

Hedysarum marashicum (Fabaceae), a new species from Turkey

TUĞBA ERTUĞRUL^{1*}, AHTER FİŞNE¹, MEVLÜDE ALEV ATEŞ² & ECEM BAĞ³

¹ Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey

² Department of Agricultural Biotechnology, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey

³ Department of Pharmaceutical Botany, School of Pharmacy, Ankara Medipol University, Ankara, Turkey

✉ tugbaekici@gazi.edu.tr;  <https://orcid.org/0000-0002-3621-6845>

✉ ahter@gazi.edu.tr;  <https://orcid.org/0000-0001-6895-8870>

✉ malev.ates@ahievran.edu.tr;  <https://orcid.org/0000-0002-2141-5438>

✉ ecem.bag@ankaramedipol.edu.tr;  <https://orcid.org/0000-0002-8409-0749>

*Correspondence: ✉ tugbaekici@gazi.edu.tr

Abstract

Hedysarum marashicum T. Ertuğrul & E. Bağ is described as a new species from Osmanoğlu Plateau in Afşin, Kahramanmaraş Province, Turkey. The new species differs from *Hedysarum erythroleucum* Schott & Kotschy ex Boiss. in loosely adpressed sericeous, greenish appearance (vs. densely adpressed sericeous, greyish-whitish appearance); branched near the base and 4–5 cm long stem (vs. not branched and absent or very short stem); longer stipule (7–10 mm vs. 3 mm); bigger obovate–oblong, 8–12 × 4–6 mm, densely sericeous whitish below and sparsely sericeous greenish above leaflets (vs. smaller ovate–rounded, 4–7 × 3–4 mm, very densely sericeous whitish on both surfaces leaflets); longer peduncle (10–15 cm vs. 3–10 cm); longer bract and bracteole (5–7 mm and 4–5 mm vs. 3 mm and 1–2 mm); bigger calyx 8–9 mm long (vs. calyx 5–8 mm long); bigger and bright pink corolla (vs. intensely purple corolla); densely pubescent pods (vs. pilose pods). Taxonomic descriptions, an identification key, palynology, fruit and seed morphology, phylogeny, the IUCN threat category, and figures for the new species were presented. Furthermore, the mapping of the geographical distribution of *H. marashicum* and *H. erythroleucum* was included.

Key words: *Hedysarum*, Kahramanmaraş, *Multicaulia*, new species, Southeastern Anatolia

Introduction

The genus *Hedysarum* L. (1753: 745), consists of species that are difficult to define like other genera as *Onobrychis* Mill. (1754: 970), *Lagoecia* L. (Perrino *et al.* 2023) within the family Fabaceae, comprises highly ornamental and fragrant species characterized by their flowers, fruits, and indumentum features (Yıldız & Aktoklu 1997). These two genera are the largest within the tribe Hedysareae (Fabaceae) and are distinguished from each other by fruit morphology, floral characters of wing size and ovary shape (Hedge 1970, Yıldız & Aktoklu 1997).

Representing by over 220 species of annual or perennial herbs, the genus *Hedysarum* is widely distributed across temperate Asia, Europe, northern Africa, and North America (Post 1896, Fedtschenko 1948, Grossheim 1952, Hedge 1970, Townsend & Guest 1974, Meikle 1977, Polunin 1980, Rechinger 1984, Vassiljeva 1987, Choi & Ohashi 2003, Ranjbar *et al.* 2006, POWO 2024).

Previous phylogenetic analyses delimited *Hedysarum* into three main clades: the first clade (the mesic group) corresponds to *H. sect. Hedysarum*; the second clade (the xeric group) is the re-defined *H. sect. Multicaulia* (Boiss.) B. Fedtsch., consisting of the core group of *H. sect. Multicaulia* plus the former genus *Sartoria* Boiss. & Heldr; and the third clade (the psychrophilic group) includes the monospecific *H. sect. Stracheya* (Benth.) B.H. Choi & H. Ohashi and the sect. The *Subacaulia* section was merged into the *Multicaulia* section and is now considered part of the expanded *H. sect. Multicaulia*, which includes the subsections *Multicaulia* and *Crinifera*. (Choi & Ohashi 2003, Duan *et al.* 2015, Liu *et al.* 2017, 2019, Nafisi *et al.* 2019).

Species of *H. sect. Multicaulia* are mostly distributed in open, arid and semi-arid areas of Southern Siberia, Central and Western Asia, Mediterranean Europe, and North America (Choi & Ohashi 2003, Duan *et al.* 2015, Yurkevich *et al.* 2024).

The genus *Hedysarum* is represented with 32 taxa (with this addition), 22 of them are endemic, and the endemism rate is 69% in Turkey (Hedge 1970, Choi & Ohashi 2003, Aktoklu 2012, Amirahmadi *et al.* 2014, Başköse *et al.* 2018, Govaerts 2018, Hoşgören & Ertekin 2018, Aytaç *et al.* 2020, Hamzaoglu & Koç 2020, Govaerts *et al.* 2021, Nafisi *et al.* 2021, Aytaç *et al.* 2022, Kandemir *et al.* 2023, Ertuğrul *et al.* 2024). Recent taxonomic and molecular studies on the genus *Hedysarum* have resulted in several changes regarding the species found in Turkey. According to Govaerts (2018), some species names have been changed due to homonymy: *H. dededaghense* Govaerts (syn. *H. laxum* Boiss.), *H. malatyanum* Govaerts (syn. *H. rotundifolium* Boiss. & Noë) and *H. erzurumianum* Govaerts (syn. *H. elegans* Boiss. & A.Huet). *H. sericeum* M. Bieb. was deemed nom. illeg. and was subsequently renamed as *H. kolenatii* K. Koch. (Govaerts *et al.* 2021). According to Choi & Ohashi (2003), *H. sect. Spinosissima* (= sect. *Hedysarum*) was transferred to the genus *Sulla* Medik. therefore, *H. spinosissimum* L. has been transferred to *Sulla spinosissima* (L.) B.H.Choi & H.Ohashi *comb. nov.* as a new combination. *H. varium* Willd. subsp. *syriacum* (Boiss.) C.C.Towns. was elevated to species rank and renamed as *H. syriacum* Boiss. following a taxonomic revision conducted in Iran (Nafisi *et al.* 2021). *Sartoria hedysaroides* Boissier & Heldreich (1849: 149), the only species of the monotypic genus *Sartoria* Boissier (1849: 109) was transferred into the genus *Hedysarum* under the new name and combination as *Hedysarum anatolicum* Amirahm. & Kaz. Osaloo by the recent molecular systematic study (Amirahmadi *et al.* 2014).

The genus *Hedysarum* has been the subject of several studies focusing on pollen morphology (Choi & Ohashi 1996, Pavlova & Manova 2000, Ghanavati & Amirabadizadeh 2012, Saatçioğlu 2017, Aytaç *et al.* 2022, Ertuğrul *et al.* 2024). Pollen of *Onobrychis* and *Hedysarum* has been ascribed to one pollen type (the *Onobrychis* type) based on the three apertures and the reticulate (suprareticulate) ornamentation of the exine. (Faegri 1956, Faegri & Iversen 1989, Moore *et al.* 1991).

