adansonia

2025 • 47 • 21

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art. 47 (21) — Published on 24 November 2025
www.adansonia.com

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Adansonia *is a fast track journal published by the Museum Science Press, Paris*

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Diffusion – Publications scientifiques Muséum national d'Histoire naturelle
CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél.: 33 (0)1 40 79 48 05 / Fax: 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / http://sciencepress.mnhn.fr

ISSN (imprimé / *print*): 1280-8571/ ISSN (électronique / *electronic*): 1639-4798

A new subspecies of *Corydalis wendelboi* Lidén (Papaveraceae) from NW Türkiye, evidence from morphological and molecular analysis

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Submitted on 28 February 2025 | accepted on 30 April 2025 | published on 24 November 2025

Olcay B., Kültür Ş. & Doğru-Koca A. 2025. — A new subspecies of *Corydalis wendelboi* Lidén (Papaveraceae) from NW Türkiye, evidence from morphological and molecular analysis. *Adansonia*, sér. 3, 47 (21): 341-351. https://doi.org/10.5252/adansonia2025v47a21. http://adansonia.com/47/21

ABSTRACT
Corydalis wendelboi subsp. *fragilis* subsp. nov. (Papaveraceae), a new subspecies is described from northwest Anatolia, Türkiye. It is closely related to *C. wendelboi* subsp. *wendelboi* Lidén and *C. wendelboi* subsp. *congesta* Lidén & Zetterl. but it differs in having very narrowly lobed leaves, in having bracts, which are smaller and divided into very thin lobes, and in more lax racemes. The ribosomal internal transcribed spacer (ITS 1, 5.8 S rRNA, ITS 2) region was employed to ascertain the phylogenetic relationship of *Corydalis* DC. species, utilising Maximum Likelihood and Bayes Inference methods. The phylogenetic results provided compelling evidence in support of the description of this new subspecies. An identification key has been created to facilitate the morphological distinction of closely related taxa.

KEY WORDS
Papaveraceae,
Corydalis,
Turkey,
ITS,
identification key,
new subspecies.

RÉSUMÉ
Une sous-espèce nouvelle de Corydalis wendelboi *Lidén (Papaveraceae) du nord-ouest de la Turquie, appuyée par des analyses morphologiques et moléculaires.*
Corydalis wendelboi subsp. *fragilis* subsp. nov. (Papaveraceae), une sous-espèce nouvelle est décrite du nord-ouest de l'Anatolie, en Turquie. Elle est étroitement apparentée à *C. wendelboi* subsp. *wendelboi* Lidén et à *C. wendelboi* subsp. *congesta* Lidén & Zetterl., mais elle s'en distingue par ses feuilles très étroitement lobées, ses bractées plus petites et divisées en lobes très fins, et ses grappes plus lâches. La région de l'espace interne transcrit ribosomique (ITS 1, 5.8 S rRNA, ITS 2) a été utilisée pour déterminer la relation phylogénétique des espèces *Corydalis* DC., en utilisant les méthodes de maximum de vraisemblance et d'inférence bayésienne. Les résultats phylogénétiques ont fourni des preuves convaincantes, soutenant la description de cette nouvelle sous-espèce. Une clé d'identification est fournie, pour faciliter la distinction morphologique des taxons étroitement apparentés.

MOTS CLÉS
Papaveraceae,
Corydalis,
Turquie,
ITS,
clé d'identification,
sous-espèce nouvelle.

INTRODUCTION

Corydalis DC. *in* Lamarck & Candolle (1805) is the largest genus in the family Papaveraceae, and comprises about 538 species (POWO 2024). *Corydalis* species are distributed in temperate regions of the Northern Hemisphere (Lidén & Zetterlund 1997). The genus *Corydalis* has consistently been regarded as one of the most taxonomically challenging due to species identification and complex polymorphisms. In recent years, extensive phylogenetic studies have enabled *Corydalis* to be classified with greater accuracy (Pérez-Gutiérrez *et al.* 2015; Ren *et al.* 2019; Xu & Wang 2021; Xu *et al.* 2022; Chen *et al.* 2023; Liu *et al.* 2024). It has been proposed that morphological data should be interpreted in conjunction with molecular data in the infrageneric classification of *Corydalis* and that the difficulties encountered in the classification of species exhibiting variation can be illuminated by molecular data (Pérez-Gutiérrez *et al.* 2015; Ren *et al.* 2019). The genus *Corydalis* is represented by 23 taxa (18 species and five subspecies) in Türkiye, including the recently published *Corydalis varolaydinii* Yıldırım. Of these 23 taxa, nine are endemic (Aslan 2012; Özdöl *et al.* 2024). The first comprehensive revision of the genus *Corydalis* in Türkiye was made by Davis & Cullen (1965) in *Flora of Turkey and the East Aegean Islands*. Later in volume 11 of this work, published in 2000, the genus *Corydalis* was revised again by Lidén (2000). He described some new species, synonymized some taxa published in the first volume of *Flora of Turkey and the East Aegean Islands*, and discussed the current taxonomic status of these taxa. Lidén (1988) described a new species, *Corydalis wendelboi* Lidén & Zetterl, from Muğla province in southwestern Türkiye. He reported that this species was distributed in western Anatolia and had previously been described as *C. solida* subsp. *solida* (L.) Clairv. by Davis & Cullen (1965) in the first volume of the *Flora of Turkey*. In later work, Lidén (1996) described the new subspecies *C. wendelboi* subsp. *congesta* Lidén & Zetterl. from Uludağ in northwestern Anatolia. He reported that this taxon is homogeneous in the whole area of its distribution, but in the province of Ankara, there are also forms with narrower leaf lobes. Additionally, Lidén elevated *C. solida* subsp. *tauricola* to the category of species.

The *Corydalis* specimens were collected as part of the first author's PhD thesis. Molecular studies as part of this thesis showed that some populations previously identified as *C. wendelboi* formed a separate lineage. A larger sample was then examined morphologically. This examination led to the conclusion that some populations represented a new subspecies.

