24	intensive (0)	scarce(1)	
Pappus length (mm)	25	4-7 (0)	8-12(1)	
Achene shape	26	heteromorphic (0)	homomorphic(1)	
Achene length (mm)	27	1-1,5 (0)	3-4,5(1)	5-6(2) 12-14(3)
Achene color	28	Dark brown (0)	light brown (1)	
Achene shape	29	Ovate(0)	Curved (1)	

Table 4 Matrix Data to be Used for Phylogenetic Analysis

↓ Type / → Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>P. hieracioides</i>	0	1	1	2	0	0	1	0	1	0	1	1	1	0	0	2	1	2	1	3	1	2	1	1	1	1	0	0	0
<i>P. olympica</i>	0	0	1	1	0	0	0	0	1	0	1	2	0	2	1	0	1	3	2	1	3	2	0	0	1	1	0	1	0
<i>P. strigosa</i>	0	0	0	0	0	3	0	0	1	1	1	0	0	1	0	0	0	1	0	1	1	2	1	0	0	1	3	0	0
<i>P. pauciflora</i>	0	1	1	0	1	0	0	0	1	1	0	0	0	2	0	2	0	0	1	0	3	1	1	0	1	1	1	0	1
<i>P. cyprica</i>	0	0	1	0	0	3	1	0	1	2	0	1	0	2	0	2	0	1	1	0	3	1	1	0	1	1	2	0	1

<i>P. campylocarpa</i>	0	1	0	1	1	0	0	0	1	0	0	0	0	2	0	0	1	3	1	0	1	2	2	0	1	0	1	1	1	
<i>P. altissima</i>	1	0	0	0	1	3	0	0	1	0	0	1	1	1	0	1	0	1	1	0	0	0	0	1	0	0	2	0	1	
<i>P. kotschy</i>	1	0	0	0	1	0	1	0	1	1	0	1	1	0	0	2	0	2	2	1	0	1	1	1	1	1	0	1	1	0
<i>Helminthotheca echioides</i>	0	0	0	0	1	2	1	1	?	2	0	2	?	?	1	0	1	1	1	0	2	0	1	0	0	1	1	1	0	
<i>Hypochoeris radicata</i>	0	0	?	0	0	0	0	0	1	2	1	2	0	2	1	0	1	2	2	1	3	2	1	0	1	1	3	0	0	
<i>Leontodon asperrimus</i>	0	0	2	0	1	2	0	2	1	2	0	1	1	2	1	0	0	3	2	1	2	1	0	1	0	1	2	0	0	



Figure 2 Tree number of most parsimonious tree of *Picris* based on morphological characters (Numbers above of branches indicate Bootstrap support)

3.2 Molecular findings

ITS and *trnL-F* regions of *Picris* species amplified by PCR reactions were aligned with ClustalW program. Then, the combined tree topology was reached by using the appropriate parameters of the PAUP* phylogenetic analysis software program in the parsimony trees generated with the Branch-and-Bound algorithm, 803 best trees were identified. 1 of them is the tree with the most reliable tree topology (Figure 2, 3). In tree topology, *Picris* species appear as a monophyletic group.

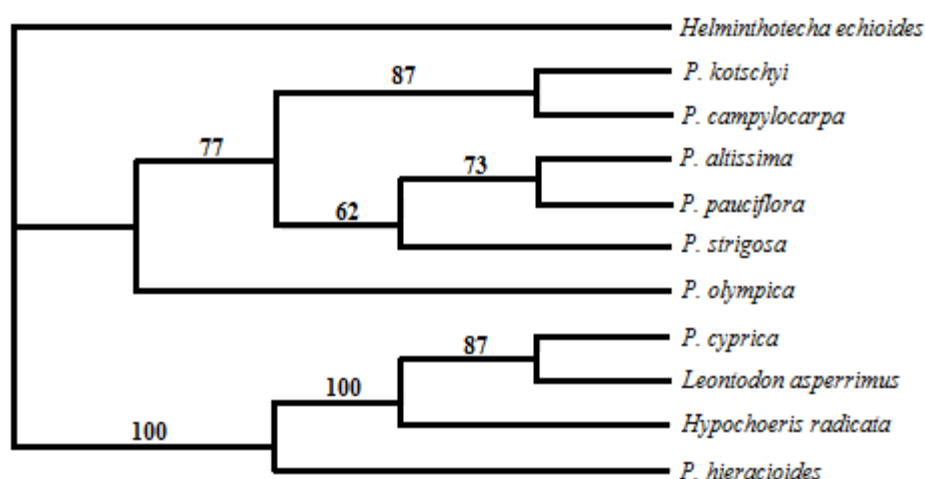


Figure 3 Tree number of 1 most parsimonious tree of *Picris* based on *ITS* and *trnL-F* characters (Numbers above of branches indicate Bootstrap support)