Studies on the fruit and seed morphology of the genus *Hedysarum* are quite limited. Sa *et al.* (2010) conducted a taxonomic revision of the part recognized as the *Hedysarum gmelinii* Ledeb. complex, resulting in the recognition of one widespread species and the proposal of two new combinations have made new systematic arrangements based on morphological studies of herbarium specimens and seed coat ornamentation characteristics. Dural & Çıtak (2015), examined the morphological, anatomical, palynological, fruit, and seed micromorphological properties of *Hedysarum pannosum* (Boiss.) Boiss.

DNA barcoding approaches, which involve the study of standardized DNA markers, have demonstrated utility in molecular characterization investigations (Zhang & Jiang 2020). Kress *et al.* (2005), Chen *et al.* (2010), the China Plant BOL Working Group (2011) and Kress (2017) elucidate the extensive application of these markers for species differentiation and the conservation of endangered species. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) is extensively utilized for barcoding of DNA in species identification, particularly in the conservation of endangered species (Kress *et al.* 2005, Kress 2017, Chen *et al.* 2010, China Plant BOL Working Group 2011, Zhang & Jiang 2019). Furthermore, the non-coding trn region (trn L-F) of chloroplast DNA has been utilized as a barcode in plant identification. Combined examining cpDNA and nrDNA in a single study yields dependable outcomes for phylogenetic research (ChinaPlant BOL Working Group 2011).

There are many molecular studies on *Hedysarum* genus by using different gene regions for characterizing and identifying new species. Nafisi *et al.* (2019) studied on molecular phylogeny of the genus *Hedysarum* (Fabaceae) with nrDNA(ITS) and cp DNA(trnL-F&matK) gene regions. Also, Juamurodov *et al.* (2023, 2024) reported an study on the genus *Hedysarum* in Uzbekistan by using ITS gene region. Additionally Liu *et al.* (2019) worked on identifying a new species *H. wangii* by using combination of molecular and morphological data. Furthermore, there were studies on naming new species at *Hedysarum* genus via both molecular and morphological data (Dehshiri & Goodarzi 2016, Başköse *et al.* 2018, Hamzaoglu & Koç 2020, Juamurodov *et al.* 2021).

In June 2024, during our fieldwork at Osmanoğlu Plateau in the Afşin district of Kahramanmaraş, we collected an interesting specimen of the genus *Hedysarum*. As a result of our detailed morphological studies, we found that although the specimen is closely related to *H. erythroleucum* Schott & Kotschy ex Boiss. in general appearance, it exhibited some distinct differences. In August 2024, we returned to the field to collect fruiting specimens, and finally, we described *H. marashicum* as a new species of the genus.

Material and methods

For the identification of the collected specimens *Heysarum* in The Flora of Turkey (Hedge 1970), Revision of *Hedysarum* in Turkey (Yıldız & Aktoklu 1997), the floras of neighboring countries and related publications (Post

1896, Fedtschenko 1948, Grossheim 1952, Townsend & Guest 1974, Meikle 1977, Polunin 1980, Rechinger 1984, Vassiljeva 1987, Ranjbar *et al.* 2006) and the original description (Boissier 1872: 515) were used. The specimens were compared with the specimens in GAZI, ANK herbaria, and type specimens (digital) of *Hedysarum erythroleucum* (G00330429, G00330430, WAG0004602, GOET005029) collected by Kotschy. Conservation assessments were made using the International Union for Conservation of Nature (IUCN 2022) guidelines.

Palynological, fruit, and seed morphological analysis: Pollen grains of the new taxon *H. marashicum* and *H. erythroleucum* were investigated using light microscopy (LM) and scanning electron microscopy (SEM). Initially, pollen slides were prepared following the Wodehouse technique (1935). Pollen grains were stained with a glycerin-jelly and basic fuchsin mixture, slightly heated, and covered with a coverslip. Measurements, including polar axis (P), equatorial axis (E), colpus length (Clg), colpus width (Clw), exine thickness (Ex), intine thickness (I) one side of the polar triangle (t), and mesocolpium diameter (Me), were taken from at least 30 fully developed grains per sample using a Leica ICC50 HD microscope. Results are presented as minimum, maximum, and mean $\pm$ standard deviation values.

Fruits and seeds of the new taxon *H. marashicum* were first examined, photographed, and measured for their length and width under an Olympus SZX7 stereoscopic microscope using the LG Micro program and scanning electron microscopy (SEM).

Dried pollen grains, fruits, and seeds were prepared for SEM analysis by mounting them on stubs and coating them with gold. Observations and imaging were conducted using a JEOL-6060 SEM (JEOL Ltd., Akishima, Tokyo, Japan) at the Prof. Dr. Zekiye Suludere Electron Microscopy Center at Gazi University, Turkey. The terminology used in this study follows the guidelines of Faegri & Iversen (1989) and Punt *et al.* (2007). The pollen shape class was identified based on the polar axis to equatorial axis (P/E) ratio, using Erdtman's classification system (Erdtman 1969).

Total DNA Extractions, PCR Amplifications, and Phylogenetic Analysis: Leaf specimens of *Hedysarum* sp. (stored at GAZI with the herbarium number: TE 2947) were collected in their natural habitat for the analysis of genetic data. Additionally, herbarium specimens of some *Hedysarum* species indigenous to Türkiye were obtained from the GAZI herbarium (codes: ZA 8657, ZA 11023, HA 5627, TE 1791, MV 8910). DNA extraction was conducted with a plant DNA extraction kit produced by EUR X. The amplification of the ITS region (ITS1+5.8S+ITS2) and trn L-F was performed utilizing the primer pairs designated by Hsiao *et al.* (1995) and Taberlet *et al.* (1991), respectively. PCR amplifications were conducted in a total volume of 20 μ l, comprising 3 μ l of 5 Hot FirePol Blend PCR Mix (Solis Biodyne) (12.5 mM MgCl₂), 0.5 μ l of each primer pair, 1 μ l of template DNA, and 15 μ l of water. PCR reactions were conducted utilizing SuperCycler™ (Kyrtec) with the subsequent optimized cycling parameters: 5 minutes at 95°C for initial denaturation, succeeded by 35 cycles of 30 seconds at 95°C for template denaturation, 30 seconds for diverse primer annealing per primer pairs, 90 seconds at 72°C for extension, and 10 minutes at 72°C for final extension. Materials were verified on an agarose gel (1.5% concentration) before being purified and sequenced by BM Labosis Company (Ankara). The Finch Tv program version 1.4.0 (Patterson *et al.* 2004, 2006), created by the Geopiza Research Team, was utilized to authenticate the obtained sequences before analysis. The MEGA (Molecular Evolutionary Genetics Analysis) version 11 software's MUSCLE (Multiple Sequence Comparison By Log Expectation) tool was used to align the sequences (Tamura *et al.* 2021). The ideal replacement model for reconstructing the species' phylogeny was determined using MEGA. The Maximum Likelihood (ML) method utilizing the Jukes-Cantor (JC) model (Jukes & Cantor 1969) for ITS and the Hasegawa-Kishino-Yano (HKY) model (Hasegawa *et al.* 1985), for trn L-F, accompanied by a bootstrap test analysis (1000 replicates) with uniform rates, was employed to construct the phylogenetic tree following the identification of the most suitable substitution model in the MEGA program. The identical substitution models (HKY for trnL-F and JC for ITS) with uniform rates for data partitions were also used with the BEAST (Bayesian Evolutionary Analysis by Sampling Trees) software tool. The Yule tree was previously utilized, and an initial tree was randomly produced by 10,000,000 Markov Chain Monte Carlo (MCMC) iterations. Furthermore, the raxmlGUI 2.0 software was employed, applying the same models for the ITS and trn L-F regions with bootstrap values methodology, to verify and confirm the reliability of the data obtained. With a posterior probability threshold of 1, the phylogenetic trees were then merged and compressed using the Tree Annotator program (Drummond *et al.* 2012, Bouckaert *et al.* 2019). The phylogenetic trees produced by the MEGA and BEAST software were analyzed comparatively and integratively.