MATERIAL AND METHODS

Plant material and morphological studies

The first author collected Plant samples in Türkiye between 2019 and 2024. The specimens were identified and their morphological characteristics were analyzed in detail. Plant samples were identified using Flora of Turkey and the East Aegean Islands Vol 11 (Lidén 2000). Prepared voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE). Many *Corydalis* specimens in other herbaria, such as ANK, GAZI, HUB, ISTF, and others in Türkiye were also investigated. The complete list of specimens examined is given in the Appendix.

DNA extraction, PCR and sequencing

The total genomic DNAs were isolated from silica gel dried leaf tissues from *Corydalis wendelboi* subsp. *fragilis* subsp. nov., *C. wendelboi* subsp. *wendelboi* and *C. wendelboi* subsp. *congesta* using Exgene™ Plant SV GeneAll® Mini Kit (Seoul, Republic of Korea). The DNA extraction protocol indicated by the manufacturer was followed. The primer pair used for amplifying the ITS region was ITS5/ITS4 (White *et al.* 1990). The relevant marker sequences of all of the other taxa used for phylogenetic analysis were obtained from GenBank. Their NCBI accession numbers are listed in Table 1.

In the present study, the ITS1, 5.8S rRNA, ITS2 region was amplified in a single reaction using ITS 4 (R) (5' TCC TCC GCT TAT TGA TAT GC 3') and ITS 5 (F) (5' GGA AGT AAA AGT CGT AAC AAG G 3') primers, following White *et al.* (1990). The thermocycling conditions were as follows: initial denaturation step of 94°C for 3 min, followed by 35 cycles: denaturation at 94°C for 60s, primer annealing at 53°C for 90s, and extension at 72°C for 90s; a final extension at 72°C for 10 min; and maintenance at 4°C before electrophoresis. PCR reactions were carried out using the T100 Thermal Cycler (Bio-Rad, Hercules, CA, United States). Sanger sequencing of PCR products was performed by BM Labosis Inc (Ankara, Türkiye).

Phylogenetic analyses

In the present study, sequences of ribosomal DNA internal transcribed spacer (ITS1, 5.8S rRNA, ITS2) were obtained from three samples belonging to two different populations of *C. wendelboi* subsp. *fragilis* subsp. nov. and from samples belonging to one population, each from five different *Corydalis* taxa distributed in Türkiye and closely related to the new subspecies. In addition to the ITS sequences of *Corydalis* taxa obtained in this study, the datasets of a total of 26 *Corydalis* taxa, the majority of which are not distributed in Türkiye, were obtained from the NCBI database and included in the study. All sequences found for the sect. *Corydalis* (with the exception of duplicates and those of doubtful identification) has been added to the phylogenetic tree. *Corydalis bungeana* Turcz. was selected as an outgroup (Jiang *et al.* 2018; Ren *et al.* 2019; Xu *et al.* 2022). The data sets taken from the NCBI database and used in the analyses are given in Table 1 with their accession numbers.

All sequences were aligned with MAFFT v.7 (Katoh *et al.* 2017) and then manually checked with BioEdit v.7.2. The evolutionary substitution model was determined as GTR-Gamma according to Akaike Information Criterion(AIC) using RaxMLGUI 2.0. Maximum Likelihood (ML) analyses of the aligned data were performed using RaxMLGUI 2.0 (Edler

TABLE 1. — Taxa, voucher numbers/reference and accession numbers of ITS sequences utilized in this study. The bold accession numbers are obtained herein.

Taxon	Voucher no./reference	Accession number
Corydalis wendelboi subsp. *fragilis* subsp. nov.	ISTE118601 (holotype)	**PP849377**
Corydalis wendelboi subsp. *fragilis* subsp. nov.	ISTE 18598	**PP849378**
Corydalis wendelboi subsp. *fragilis* subsp. nov.	ISTE118781	**PQ656721**
Corydalis wendelboi subsp. *congesta* Lidén & Zetterl.	ISTE118591	**PP849382**
Corydalis wendelboi subsp. *wendelboi* Lidén & Zetterl.	ISTE118780	**PQ656720**
Corydalis wendelboi subsp. *wendelboi* Lidén & Zetterl.	ISTE118585	**PP849381**
Corydalis tauricola (Cullen & Davis) Lidén	ISTE94344	**PP849383**
Corydalis "*caucasica alba*"	Lidén *et al.* 1995	X85468
Corydalis intermedia Mérat	V-92966	MN662998
Corydalis henrikii Lidén	Lidén *et al.* 1995	X85476, X85487
Corydalis solida subsp. *incisa* Lidén	ISTE117077	**PP849380**
Corydalis solida subsp. *solida* (L.) Clairv.	ISTE117072	**PP849379**
Corydalis pumila W.D.J. Koch	O-392657	MN662999
Corydalis solida subsp. *solida* (L.) Clairv.	Lidén *et al.* 1995	X85470, X85472
Corydalis solida subsp. *incisa* Lidén	Lidén *et al.* 1995	X85464, X85471
Corydalis decumbens Pers.	ZJ006MT01	MH349117
Corydalis decumbens Pers.	ZJ006MT03	MH349119
Corydalis tauricola (Cullen & Davis) Lidén	Lidén *et al.* 1995	X85478, X85480
Corydalis repens var. *repens* Mandl & Muehld.	ZJ013MT02	MH349154
Corydalis repens var. *repens* Mandl & Muehld.	ZJ013MT01	MH349153
Corydalis repens var. *watanabei* (Kitag.) Y.C.Chu	ZJ014MT01	MH349156
Corydalis repens var. *watanabei* (Kitag.) Y.C.Chu	ZJ014MT02	MH349157
Corydalis repens var. *repens* Mandl & Muehld.	ZJ013MT03	MH349155
Corydalis ambigua Cham. & Schltdl.	–	FJ469598
Corydalis linjiangensis Z.Y.Su ex Lidén	ZJ009MT02	MH349137
Corydalis linjiangensis Z.Y.Su ex Lidén	ZJ009MT03	MH349138
Corydalis turtschaninovii Besser	ZJ020MT04	MH349205
Corydalis turtschaninovii Besser	NICOMVP0000	KY305172
Corydalis yanhusuo W.T.Wang	ZJ022MT06	MH349215
Corydalis yanhusuo W.T.Wang	ZJ022MT01	MH349210
Corydalis dajingensis C.Y.Wu & Z.Y.Su	39676	MH293121
Corydalis benecincta W.W.Sm.	ZYZ90	MH293114
Corydalis glaucescens Regel	Q-010	MT924005
Corydalis nariniana Fed.	Lidén *et al.* 1995	X85460, X85463
Corydalis bungeana Turcz.	Ren *et al.* 2019	MH349108