4. Discussion

The genus *Picris* in the Asteraceae family has been the subject of our research as a problematic genus. It is difficult to establish clear morphological boundaries between species within the genus *Picris*. The tendency for hybridization within species makes species boundaries unclear. It can be confused with close genera due to their similarities. It is a genus sensitive to ecological factors. Phenotypic plasticity can be seen within the same species. This makes species identification difficult. The phylogenetic position of the genus *Picris* has been controversial for many years. Genetic studies show that some species may actually be more closely related to other genera. In molecular phylogenetic analyses, some *Picris* species give different results than the traditionally expected groupings. For these reasons, *Picris* is considered a problematic group and is a genus that still needs detailed taxonomic and genetic studies [26,29,31,32]. *Picris* species with natural distribution in Türkiye were used in this study. When we look at the distribution areas of the genus in Türkiye, it is seen that it has a natural distribution in all regions. The localities of the species belonging to the genus registered in the visited herbariums were combined with the localities given in Flora of Türkiye and the current distribution limits were tried to be determined. It was observed that the *Picris strigosa* taxon had the widest distribution.

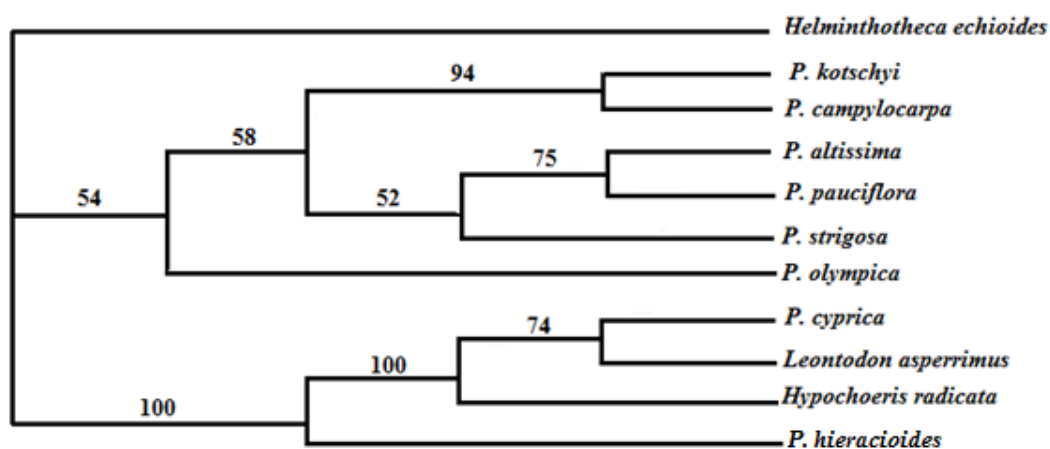


Figure 4 The most parsimonious tree of the genus *Picris* based on morphological, ITS and trnL-F characters (Numbers above branches indicate Bootstrap support)

Firstly, morphological studies were carried out on *Picris* species distributed in Türkiye. The capitulum, leaf characteristics, pubescence and fruit characteristics of the genus were reviewed. In diagnostics, there are problems with peduncle thickness, achene and pappus hair growth. The most problems are experienced between *P. kotschyi*, *P. campylocarpa*, and *P. altissima*.

Molecular analyzes were performed using *ITS* and *trnL-F* primers to support the morphological data. A total of 1204 criteria in the morphology, *ITS* and *trnL-F* combined dataset were used for analysis. Variable character 163, unchanging character 598, informative character 443, best tree score 914, and the most reliable tree was the number 1. The expected results were achieved in the combined tree topology created by interpreting molecular analysis and morphological data together (Figure 4).

As expected, *Helminthotecha* and *Hypochoeris*, identified as outgroups, were separated from *Picris* species with %100 strong support. *Leontodon*, like other outgroups, was separated from *Picris* taxa with 74% support. While making morphological observations, the difficulties in diagnosis, especially due to the number of glochid and peduncle thickness characteristics in the diagnostic key, were also reflected in the tree topology. *P. kotschyi* plant resembles *P. olympica* with its short height and resembles *P. strigosa* with its gray body color and 4 glochid hairs.