The sequences of *Hedysarum* species and chosen species from related genera, including Ebenus and Onobrychis within the Fabaceae family, were retrieved from the NCBI database. The sequences were included with the samples utilized in the analysis to provide an evolutionary context (accession numbers were indicated on the phylogenetic trees and references of the obtained samples given in the appendix).

Taxonomic treatment

Hedysarum marashicum T. Ertuğrul & E. Bağ *sp. nov.* Sect. *Multicaulia* (Figure 1–3).

Type:—Turkey. Kahramanmaraş: Afşin, Osmanoğlu Plateau, rocky limestone, 2385–2400 m, 30 June 2024, *T. Ertuğrul* 2947 and *E. Bağ* (holotype GAZI, isotypes ANK, HUB).

Paratype:—Turkey. Kahramanmaraş: Afşin, Osmanoğlu Plateau, rocky limestone, 2385–2400 m, 11 August 2024, *T. Ertuğrul* 2958 and *E. Bağ*.



FIGURE 1. Photos of *Hedysarum marashicum* from the field a—Habitat, b–c—habitus, d—inflorescence.

Diagnosis: This new species is morphologically similar to *H. erythroleucum* but can be distinguished by the whole plant being loosely adpressed sericeous with a greenish appearance (vs. densely adpressed sericeous with a greyish-whitish appearance), ascending to erect, branched near the base and 4–5 cm long stem (vs. not branched and absent or very short stem), longer stipule (7–10 mm vs. 3 mm), obovate–oblong, bigger (8–12 × 4–6 mm), densely sericeous whitish below and sparsely sericeous greenish above leaflets (vs. ovate–rounded, smaller (4–7 × 3–4 mm), very densely sericeous whitish on both surfaces leaflets), more flowered (5–10) inflorescence (vs. 3–5 flowered), longer peduncle (10–15 cm vs. 3–10 cm), longer bract and bracteole (5–7 mm and 4–5 mm vs. 3 mm and 1–2 mm), bigger calyx 8–9 mm long, teeth 5–6 mm (vs. calyx 5–8 mm long, teeth 4–5 mm), bright pink corolla (vs. intensely purple corolla), 17–20 × 11–13 mm, obovate to oblanceolate standard (vs. 8–15 × 8–11 mm, obovate standard), longer keel (16–18,5 mm vs. 9–13 mm), wings 12–15 × 5 mm, broadly angled and obtuse at apex (vs. wings 8–12 (–14) × 3–4 mm, ligulate,

rounded at apex), pods 1–2 jointed and joints 4–4,7 × 3,8–4 mm, densely pubescent (vs. pods 1–3 jointed and joints 6 × 5 mm, pilose) (Table 1).

TABLE 1. Morphological comparison of *H. marashicum* and *H. erythroleucum*.

Characters	<i>H. marashicum</i>	<i>H. erythroleucum</i>
Stem	4–5 cm long; branched near the base, loosely adpressed sericeous, greenish	Absent or very short; not branched, densely adpressed sericeous, greyish-whitish
Stipule	7–10 mm long	3 mm long
Leaves	3–4 paired	1–4 paired
Leaflets	Obovate–oblong, 8–12 × 4–6 mm; densely sericeous below and sparsely sericeous above, greenish above and whitish below	Ovate–rounded; 4–7 × 3–4 mm; very densely sericeous on both surfaces, whitish on both surfaces
Inflorescence	5–10-flowered	3–5 (–10)-flowered
Peduncle	10–15 cm long	3–10 cm long
Bract	5–7 mm long	3 mm long
Bracteoles	4–5 mm long	1–2 mm long
Calyx	8–9 mm long, teeth 5–6 mm	5–8 mm long, teeth 4–5 mm
Corolla	Bright pink	Intensely purple
Standard	17–20 × 11–13 mm, obovate to oblanceolate	8–15 × 8–11 mm, obovate
Keel	16–18.5 mm long	9–13 mm long
Wings	12–15 × 5 mm, broadly angled and obtuse at apex	8–12 (–14) × 3–4 mm, ligulate, rounded at apex
Pods	1–2 jointed and joints 4–4.7 × 3.8–4 mm; pubescent	1–3 jointed and joints 6 × 5 mm; pilose
Seed	3.3 × 3 mm, rugulate–reticulate, dark brown	3 × 2.5 mm, smooth, light brown



FIGURE 2. a—*Hedysarum marashicum* T. Ertuğrul & E. Bağ (holotype), b—*H. erythroleucum* Schott & Kotschy ex Boiss (T. Ertuğrul 2771).