et al. 2021). In 500 simultaneously conducted analyses, the 'Bootstrap' analysis was performed with 1000 replications. Bayesian analysis (BI) was performed at 10×10^6 generations using BEAUti and BEAST 2.7.4 programs. The maximum clade credibility (MCC) tree was constructed using TreeAnnotator 2.7.4 (Drummond & Rambaut 2007). The resulting trees were visualized and edited in FigTree v1.4.4. (Rambaut 2018). The first 10% of the resulting trees were burned. Log files were checked in Tracer v.1.7.1 (Rambaut *et al.* 2018). Effective sample size (ESS) values were greater than 200 for each run. Posterior probabilities (PP) and bootstrap support values (BS) were added to the phylogenetic tree (Figure 1).

ABBREVIATIONS

Herbarium acronyms

AEF	Herbarium, Faculty of Pharmacy, Ankara University;
ANK	Herbarium, Faculty of Science, Ankara University
DUOF	Herbarium of Duzce University Faculty of Forestry
E	Herbarium, Royal Botanic Garden Edinburgh;
EGE	Herbarium, Faculty of Science, Ege University;
GAZI	Herbarium, Faculty of Science, Gazi University, Ankara;
HUB	Herbarium, Faculty of Science, Hacettepe University, Ankara;
ISTE	Herbarium, Faculty of Pharmacy, Istanbul University;
ISTF	Herbarium, Faculty of Science, Istanbul University;
NGBB	Herbarium, Nezahat Gökyiğit Botanical Garden, Istanbul.

TAXONOMIC TREATMENT

Family PAPAVERACEAE Juss.
Genus *Corydalis* DC.

Corydalis wendelboi subsp. ***fragilis***
Olcay, Doğru-Koca & Kültür, subsp. nov.
(Figs 2; 3)

TYPE MATERIAL. — **Turkey** • A3 Ankara, Kızılcahamam, Şahinler Nature Park, woodlands; 40°38'10.2"N, 32°26'10.3"E; 1480 m a.s.l.; 7.V.2021; *B. Olcay s.n.*; holotype: ISTE[ISTE118601]!; isotype: HUB! • A3 Bolu, Near Aladağ Lake; 1391 m a.s.l.; 7.V.2021; *B. Olcay s.n.*; paratype: ISTE[ISTE118598]!).

DIAGNOSIS. — *Corydalis wendelboi* subsp. *fragilis* subsp. nov. is closely related to *C. wendelboi* subsp. *wendelboi*, *C. wendelboi* subsp. *congesta* and *C. tauricola*, because of its divided bracts; a ratio of pedicel to bract length and obtuse lower petals. However, it differs from *C. tauricola* by its short pedicels, 1-2 mm (not 7-15 mm) and its slightly incised ± equal lobes of bracts (not central lobe of bract largest). It differs from *C. wendelboi* taxa by its narrowly lobed leaves, 2-3 mm broad (not 3.5-5 mm); by its smaller bracts, 5-6 mm long (not 9-10 mm), which are divided into very thin lobes (not divided into thick lobes as in subsp. *wendelboi* or into moderately thin lobes as in subsp. *congesta*) and by its lax racemes (not dense). *C. wendelboi* subsp. *fragilis* subsp. nov. also differs from subsp. *wendelboi* because of its smaller seeds, 1-1.2 mm in diameter (*c.* 1.5 mm in diameter in subsp. *wendelboi*) (Table 2; Figs 3; 4).