P. altissima, *P. pauciflora* and *P. strigosa* were separated from other *Picris* species by supporting 52% medium resolution. *Strigosa* differs from these two species in that its body color is gray, its fillaries have different plumage, the size of the capitulum and the fact that it has 4 glochid hairs. The capitulum sizes of *P. altissima* and *P. pauciflora* being close to each other and their body thickness being thicker than other species are the striking features in the first external morphological observations. Additionally, the peduncle becomes thinner in fruit in both species. It is separated from other species by being strongly supported like 75%. When we look at the external morphology of *P. cyprica*, its first distinctive feature is that it has the largest capitulum. It is distinguished from other *Picris* species by having 2 glochid hairs and a thickening of the peduncle in the fruit, along with *Leontodon asperimus* and *Hypochoeris radicata*, which were selected as outgroups, and 100% strong support. *L. asperimus* and *Hypochoeris radicata* are easily distinguished from the other outgroup *Helminthotecha echioides* by external morphological features. In addition, they are the outgroup examples with the largest capitulum among the *Picris* species, together with *P. cyprica*. Especially the similarity of the basal leaves of *L. asperimus* and *Hypochoeris radicata* and the shape of the pappus are very close to each other. The fact that the achene features and plumage conditions are not similar to the *Picris* samples again enabled the separation of these two outgroups with 100% strong support.

P. olympica differs from other *Picris* species by its shorter stem length, denser basal leaves, and branching of the stem from the base. It is distinguished from other *Picris* species by its features such as the same peduncle fruit, capitulum homomorphic and capitulum size. In addition, it is close to the *Helminthotecha echioides* species; It is due to the similarity of morphological features such as achene, papus and basal leaf shapes and is separated from other species with a good support of 54%. *Picris* species and *Leontodon asperimus*, *Hypochoeris radicata*, *Helminthotecha echioides* species, which were determined as outgroups, were separated with a strong support of 100%. In general, *P. kotschyi* is separated into a heteromorphic group; It distinguishes *P. altissima* and *P. campylocarpa* species from the homomorphic group by having a thicker peduncle, absence of the capitulum and having a shorter body. When we look at the distribution areas of the *Picris* genus, *P. pauciflora* has a distribution that sometimes overlaps

with *P. strigosa* and *P. altissima*. It can be thought that this situation may be due to the effect of close similarities. When looking at the tree topology, close similarities in peduncle thickness, achene and capitulum sizes make discrimination in the homomorphic group somewhat difficult [31].

Again, the difficulty in interpreting the same features gives the impression that *P. pauciflora* species are separated in tree topology as if they were in a heteromorphic group. Heteromorphic group, especially peduncle thickness and stem color, *P. strigosa* with a good support of 52% and *P. olympica* with a good support of 54%, separated from *P. cyprica*., *P. cyprica* and *P. hierocioides* were separated in a tree topology with strong support of 100%.

5. Conclusion

In this study, the phylogenetic relationships of the taxonomically complex genus *Picris* were investigated using an integrative approach that combines morphological traits with nuclear (*ITS*) and chloroplast (*trnL-F*) DNA regions. Phylogenetic analyses revealed that *Picris* forms a monophyletic group and is closely related to *Leontodon*, *Helminthotheca*, and *Hypochaeris*, consistent with its placement in the *Hypochaeridinae* subtribe.

While overlapping morphological characters complicated species delimitation, molecular data provided high-resolution results that clarified interspecific relationships. The combined dataset improved the understanding of species boundaries and highlighted inconsistencies in traditional classifications. These findings contribute valuable insights into the taxonomy, distribution, and evolutionary relationships of *Picris* in the Flora of Türkiye and underscore the importance of integrating molecular tools in plant systematics.

At the same time, it constitutes a methodological example in terms of demonstrating the effectiveness of molecular techniques in systematics in groups that show morphological similarity and include homoplasy. In this respect, the study offers a research approach that can be applied to other plant groups with similar structural difficulties.

In conclusion, this study not only sheds light on the phylogenetic structure of the genus *Picris*, but also provides important contributions to future studies on the re-evaluation, conservation and classification of problematic taxonomic groups in the Flora of Türkiye.

GenBank Submitter: *Picris* MK264354 (*Picris altissima*), *Picris* MK264355 (*Picris campylocarpa*), *Picris* MK264356 (*Picris hieracioides*), *Picris* MK264357 (*Picris pauciflora*), *Picris* MK264358 (*Picris cyprica*), *Picris* MK264359 (*Picris kotschyi*), *Picris* MK264360 (*Picris olympica*), *Picris* MK264361 (*Picris strigosa*)

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Field studies and species identification were carried out by two authors. Morphological and molecular analyses and writing of the article were done by Berna Sanon.

Acknowledgements

This study was conducted with the support of Balıkesir University BAP project no. 2013/75.

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