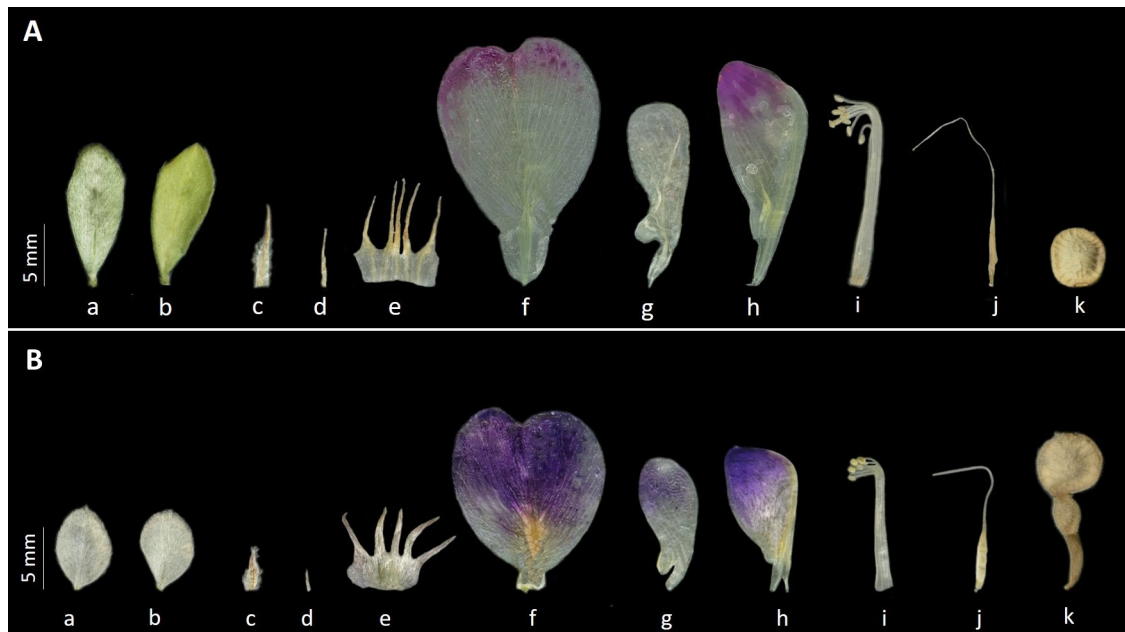


FIGURE 3. A—*Hedysarum marashicum*, B—*H. erythroleucum*. a—lower surface of leaflets, b—upper surface of leaflets, c—bract, d—bracteol, e—calyx, f—standard, g—wing, h—keel, i—stamen, j—ovary, k—pods.

Description: Perennial herbs; stem extremely short, ascending to erect, 4–5 cm long, branched near the base, loosely adpressed sericeous, greenish. Stipules scarious, united 2-toothed, 7–10 mm. Leaves imparipinnate, 2.5–4.5 cm long; leaflets 3–4 pairs, obovate-oblong, mucronulate, $8\text{--}12 \times 4\text{--}6$ mm, densely whitish sericeous below and loosely sericeous above, greenish. Inflorescence capitate, 5–10-flowered, 1.5–3.5 cm long; peduncles 10–15 cm long, much longer than leaves. Bracts 1, lanceolate, membranous with brown midvein, 5–7 mm long, spreading white hairy. Bracteoles 2, subulate, brown, 4–5 mm long, spreading white hairy. Calyx campanulate, 8–9 mm long, sericeous; teeth unequal, two of them subulate and three of them narrowly subulate, 5–6 mm long, calyx teeth 1–3 times as long as the tube. Corolla bright pink; standard glabrous, $17\text{--}20 \times 11\text{--}13$ mm, obovate to oblanceolate, emarginate, attenuate at base; wings $12\text{--}15 \times 5$ mm, broadly angled and obtuse at apex, two-thirds to nearly equal the length of the keel; keels $16\text{--}18.5$ mm long, shorter than standard. Stamen diadelphous, 15–17.5 mm. Ovary linear, 5–6 mm adpressed hairy. Pods 1–2 jointed; joints orbicular with prominent transverse nervation, $4\text{--}4.7 \times 3.8\text{--}4$ mm; densely pubescent, without setae. Seeds reniform, 3.3×3 mm, rugulate-reticulate, dark brown.

Phenology: Flowering in June–July and fruiting in August.

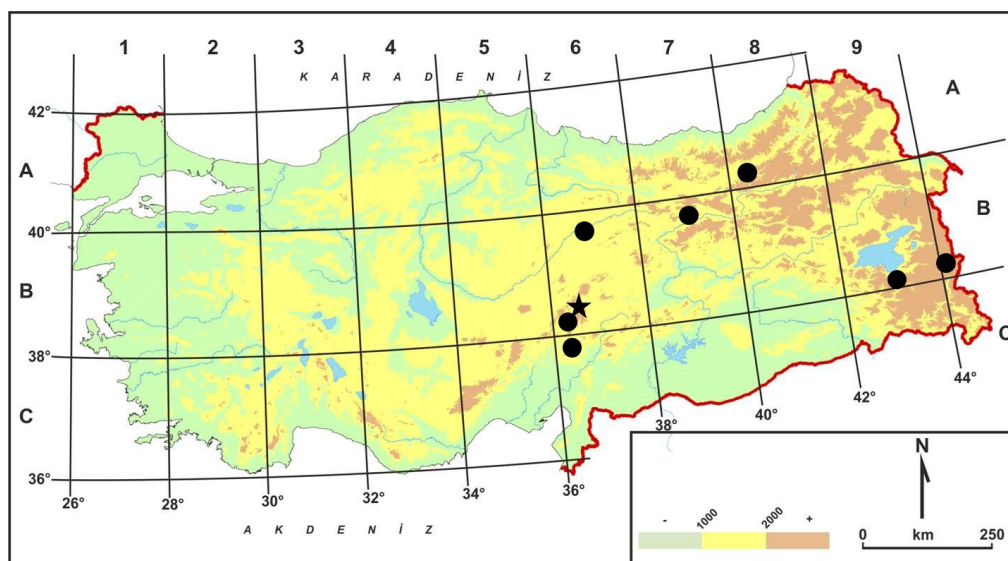


FIGURE 4. Distribution map of *H. marashicum* (●), *H. erythroleucum* (★).

Distribution and Ecology: The new species grows in Southeastern Anatolia, around Kahramanmaraş province. It is an endemic and Irano-Turanian element (Figure 4). *H. marashicum* grows on open calcareous slopes on high mountain steppe together with *Marrubium astracanicum* Jacq., *Marrubium globosum* Montbret & Aucher ex Benth., *Astragalus microcephalus* Willd., *Thymus* sp., *Silene odontopetala* Fenzl, *Verbascum* sp., *Medicago* sp., *Festuca* sp., *Poa* sp., *Carduus nutans* L., *Scrophularia* sp., *Anthemis* sp.

Conservation status: The newly discovered species is currently identified at a single location in Kahramanmaraş. The population comprises fewer than 50 individuals, and both the extent of occurrence (EOO) and area of occupancy (AOO) measure less than 4 km² (calculated using ArcGIS and spatial analysis programs). The AOO, coupled with declining habitat quality due to intensive grazing, categorizes the species as “critically endangered” CR B1ab (i, ii, iii, v) + 2ab (i, ii, iii, v), D1 (IUCN 2022).

Etymology: The scientific epithet of the new species is derived from Kahramanmaraş Province, the location where the type specimen was collected. We suggest the vernacular name “Maraş Batalağı” for *Hedysarum marashicum* (Menemen *et al.* 2016).