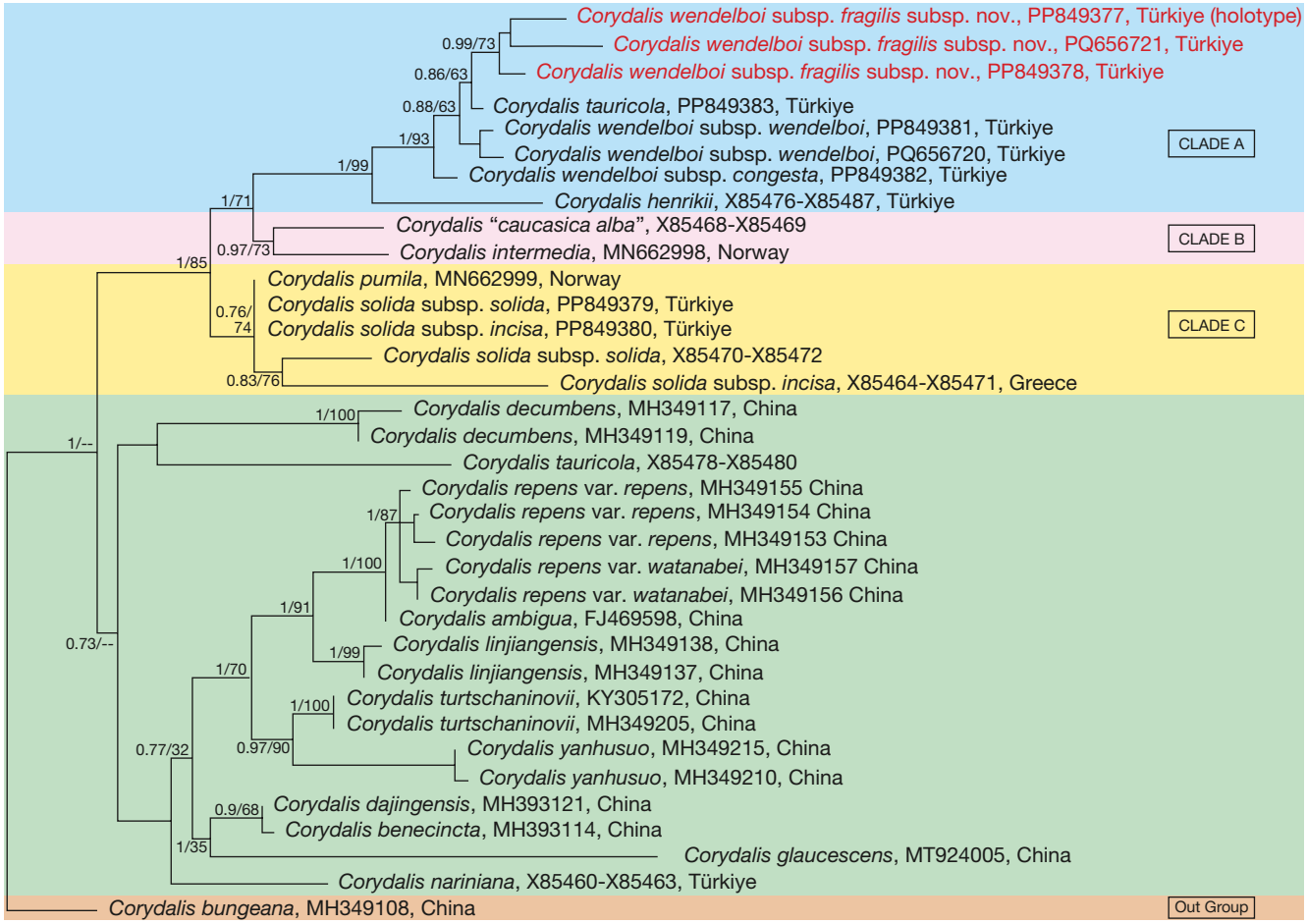

Fig. 1. — Bayesian consensus tree of *Corydalis* DC. species based on ribosomal DNA internal transcribed spacer (ITS 1, 5.8S, ITS 2). The numbers adjacent to the nodes indicate posterior probabilities and bootstrap values obtained, respectively, derived from BI and ML analysis.

Etymology. — The epithet 'fragilis' alludes to the delicate and fragile nature of the plant.

Phenology. — Flowering commences at the beginning of April and continues until the end of May. Mature fruits have been collected in May.

Distribution and ecology. — *C. wendelboi* subsp. *fragilis* subsp. nov. is endemic to Türkiye and is known from only two localities near the border between the provinces of Bolu and Ankara (Figs 5; 6). It occurs in the subalpine zone, at altitudes of 1400-1600 m a.s.l, in rocky habitats in sparse *Pinus nigra* forests and areas sheltered by *Juniperus communis* subsp. *nana*.

IUCN status. — *C. wendelboi* subsp. *fragilis* subsp. nov. is known from only two locations in Bolu and Ankara provinces (Criterion B2a), with an estimated area of occupancy that is less than 10 km^2 (Criterion B2b). A small population of between 30 and 40 individuals has been observed at both locations. Furthermore, no subpopulation was found to contain more than 50 mature individuals, with the expected continuing decline observed in all cases (Criterion C2). The number of mature individuals is less than 50 (Criterion D). Furthermore, the species' habitat has been affected by factors such as road construction, overgrazing and human pressure. Therefore, it should be classified as "Critically Endangered (CR)" according to the IUCN Red List criteria (IUCN 2022). Category: CR. B2ab(ii, iv, v); C2a(i); D.

Local name. — The Turkish name "Narin tarlakuşu" is proposed for this subspecies, according to the guidelines of Menemen *et al.* (2016).

Additional specimens examined. — *C. wendelboi* subsp. *fragilis* subsp. nov.: **Turkey** • A3 Ankara, Kızılcahamam, Şahinler Nature Park, woodlands; 40°38'10.2"N, 32°26'10.3"E; 1450 m a.s.l.; 22.IV.2024; *B. Olcay & F. Nur Olcay s.n.*; ISTE[ISTE118781]!).
C. wendelboi subsp. *wendelboi*: **Turkey** • C2 Denizli, Mount Honaz, Tuzluca Plateau; 1320 m a.s.l.; 8.IV.1973; *E. Tuzlacı s.n.*; ISTE[ISTE24024]! • C2 Muğla, Muğla-Göktepe TV transmitting station; 1450 m a.s.l.; 20.IV.1981; *M. A. Önür 483*; ISTE[ISTE47753]! • C4 Konya, Ermenek; 1.IV.1993; *A. Byfield 151*; ISTE[ISTE65756]! • C3 Antalya, Elmalı, Çam Kuyusu district; 1580 m a.s.l.; 16.IV.1993; *N. Özhatay & A. Byfield s.n.*; ISTE[ISTE65037] • B3 Afyon, Sultandağları, above Dereçine; 1450 m a.s.l.; 19 IV.1976; *A. Baytop s.n.*; ISTE[ISTE34558]! • B1 İzmir, Mount Nif; 990 m a.s.l.; 26.III.1981; *A.&T. Baytop s.n.*; ISTE[ISTE46151]! • C4 Konya, Ermenek, Mount Yeşilgöl; 1500 m a.s.l.; *Remark & Wendelbo s.n.*; ISTE [ISTE101596]! • C3 Konya, Beyşehir, Kurucova, Suludere district; 1500 m a.s.l.; 8.VI.1975; *H. Peşmen & A. Güner 2183*; HUB[HUB01517]! • C4 Konya, Seydişehir, Şahinkayası district; 1500 m a.s.l.; 14.IV.1982; *H. Ocakverdi s.n.*; ANK[ANK1358]! • C2 Muğla, Köyceğiz, Sultaniye district; 17.IV.1992; *A. Güner H. Duman & A. Dönmez 10450*; GAZI[GAZI 210919]! • A3 Ankara, Ayaşbeli Çal Hill; 1380 m a.s.l.; 13.IV.1986; *M. Vural s.n.*; GAZI[GAZI4030]! • C3 Konya, Beyşehir-Akseki Plateau, Baharan district; 1400 m a.s.l.; 21.IV.1983, *M. Koyuncu 5934*; AEF[AEF13415]! • A3 Bolu, Göynük, Sünnetgölü district; 965 m a.s.l.; 7.IV.2009; *A. Güner, M. Johnson & N. İkinci 15387*; NGBB[NGBB001980]! • B4 Ankara, Beypazarı, Sığıryatağı Hill; 1400 m a.s.l.; 24.IV.2013; *A.Ö. Çimen s.n.*; NGBB[NGBB009180]! • B2 İzmir, Ödemiş, above Bozdağ

FIG. 2. — *Corydalis wendelboi* subsp. *fragilis* subsp. nov.: **A**, habit; **B**, inflorescence; **C**, fruits and bracts. Scale bars: A, 3 cm; B, C, 1 cm. Photos by B. Olcay.

TABLE 2. — Morphological features comparisons of *Corydalis wendelboi* subsp. *fragilis* subsp. nov., *C. wendelboi* subsp. *wendelboi* Lidén, *C. wendelboi* subsp. *congesta* Lidén & Zetterl., and *C. tauricola* (Cullen & P.H.Davis) Lidén.

	C. wendelboi* subsp. *congesta	***C. wendelboi* subsp. *wendelboi***	***C. wendelboi* subsp. *fragilis* subsp. nov.**	***C. tauricola***
Leaf lobes	4-5 mm wide	4-5 mm wide	less than 3mm wide	4-5 mm wide
Inflorescence	very dense, 10-25 flowered	moderately dense, 10-15 flowered	lax, 5-15 flowered	moderately dense, 5-15 flowered
Bracts	*c.* 10 mm long, broadly flabellate, divided into unequal sized lobes	*c.* 10 mm long, broadly obovate, divided into ± equal sized lobes	*c.* 5 mm long, obovate, deeply divided into dense, unequal sized lobes	*c.* 10 mm long, obovate, divided, central lobe of bracts usually largest
Pedicels	2-4 mm long	2-4 mm long	1-2 mm long	7-15 mm long
Corolla	lower petal 4-6 mm broad, not emarginate at the apex, spur 9-13 mm long	lower petal 2-4 mm broad, not or slightly emarginate at the apex, spur 9-13 mm long	lower petal 2-4 mm broad, not emarginate at the apex, spur 7-12 mm long	lower petal 4-6 mm broad, slightly emarginate at the apex, spur 12-14 mm long
Capsule	narrowly lanceolate, 6-11 seeded, (8)10-18 × 2.5-5 mm	narrowly lanceolate, 6-11 seeded, (12)14-24 × 3-5 mm	lanceolate, 10-17 seeded, (12)13-18 × 2.5-4 mm	lanceolate, 3-8 seeded, 18-28 × 2 mm
Seed	1.5-1.8 mm in diam., elaiosome spirally twisted	1.5-1.9 mm in diam., elaiosome spirally twisted	1.2-1.4 mm in diam., elaiosome spirally twisted	1.6-2.2 mm in diam., elaiosome spirally twisted

village; 1300 m a.s.l.; 3.IV.1972; *E. Leblebici s.n.*; EGE[EGE15109]!. *Corydalis wendelboi* subsp. *congesta*: **Turkey** • A3 Bolu, around Lake Abant; 1350 m a.s.l.; 28.IV.2019; *M. Koçyiğit s.n.*; ISTE[ISTE116560]! • A2 (A) Bursa, Uludağ, Kirazlıyayla district; 24.IV.1964; *A & T. Baytop s.n.*; ISTE[ISTE7587]! • B3 Eskişehir, between the junction of Kütahya and Eskişehir roads and Bozüyük; 17.IV.1965; *A & T. Baytop s.n.*; ISTE[ISTE7878]! • A5 Amasya, Doğantepe Kayacık village; 950 m a.s.l.; 11.IV.1982; *A & T. Baytop s.n.*; ISTE[ISTE48459]! • A2 Kocaeli,