Pollen Morphology: The pollen grains of *H. marashicum* and *H. erythroleucum* are isopolar, symmetric, and tricolpate. Pollen grains are triangular-obtuse in polar view and rectangular-obtuse (*H. marashicum*) to elliptic (*H. erythroleucum*) in equatorial view. The pollen shape is subprolate (P/E:1.15) in *H. marashicum* while prolate (P/E:1.42) in *H. erythroleucum*. The pollen size of *H. marashicum* is P: 19.1 ± 1.59 µm, E: 16.58 ± 0.98 µm and P: 21.90 ± 0.98 µm, E: 15.35 ± 0.63 µm in *H. erythroleucum*. Ectocolpi is long and shallow with acute ends. The colpus membrane is covered by large and small sculptural elements in both taxa. Colpus length (Clg) is 19.32 ± 0.95 µm, and colpus width (Clw) is 2.1 ± 0.94 in *H. marashicum* and 21.08 ± 0.94 and 3.1 ± 1.01 in *H. erythroleucum*. Exine ornamentation is reticulate in both the equatorial and polar views. The reticules at the edge of the colpus and in the polar area are smaller than in the mesocolpium region. In addition to this, lumina diameter of the reticules in the mesocolpium of *H. marashicum* is shorter than that of *H. erythroleucum* (Table 2, Figure 5). Apocolpium diameter (t) is 6.00 ± 0.42 µm in *H. marashicum* while 3.45 ± 0.44 in *H. erythroleucum*. Detailed pollen morphological characters of the examined two taxa are given in Table 2.

TABLE 2. Pollen morphological data of *Hedysarum marashicum* and *H. erythroleucum* (values in µm; minimum, maximum, mean ± standard deviation).

Species/Characters		<i>H. marashicum</i>	<i>H. erythroleucum</i>
Polar axis	Min.	17.8	20.1
	Max.	24.2	24.5
	Mean	19.1 ± 1.59	21.90 ± 0.98
Equatorial axis	Min.	14.1	14.2
	Max.	17.6	16.9
	Mean	16.58 ± 0.98	15.35 ± 0.63
Pollen shape		subprolate	prolate
Aperture type		tricolpate	tricolpate
Ornamentation		reticulate	reticulate
Colpus (Cl)	Colpus length (Clg)	19.32 ± 0.95	21.08 ± 0.94
	Colpus width (Clw)	2.1 ± 0.94	3.1 ± 1.01
Exine thickness		0.73 ± 0.06	0.42 ± 0.02
Intine thickness		0.52 ± 0.02	0.62 ± 0.01
Apocolpium diameter (t)		6.00±0.42	3.45 ± 0.44
Mesocolpium diameter (Me)		11.61 ± 0.55	11.09 ± 0.79

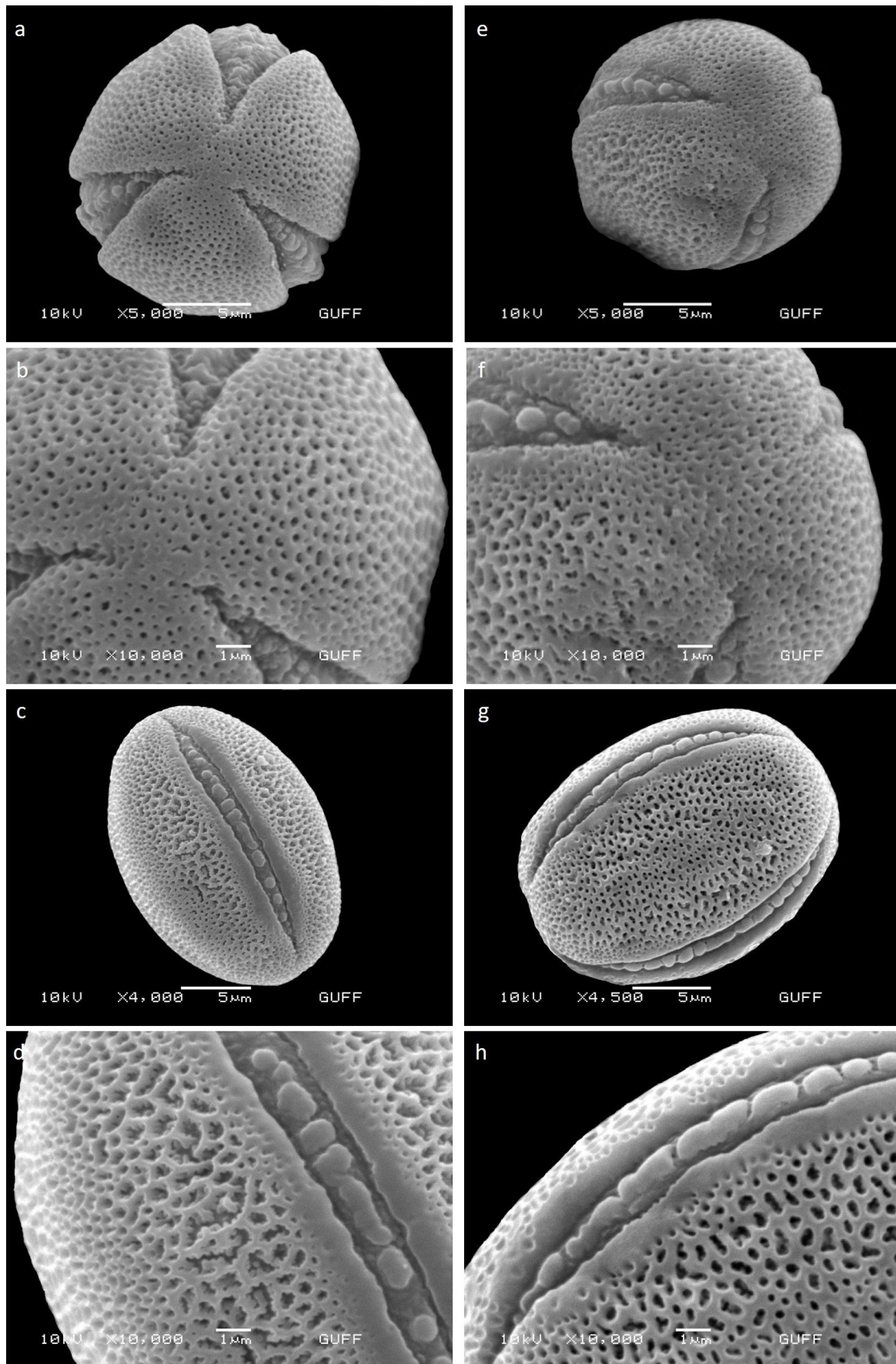


FIGURE 5. Pollen grains SEM micrographs of *Hedysarum marashicum* (a—d) and *H. erythroleucum* (e—h) a,e—polar view, b,f—exine ornamentation, c,g—equatorial view, d,h—exine ornamentation.

Fruit and Seed Morphology: Mature lomentums of *H. marashicum* have 1–2 joints; joints orbicular with prominent transverse nervation, 4–4.7 × 3.8–4 mm; densely pubescent, without setae. Mature seeds are reniform, 3.3 × 3 mm, dark brown, seed surface ornamentation is rugulate-reticulate (Figure 6).