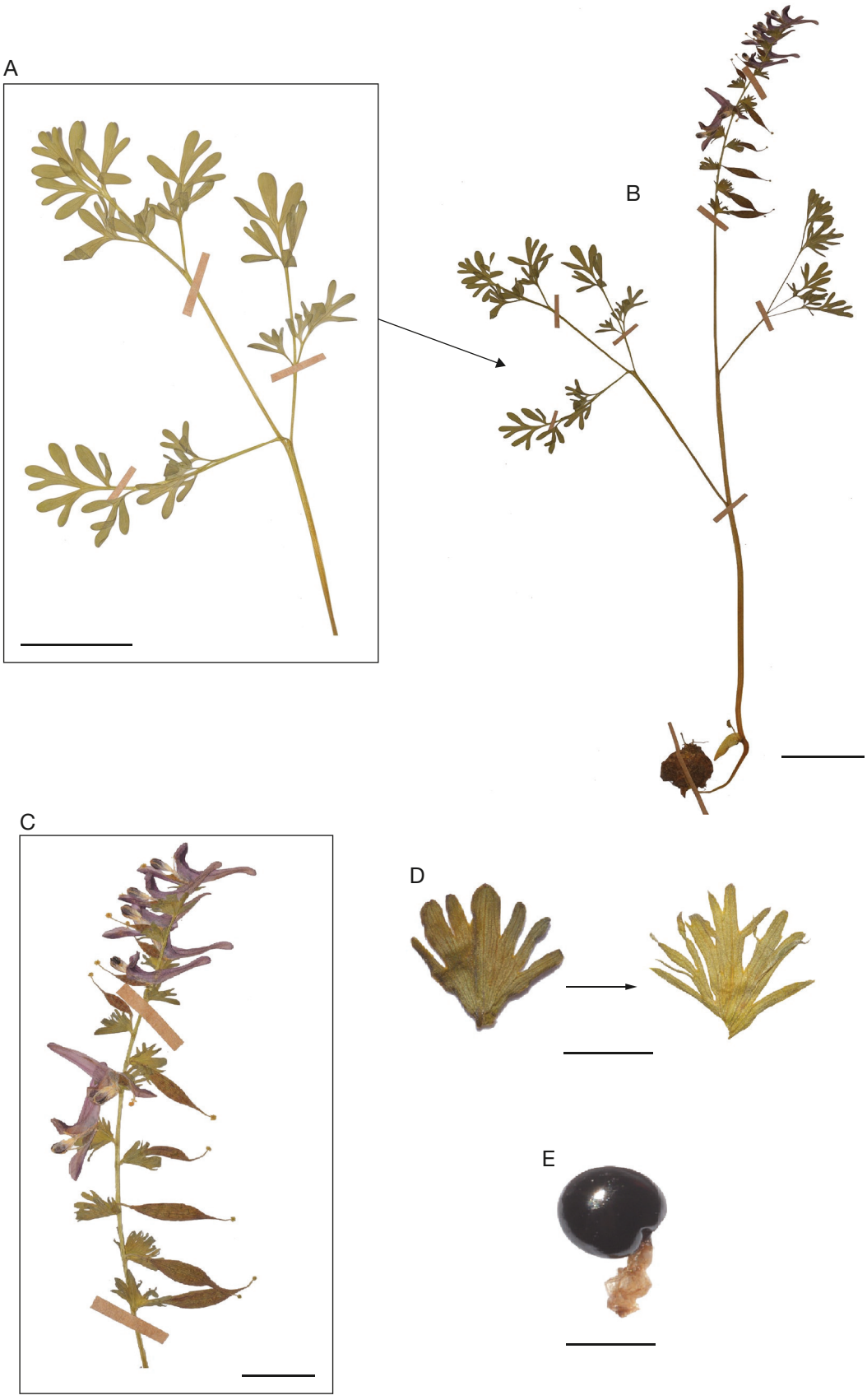

Fig. 3. — Herbarium specimen of *Corydalis wendelboi* subsp. *fragilis* subsp. nov., holotype ISTE118601: **A**, leaf; **B**, plant; **C**, raceme, flowers, fruits and bracts; **D**, bracts (more divided in maturity); **E**, seed. Scale bars: A, 2 cm; B, 3 cm; C, 1 cm; D, 3 mm; E, 1 mm. Photos by B. Olcay.

Fig. 4. — Herbarium specimens of: **A**, *Corydalis wendelboi* subsp. *fragilis* subsp. nov., holotype ISTE118601; **B**, *C. wendelboi* subsp. *wendelboi* Lidén, specimen ISTE118585; **C**, *C. wendelboi* subsp. *congesta* Lidén & Zetterl., specimen ISTE118591; **D**, *C. tauricola* (Cullen & P.H.Davis) Lidén, specimen ISTE94344. Bracts are magnified and shown in a circle. Scale bars: full specimens, 3 cm; details; 5 mm. Photos by B. Olcay.

Fig. 5. — Habitat of the holotype specimen of *Corydalis wendelboi* subsp. *fragilis* subsp. nov. Photo by B. Olcay.

Maşukiye- Kartepe (Keltepe); 30.V.1987; *N. Özhatay, E. Özhatay* & *M. Johnson s.n.*; ISTE[ISTE57794]! • A4 Kastamonu, Ilgaz passage, 40 km to Kastamonu; 1770 m a.s.l.; 18.V.1972; *A. & T. Baytop s.n.*; ISTE[ISTE21751]! • A3 Bolu, Abant Lake side, under *Pinus* forest; 11.IV.1973; *T. Baytop s.n.*; ISTE[ISTE24198]! • A5 Amasya, Akdağ, above Ormanözü village, Eğricepınar district, wetlands; 1200 m a.s.l.; 29.III.1977; *T. Baytop s.n.*; ISTE[ISTE36611]! • A1 (A) Balıkesir, Marmara Island, hills around the radar facility; 700 m a.s.l.; 16.III.1977; *E. Tuzlacı s.n.*; ISTE[ISTE36511]! • B2 Kütahya, Mount Murat, Kesik Söğüt district; 1600 m a.s.l.; 18.V.1978; *A. Baytop s.n.*; ISTE[ISTE39400]! • A4 Ankara, between Kızılcahamam and Gerede, near the passage; 1512 m a.s.l.; 30.III.2011; *E. Kaya 1807*; ISTE[ISTE99393]! • A3 Bolu, near Abant Lake; 1320 m a.s.l.; 28.III.2011; *E. Kaya 1782*; ISTE[ISTE95481]! • A3 Bolu, Sarıalan-Yazıören Plateau, rocky area; 1677 m a.s.l.; 4.V.2014*; H. Arslan 1024*; DUOF[DUOF0002247]! • A3 Düzce, Sinekli Plateau; 1399 m a.s.l.; 2.IV.2010; *N. Koçer 1082*; DUOF[DUOF0003500]! • A3 Bolu, Yedigöller National Park, yellow pine forest clearings; 1730 m a.s.l.; 15.IV.2018; *A. Ayteğin & Y. Zorlu 2339*; DUOF[DUOF0007674]! • B4 Ankara, Kızılcahamam, Mount Işık; 1500 m a.s.l.; *F. Demircioğlu & M. Koyuncu s.n.*; AEF[AEF7359]! • Kastamonu, Mount Ilgaz; 1800 m a.s.l.; 11.V.1982; *Y. Akman & E. Yurdakulol s.n.*; ANK[ANK12457]!