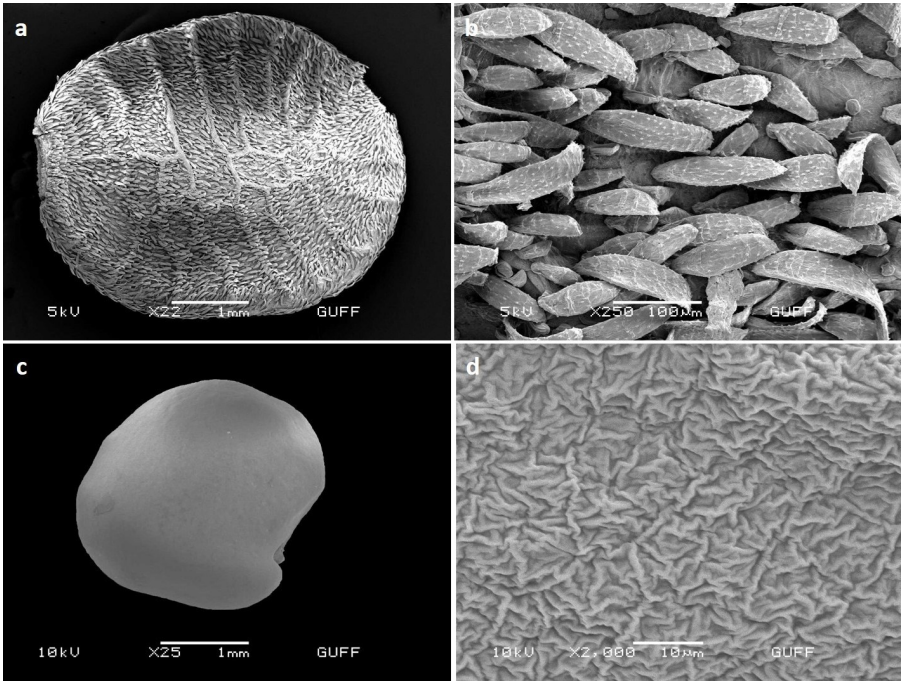


FIGURE 6. Fruit and seed SEM micrographs of *Hedysarum marashicum* a—pod, b—indumentum, c—seed, d—seed-surface details.

Phylogeny: ITS sequences were obtained as a total of 640 bp in length (after the exclusion of gap-rich and ambiguous bases), and 52 of them were variable. Moreover, trn L-F sequences (after exclusion of gap-rich and ambiguous bases) were obtained as 799 bp in length, and 60 of them were variable among *Hedysarum* species. Overall mean genetic divergence among species based on ITS, and trn L-F regions were calculated as 0.020 and 0.030, respectively (All statistic values were given in the Table 3). According to phylogenetic tree which was constructed by using ITS region (Figure 7), there are 2 main clusters with a bootstrap value of 98 and a posterior probability value of 1. One of the cluster is composed of *H.syriacum* and *H.huteii* species and other cluster composed of other studied species (*H.sericeum*, *H.elegans*, *H.cappadocicum*) additional with newly discovered species (*Hedysarum* sp) and morphologically closely related species, *H. erythroleucum*. In the phylogenetic tree the newly named species *Hedysarum* sp was separated from other species with sufficient bootstrap and pp values (98 & 1 respectively). Even if overall mean genetic divergence is higher than ITS region genetic divergence value, the phylogenetic tree of trn L-F region (Figure 8) indicates phylogenetic separations parallel with ITS gene region data. Again, *Hedysarum* species are divided into two main clusters (bs 100 & pp 1). One of the clusters included *H.huteii* and *H.syriacum*; the other cluster was composed of other studied samples. Like ITS region tree newly named *Hedysarum* sp. was positioned under same subcluster with *H. erythroleucum* species but separated from this with sufficient values (100 bs& 1 pp).

TABLE 3. Statistical values among *Hedysarum* species via MEGA 11 program (using Kimura-2 parameters for R & overall genetic diversity).

Statistics (within <i>Hedysarum</i> species)	ITS (Total: 640 bp)	Trn L-F (Total:799 bp)
Conserved sites	573 bp	615 bp
Variable sites	52 bp	60 bp
Singleton	4 bp	17 bp
Parsimony informative	48 bp	43 bp
Overall genetic diversity	0.020	0.030
R (Transition/Transversion Bias)	1.71	0.52
% GC content	53.5	32.5

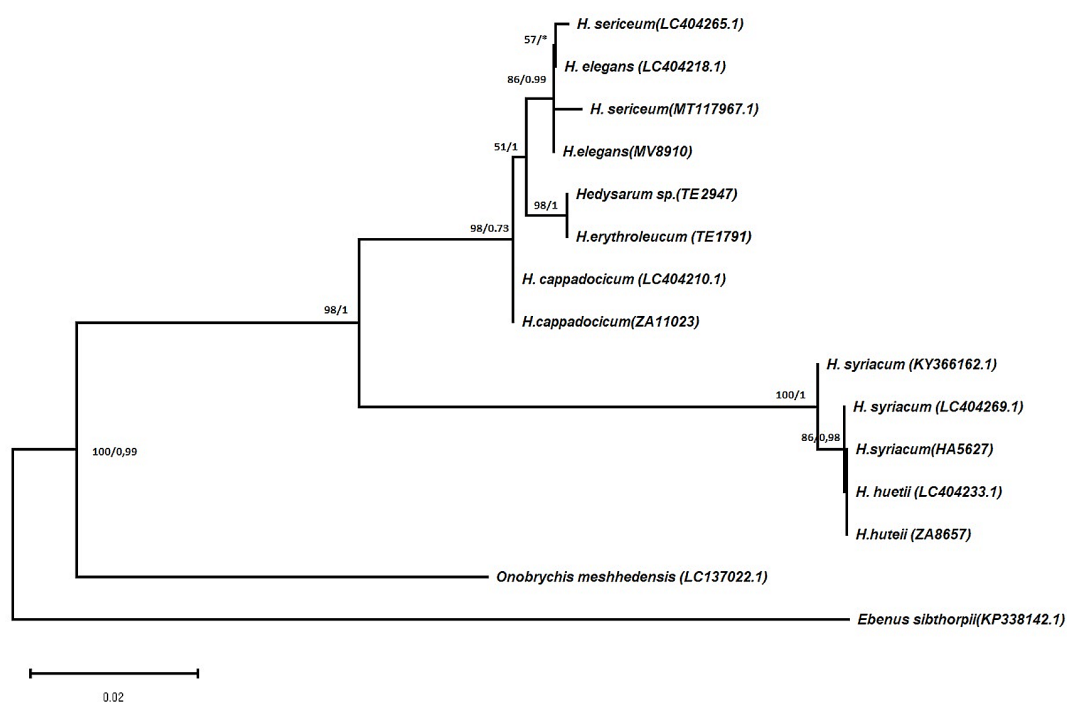


FIGURE 7. The evolutionary history obtained from the ITS gene region was inferred utilizing the Maximum Likelihood method and Bayesian approaches using the Jukes-Cantor model. The tree is rendered to scale, with branch lengths quantified by the number of substitutions per site. The outcomes of the ML analysis, along by subsequent optimization (bootstrap values from 1000 repeats) and posterior probabilities (PP) above one, are presented adjacent to the nodes and separated by slashes (bootstrap values under 50 are marked with *).