C. tauricola: **Turkey** • C6 Adana, Seyhan, Düldül Dağı, Haruniye; 1300 m a.s.l.; *P.H.Davis s.n.*; ANK[ANK26094]! • C5 Adana, Seyhan, Pozantı, Bürücek; 1300 m a.s.l.; 3.IV.1957; *Davis &Hedge s.n.*; ANK[ANK26350]! • C5 İçel, Namrun; 1000-1800 m a.s.l.; *Balls s.n.*; ANK[ANK176]! • C6 Adana, Saimbeyli, Bozoğlan Mt, Obruk Plateau; 1400 m a.s.l.; 13.IV.1957; *Davis & Hedge s.n.*; ANK[ANK26683]! • C6 Kahramanmaraş, Akkar Mt; 1524 m a.s.l.; 1.V.1934; *Balls s.n.*; ANK[ANK942]! • C4 İçel, Mut to Ermenek; 1800 m a.s.l.; 14.V.1965; *M.J.E. Coode & B.M.G. Jones s.n.*; E[E00410736]! • C4 Konya, Ermenek, Damlaçal, Sedir ormanı; 1750 m a.s.l.; 4.IV.1979; *M. Vural s.n.*; ANK[ANK1221]!, E[E00410737] • C6 Hatay, Amanus Mts, Karlik tepe from sogukoluk; 900 m a.s.l.; 26.III.1967; *M.J. Cheese s.n.*; E[E00410739] • C6 Kahramanmaraş, Engirek Mt, Biçmeoluk district; 1500 m a.s.l.; 21.IV.1987; *H. Duman s.n.*; GAZI[GAZI2485]! • B6 Kahramanmaraş, Göksun, Acalmak, Cennet river; 1800 m a.s.l.; 25.IV.1987; *B. Yıldız s.n.*; HUB[HUB01530]! • C4 Mersin, between Silifke and Gülnar, rocky places; 750 m a.s.l.; 7.III.1973; *M. Liden s.n.*; ISTE[ISTE23911]! • C5 Niğde, Çamardı district; 1740 m a.s.l.; *M. Liden s.n.*; ISTE[ISTE101598]! • C6 Hatay, Antakya, Harbiye- Yayladağı, near Şenköy village; 800 m a.s.l.; 20.III.1955; *H. Demiriz s.n.*; ISTF[ISTF13986]! • C6 Kahramanmaraş, Hartlap, between Fatih and Hacılar district, Bozarmut passage; 1580 m a.s.l.; 30.IV.2007; *A. Güner s.n.*; NGBB[NGBB00240]! • C6 Adana, Saimbeyli, between Karakuyu village and Sarıçiçek plateau; 1530 m a.s.l.; 22.III.2014; *A. Güner s.n.*; NGBB[NGBB006377]!

Description

Perennial, herbs, tuber simple ± spherical, 10-15(-20) mm in diameter. Stem very slender, erect 10-20(-25) cm long with one yellowish scale leaf at the base. Cauline leaves 2, green, alternate, 3(-4) × ternate, long-stalked with deeply divided leaflets. The segments very narrow, divided into obovate to obtuse laciniae, *c.* 3 mm wide at the mid-point. Racemes lax, 5-15 flowered. Bracts 5(-6) mm long, obovate, semiamplexicaul, divided deeply into numerous unequal acute to obtuse thin lobes. Bracts always longer than pedicels. Pedicels very short, 1-2(3) mm, patent to recurved in fruit. Corolla pale pink, pale lilac to reddish purple, with inner petals internally

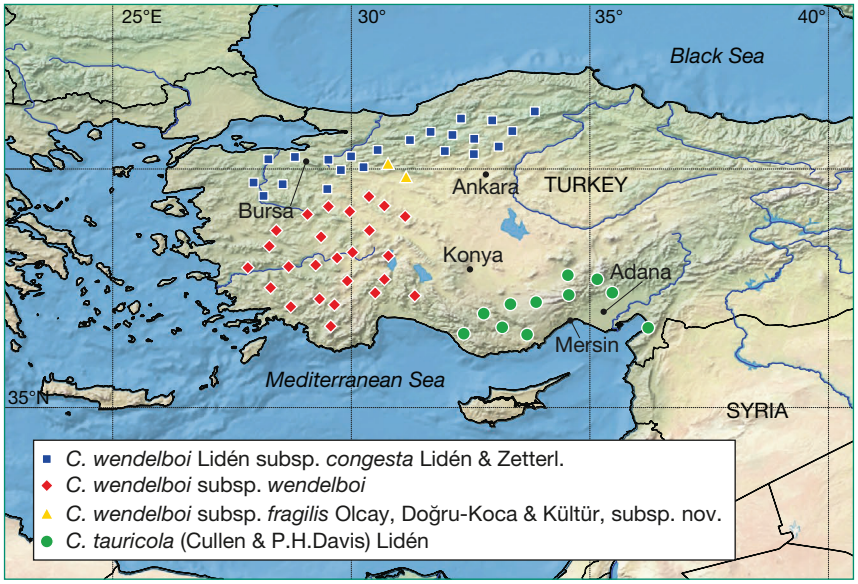

FIG. 6. — Distribution of *Corydalis wendelboi* subsp. *fragilis* subsp. nov. and closely allied taxa: *C. wendelboi* subsp. *wendelboi* Lidén, *C. wendelboi* subsp. *congesta* Lidén & Zetterl. and *C. tauricola* (Cullen & P.H.Davis) Lidén.

IDENTIFICATION KEY TO SPECIES MORPHOLOGICALLY RELATED TO *CORYDALIS WENDELBOI* SUBSP. *FRAGILIS* SUBSP. NOV. IN TÜRKIYE

1. Pedicels 7-15(20) mm, longer than bracts 2
— Pedicels 2-7 mm, shorter than bracts 3

2. Bracts divided into lax to dense ± equal lobes ..... *C. solida* (L.) Clairv.
— Bracts divided into lax unequal lobes, central lobe of bracts usually largest ..... *C. tauricola* (Cullen & P.H.Davis) Lidén

3. Racemes very dense 10-25 flowered, lower petal 4-6 mm broad ..... *C. wendelboi* subsp. *congesta* Lidén & Zetterl.
— Racemes moderately dense to lax 5-15 flowered, lower petal 2-4 mm broad 4

4. Racemes moderately dense 10-15 flowered, bracts *c.* 10 mm long, leaf lobes more than 5 mm wide, seeds more than 1.5 mm in diameter ..... *C. wendelboi* subsp. *wendelboi* Lidén
— Racemes lax 5-15 fowered, bracts *c.* 5 mm long, leaf lobes less than 3 mm wide, seeds less than 1.5 mm in diameter ..... *C. wendelboi* subsp. *fragilis* Olcay, Doğru-Koca & Kültür, subsp. nov.

tipped dark purple at the apex. Spur 7-12 mm long, straight to slightly curved; outer petals subspathulate, obtuse; lower petal 2-4 mm broad. Stigma flattened with simple lateral papillae. Nectary 4-6 mm long, *c.* ⅓-⅖ as long as spur. Capsule lanceolate, 10-17 seeded, (12-)13-18 × 2.5-4 mm. Seeds *c.* 1.2 mm in diameter, with spirally twisted elaiosome (Figs 2; 3).

MOLECULAR PHYLOGENY

The samples of the new subspecies *Corydalis wendelboi* subsp. *fragilis* subsp. nov. occurred in the clade A. The phylogenetic tree displayed that clade A was strongly supported by posterior probability and high bootstrap value (PP = 1, BS = 99) (Fig. 1). This clade was subdivided into subclades: three samples from two different populations of *C. wendelboi* subsp. *fragilis* subsp. nov. formed a monophyletic subclade with strong posterior probability and moderate bootstrap value (PP = 0.99 BS = 73). *C. tauricola* was a sister group of the *C. wendelboi* subsp. *fragilis* subsp. nov. lineage, with branch support values (PP = 0.86, weak BS = 63). In the second subclade, two samples from different populations of *C. wendelboi* subsp. *wendelboi* were on the same lineage with moderate posterior probability and weak bootstrap value (PP = 0.88, weak BS = 63). *C. wendelboi* subsp. *congesta* was a sister species of the *C. wendelboi* subsp. *wendelboi* lineage group was on the same lineage with strong posterior probability and high bootstrap value (PP = 1, BS = 93). *C. henrikii* was sister to *C. wendelboi* group. Regarding clade B, *C. intermedia* Mérat, and *C.* '*caucasica alba*', which is a garden-origin specimen and whose identification is uncertain (Lidén *et al.* 1995) conducted a clade with a high posterior probability and medium bootstrap value (PP = 0.97, BS = 73). In clade C, *C. pumila* and the intra-specific taxa of *C. solida* generate a monophyletic clade supported by a moderate posterior probability and bootstrap value (PP = 0.76, BS = 74). Sequences obtained from the GenBank and in this study demonstrate consistency.

DISCUSSION

Molecular studies conducted on the internal transcribed spacer (ITS) region have yielded data that can be employed to distinguish between certain species of *Corydalis* (Lidén *et al.* 1995; Ren *et al.* 2019). Nevertheless, it has been noted that some of the section classifications and different subgenus classification groups cannot be fully elucidated based on ITS data alone. Herein, sect. *Dublotuber* nested in sect. *Corydalis*. However, recently, the phylogenomic studies have provided definitive evidence that sect. *Corydalis* constitutes a monophyletic lineage (Chen *et al.* 2023; Liu *et al.* 2024). Jiang *et al.* (2018) also reconstructed the phylogeny of the *Corydalis* section and suggested that the ITS region is not an optimal marker for phylogenetic analyses. The failure of the ITS marker to yield section classification of the genus *Corydalis* in our study follows the findings of Jiang *et al.* (2018) and Chen *et al.* (2023). The *C. tauricola* sample (GenBank accession no: X85478, X85479, X85480) is nested within the Chinese *C. decumbens* (Thunb.) Pers., which is classified in the sect. *Duplotuber* Ryberg. The fact that this species occurs in the same clade as the Chinese species *C. decumbens*, although it is distributed in an area geographically distant from the former, and that no herbarium record of this sequence has been found, suggests that the specimen is suspect. The comprehensive herbarium investigations and field studies reveal that some plants previously identified as *Corydalis wendelboi* show morphological differences. These plants are morphologically smaller than *C. wendelboi* regarding both vegetative and generative characteristics. The diagnostic characteristics such as narrower leaf segments, smaller seeds, lax inflorescence, and smaller bracts (Figs 3; 4) indicated that these populations could be a new species of *Corydalis*. In the study conducted by Mikhailova (2023), a novel species of *Corydalis* is described. Mikhailova's (2023) study stated that the most evident morphological distinction of the new species from its closely related species is the presence of smaller bracts. Evidence has been provided to demonstrate that bract size is a significant factor in distinguishing between *Corydalis* species. The bract size differences observed in this study corroborate the new subspecies hypothesis. These morphological differences were also genetically supported (Fig. 1). Three ITS sequences belonging to two populations of *C. wendelboi* subsp. *fragilis* subsp. nov. generate a well-supported clade and differentiated *C. wendelboi* subsp. *wendelboi* population which is a species that is morphologically separated. The morphologically closest species to *C. wendelboi* subsp. *fragilis* subsp. nov. is *C. wendelboi* subsp. *wendelboi*. However, in terms of species distribution, it is isolated from *C. wendelboi* subsp. *wendelboi*. Its distribution lies within the range of the phylogenetically distant taxon *C. wendelboi* subsp. *congesta*. Considering the morphological data and phylogenetic results, we believe this evidence meets the diagnostic criteria for accepting *Corydalis wendelboi* subsp. *fragilis* subsp. nov. as a new subspecies.

Acknowledgements
We would like to thank Prof. Dr. Magnus Lidén for his valuable comments and contributions on the manuscript. Prof. Hasan Yıldırım, Dr Thierry Deroin and an anonymous reviewers are also thanked for their help in improving the first version of the manuscript. We would like to express our gratitude to the ANK, AEF, DUOF, GAZI, HUB, ISTE, ISTF and NGBB herbaria curators for allowing us to study their *Corydalis* specimens.

The present work was supported by the Scientific Investigation Project Coordinator of Istanbul University. Project No: 36281.

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Submitted on 28 February 2025;
accepted on 30 April 2025;
published on 24 November 2025.