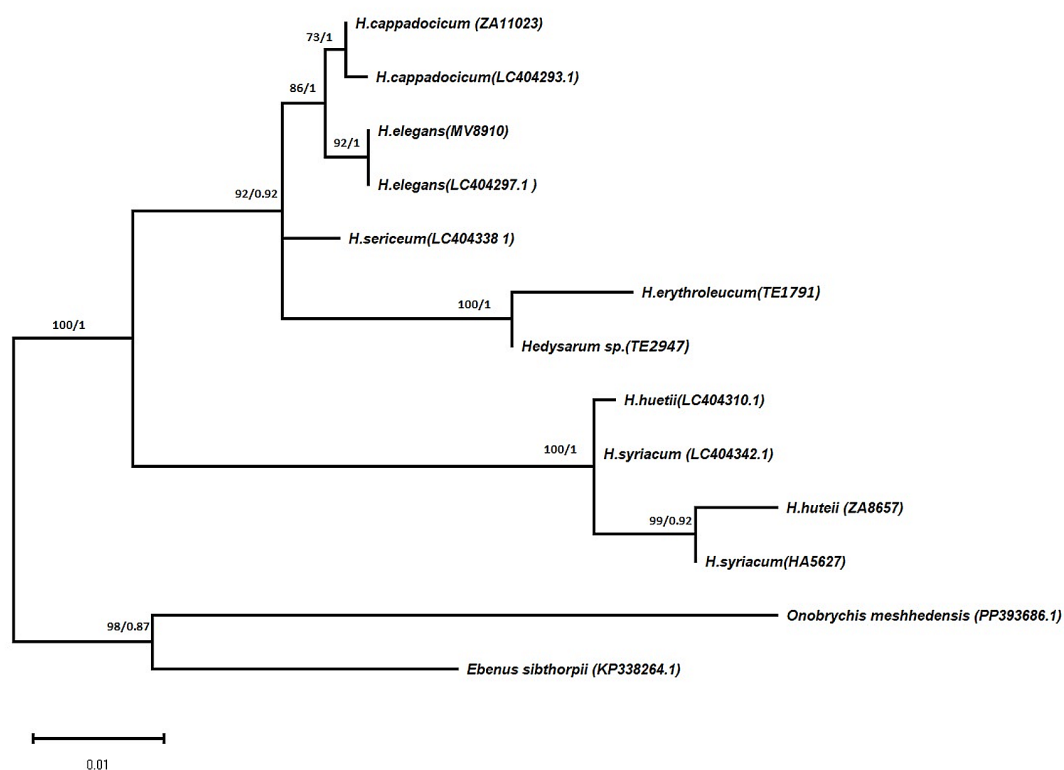


FIGURE 8. The evolutionary history obtained from the trn L-F gene region was inferred utilizing the Maximum Likelihood method and Bayesian approaches using the Hasegawa-Kishino-Yano model. The tree is rendered to scale, with branch lengths quantified by the number of substitutions per site. The outcomes of the ML analysis, along by subsequent optimization (bootstrap values from 1000 repeats) and posterior probabilities (PP) above one, are presented adjacent to the nodes and separated by slashes (bootstrap values under 50 are marked with *).

Discussion

Hedysarum marashicum is closely related to the species previously classified under the section known as *Subacaulia* due to its very short stem length. It is similar, and probably most closely related to *H. erythroleucum* in terms of many morphological characters such as stem length, number of leaflet pairs, inflorescence, calyx teeth /tube ratio, segment number and size of pod. But they are distinguished from each other by indumentum, bract and bracteole length, calyx length, corolla color and length, the shape and size of their leaflets and indumentum of pod (Table 1). *H. marashicum* can be distinguished from *H. cappadocicum* by its corolla, which is noticeably longer than the calyx. Additionally, the new species is distinguished from other closely related species by its calyx teeth, which are 1–3 times longer than the calyx tube, and its capitate inflorescence.

The species closely related to the newly described taxon in the neighboring countries (Greece, Bulgaria, Armenia, Azerbaijan, Georgia, Iran, Iraq, Syria, and Cyprus) have already been examined according to the floras and publications. *H. marashicum* differs markedly from the Cypriot endemic *H. cyprium* Boiss. in several morphological characters: it has a much shorter stem, lacks the subshrubby growth habit, possesses a distinct type of inflorescence, a significantly longer calyx, and a notably bigger corolla (Meikle 1977).

The new species differs from the acaulescent or subacaulescent species of the Flora of Iran such as *H. renzii* Rech. f., *H. monophyllum* Boriss., *H. lehmannianum* Bunge, *H. brahuicum* Boiss., *H. sericeum* M.Bieb. and *H. plumosum* Boiss. & Hausskn. by having 3–4 pairs of leaflets; from *H. minjanense* Rech.f. by its obovate–oblong leaflets; from *H. volkii* Rech.f. by the calyx length, the calyx teeth length, and the unstriated corolla and from *H. papillosum* Boiss. and *H. elbursense* Bornm. & Gauba by the calyx length, the calyx teeth being 1–3 times as long as the tube, corolla length, and the dense sericeous indumentum restricted to the lower surface of the leaflets (Rechinger 1984, Amirabadi-zadeh 2011). It differs from *H. johartchii* Ranjbar by its greenish and smaller and different shape leaflets, a higher number of leaflet pairs, capitate inflorescence, and different corolla colour (Ranjbar *et al.* 2006).

It is distinguished from the *Hedysarum* species recorded in Iraq (*H. pannosum* (Boiss.) Boiss., *H. singarense* Boiss. & Hausskn., *H. kotschyi* Boiss., *H. varium* Willd.) by its markedly reduced stem (subacaulescent habit) and the standard that clearly exceeds the keel in length (Townsend & Guest 1974).

The new species shows resemblance to *H. coelesyriacum* Sam. ex Rech.f., which is distributed in Syria, particularly due to its short stem (0.5–8 cm). However, it differs by its capitate inflorescence, longer and glabrous corolla, standard being longer than the keel, and distinct fruit size and indumentum (Post 1896, Rechinger 1950).

Compared to the closely related Caucasian species, the new taxon differs from *H. bordzilovskyi* Grossh. by having a greater number of leaflet pairs, leaflet indumentum, inflorescence, bigger corolla, and glabrous standard. It also differs from *H. argenteum* M. Bieb. by its shorter habit, fewer leaflet pairs, indumentum, calyx shorter than the corolla, corolla color, and wings shorter than the standard (Grossheim 1952).

Among the *Hedysarum* species distributed in Greece and Bulgaria, the new taxon is morphologically close to *H. grandiflorum* Pall. subsp. *bulgaricum* Kožuharov, but differs by having smaller leaflets, a calyx that is not green tinged red and has a distinct indumentum, shorter calyx teeth, and corolla color (Polunin 1980, Constantinidis 2012).

The pollen grains of *H. marashicum* and the closely related species *H. erythroleucum* were compared in detail. The results of pollen morphology investigations show that *H. marashicum* and *H. erythroleucum* pollens are confirmed the general description of *Hedysarum* presented by Ohashi (1971), Faegri & Iversen (1989), Moore *et al.* (1992), Choi & Ohashi (1996), Pavlova & Manova (2000), Ghanavati & Amirabadizadeh (2012), Saatçioğlu (2017), Aytaç *et al.* (2022), and Ertuğrul *et al.* (2024). Choi & Ohashi (1996) reported that tricolpate pollen type and reticulate sculpture is the most common in the tribe Hedysareae. Similarly, in our study, the pollen type of the two examined species was identified as tricolpate and the sculpture is reticulate. Saatçioğlu (2017) conducted a detailed study of the pollen morphology of 31 *Hedysarum* taxa. Among them, three taxa from the section *Subacaulia*; *H. elegans*, *H. erythroleucum*, and *H. cappadocicum* were analyzed, including the section to which the newly described taxon also belongs. It was found that all three have prolate-shaped pollen. However, when the palynological comparison of *H. marashicum* and *H. erythroleucum* is conducted, the first noticeable difference emerges in the shape of their pollen. While *H. marashicum* has subprolate pollen, the pollen shape of *H. erythroleucum* is identified as prolate, consistent with the findings of Saatçioğlu (2017). Additionally, the pollen of *H. marashicum* is rectangular—*H. erythroleucum* obtuse in equatorial view, whereas *H. erythroleucum* is elliptical. One of the most significant differences between the pollen of these two species is the variation in apocolpium diameter. The apocolpium diameter of the new described taxon is significantly longer than that of *H. erythroleucum*. Furthermore, the size of the reticules in the mesocolpium also differs between the two species. The lumina diameter of the reticules in the mesocolpium of *H. marashicum* is shorter than that of *H. erythroleucum*.

In the majority of angiosperms, the plastid genome is transmitted from one parent (uniparentally), while the nuclear genome is inherited from both parents (biparentally) (Wicke *et al.* 2011). Following numerous generations of backcrossing, the introgressed plant acquired the plastid genome from one progenitor and predominantly all nuclear genes from the alternate progenitor (Nafisi *et al.* 2019). A key features of chloroplast capture is that plastid genome introgression typically occurs without nuclear introgression (Wendel & Doyle 1998). The using multiple DNA regions in this study significantly enhance our comprehension of the evolutionary relationships among the examined *Hedysarum* species and the association of newly identified species (*Hedysarum marashicum*). In agreement with the previous morphological taxonomic (Dehshiri & Goodarzi 2016, Başköse *et al.* 2018, Hamzaoglu & Koç 2020, Juramurodov *et al.* 2021) and molecular revision studies (Nafisi *et al.* 2019, Liu *et al.* 2019, Juramurodov *et al.* 2023, Juamurodov *et al.* 2024) using the combination of data always reliable for identifying the new species. Juramurodov *et al.* (2023) conducted a comprehensive study on the phylogenetic relationships of *Hedysarum*. Consistent findings were achieved in both datasets from different gene areas (nr and cp DNA) about morphological distinctions, especially with the closely related species. In this study, the *trn* portions of cpDNA and the ITS gene region of nrDNA were used, similar to our present investigation and In the phylogenetic trees, based on a combination of cpDNA gene regions, both *H. cappadocicum*, *H. elegans*, and *H. erythroleucum* were situated in one clade, whereas *H. huetii* and *H. syriacum* were placed in another clade, consistent with our present results. Furthermore, the phylogenetic tree of the ITS gene region in that study suggested similar separations, which were corroborated by the findings of our present research. Furthermore, our newly diverged species *H. marashicum* was distinguished from these species in our phylogenetic trees with its closely related taxa (both cpDNA and nrDNA). Consequently, it could be inferred that the molecular data corroborates the morphological data in the context of species discrimination.

In conclusion, the findings of this study indicate that the morphological, palynological, and phylogenetic differences provide sufficient evidence to establish *Hedysarum marashicum* as a new species.

An identification key of new species to most closely related taxa:

1. Stem absent or very short (1–5 cm)
2. Corolla ± as long as calyx, slightly exerted *H. cappadocicum*
2. Corolla ½–¾ longer than calyx, clearly exerted
3. Calyx teeth 1–3 × longer than calyx tube; inflorescence capitate
4. Leaflets ovate–rounded, 4–7 × 3–4 mm, very densely sericeous on both surfaces; corolla intensely purple, standard 8–15 × 8–11 mm *H. erythroleucum*
4. Leaflets obovate–oblong, 8–12 × 4–6 mm, densely sericeous below and sparsely sericeous above; corolla bright pink, standard 17–20 × 11–13 mm *H. marashicum*
3. Calyx teeth 3–4 × longer than calyx tube; inflorescence elongated or loosely capitate
5. Peduncle up to 17 cm long
6. Bracts 5–6 mm long; bracteoles 3.5–4.5 mm long; corolla white or sometimes very pale pinkish *H. turcicum*
6. Bracts 8–15 mm long; bracteoles (7–)8–11 mm long; corolla purplish *H. elegans*
5. Peduncle 25–40 cm long *H. sericeum*
1. Stem more than 5 cm other *Hedysarum* species

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Appendix

Examined specimens: *Hedysarum erythroleucum*: Van: Bahçesaray, Bahçesaray—Van yolu, Karabel (Karpit) geçidi, yamaçlar, 3000–3200 m, N. Demirkuş 6026 (GAZI!). Kahramanmaraş: Göksun, Berit dağı, Arpa çukuru mevki, step, 2400–2450 m, 02.06.1989, Z. Aytac 2651 (GAZI!). Kahramanmaraş: Binboğa Dağı, 2750 m, P. H. Davis 19990 (ANK!). Hakkari: Cilo Dağı, 3500 m, 09.08.1954, P. H. Davis 24209 & O. Polunin (ANK!). Bitlis-Van, 2652 m, 08.08.1954, P. H. Davis 22526. Van: Gevaş, Artos Dağı, yamaçlar, 3353 m, 15.07.1954, P. H. Davis 22891 & O. Polunin (ANK!). Van: Çatak, Kavuşşahap Dağı, yamaçlar, 3300 m, 23.07.1954, P. H. Davis 23211 & O. Polunin.

Hedysarum cappadocicum: Afyon: Afyon-Şuhut yolu, 15. km, hareketli kayalar, 1100 m, 18.05.2001, E. Akçiçek 3540 (GAZI!). Ankara: Polatlı—Sivrihisar arası, 18. km, Acıkır mevki, Karaemin tepe çevresi, 840–860 m, 04.06.1991, H. Duman 3824 (GAZI!). Sivas: Sivas-Gürün, 13 km SW Kangal, gipsberg, 1580 m, 14.06.1992, M. Nydegger 46273 (GAZI!).

Hedysarum turcicum: Yozgat: Boğazlıyan, Around Yazıkışla village, marly steppe, 1280 m, 06.07.2017, E. Hamzaoglu 2942 & M. Koç holo and isotype (GAZI!).

Hedysarum elegans: Erzurum: Olur, Akdağ, Ceviz dereleri mevki, seyrek çalılık, 1400 m, 26.05.1996, E. Kaya 9024 (GAZI!). Erzurum: Göle, Göle—Oltu 6 km N Oltu, versteppter hügel, 1270 m, 22.05.1990, M. Nydegger 45504 (GAZI!).

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