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Dianthus berkayii (Caryophyllaceae), a new species from Turkey

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Abstract: *Dianthus berkayii* belongs to sect. *Fimbriati* was described and illustrated as a new species from Erzincan Province in Eastern Anatolia, Turkey. In the current study morphological, palynological, and molecular characters were compared and discussed with its closely related species. The new species is similar to *D. crinitus* with fimbriate corolla, linear, acuminate, ± rigid leaves. Also similar to *D. vanensis* with fimbriate corolla, linear, acuminate, ± rigid, curved divaricate leaves. But distinctly different from *D. crinitus* by having linear and curved divaricate leaves, stem with 3–4(–5) internodes (not 5–8), a style much longer than petals, and different from *D. vanensis* with narrower leaves, ebarbulate and long fimbriate corolla. The bracts of *D. berkayii* are 1/3 of the length of the calyx, but not equal to the calyx length. The IUCN threat category of *D. berkayii* was determined to be "CR (Critically Endangered)" therewithal it is an Irano-Turanian element. In the study, the geographical distributions of *D. berkayii, D. crinitus*, and *D. vanensis* are also mapped, the identification key of these species was provided, pollen morphology and phylogenetic analyses based on ITS region of rDNA and ecological notes are also presented.

Key words: *Dianthus,* taxonomy, palynology, phylogeny, Turkey

1. Introduction

Caryophyllaceae is one of the largest family with 86 genera and 2200 species in the world and usually found in all temperate regions of the world; generally, its distribution areas are Mediterranean and adjoining parts of Europe and southwestern Asia. Caryophyllaceae are systematically divided into 3 subfamilies: *Alsinoideae* "stipules absent and sepals free from each other", *Silenoideae* "stipules absent and sepals connate", and *Paronychioideae* "stipules present and usually scarious and sepals free or connate" (Heywood et al., 2007). Moreover, the genus *Dianthus* L. belongs to the *Silenoideae* subfamily, because of its opposite and exstipulate leaves and at least at the base connate sepals.

The Caryophyllaceae family is represented by 37 genera and 645 taxa in Turkey (Hamzaoğlu, 2012). *Silene* L., *Arenaria* L., *Minuartia* L., *Gypsophila* L., and *Dianthus* are represented by many taxa in Turkey (Davis, 1967). According to Reeve, who revised the genus *Flora of Turkey*, 67 species were reported (Reeve, 1967). Although according Plant List of Turkey, the genus *Dianthus*, has 86 taxa and 36 of them are endemic (Hamzaoğlu, 2012), it is represented by 82 taxa in the revision study by Hamzaoğlu (Hamzaoğlu, 2015[1]). Since 2015, 16 taxa have been added to the list and 13 of them were new and three of them were new records for the Turkish flora (Gökhan et al., 2016; Hamzaoğlu et al., 2017, 2018; Oskay, 2018). After the last studies, the number of taxa in Turkey has been recorded as 93 (Hamzaoğlu 2020, Hamzaoğlu et al., 2020, 2021).

The genus represented by 344 taxa in the world, when looking at the neighboring flora 78 in Russia (Shishkin, 1995), 49 in Iran (Rechinger, 1988), 121 in Europe (Tutin, 1964), 19 in Flora Palestina (Post, 1932) and 3 in Iraq (Rechinger, 1964). As a result, it can easily see that most of *Dianthus* taxa are in Turkey.

In the Flora of Turkey, the genus *Dianthus* is divided into 5 groups with the absence or presence of verrucous calyx; the leaf sheaths are longer 3 ´ the stem diameter or not; the petals are fimbriate or not, and barbullate or not.

According to pollen morphology palynological evidence has played an important role in discerning natural groups and understanding phylogenetic relationships (Erdtman, 1969; Walker and Doyle, 1975). Clark et al. (1980) reported that the pollen morphology can be useful in supporting taxonomic suggestions. It provides useful

[1]Hamzaoğlu E, (2015). Türkiye Dianthus (Caryophyllaceae) Cinsinin Revizyonu. TÜBİTAK Proje No: 111T873.

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taxonomic characters for the identification and classification of taxa of the family Caryophyllaceae. Pollen morphological characters are important in species delimitation. These are considered supplementary to the general plant morphology and play a critical role in the taxonomic and evolutionary debate. Yıldız (2001) studied the pollen morphology of 45 species belonging to 15 genera of Caryophyllaceae using light microscopy (LM) and scanning electron microscopy (SEM). On the basis of exine structure, shape, and ornamentation, 10 *Dianthus* species were examined in that study, and their pollen grains were grouped under the *Dianthus* type. The pollen morphology of seven species of *Dianthus* from Pakistan was investigated by Sahreen et al. (2008) and they use ornamentation, exine thickness, presence of prominent columella, pollen size, pore number, pore diameter, and the distance between pores to distinguish the species. The pollen morphology of *D. vanensis* Behçet & İlçim, which is endemic to Turkey was examined by Karaismailoğlu et al. (2019).

Generally, due to some different morphological features of *Dianthus* genus like its bracteoles with adpressed to the calyx, the genus is discerned from its related genera (Reeve, 1967). Especially *Dianthus* and *Acanthophyllum* C.A.Mey. are the most approved genus as monophyletic tribe of Caryophyllaceae family via morphological and molecular data (Fior et al., 2006; Harbaugh et al., 2010; Pirani et al., 2014). Moreover, for that reason, synonyms are still needed to make the *Dianthus* completely monophyletic (Greenberg and Donoghue, 2011; Harbaugh et al., 2010; Hamzaoğlu et al., 2021). In our study, to evaluate the identification of new species in the genus the combination of morphological and molecular data was used. Thus, the nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS) region of rDNA was agreed to apply for phylogenetic reconstructions. Particularly, because of its beneficial properties in DNA barcoding methods to identify species in the conservation studies, ITS regions of rDNA were mostly used (Kress et al., 2005; Kress, 2017; Chen et al., 2010; China Plant BOL Working Group (CBOL), 2011; Zhang and Jiang, 2020). Therefore, using both molecular phylogenetic analysis and morphological features could suggest clear information for speciation studies. After accepting this newly discovered species in the current study, the number of *Dianthus* species in Turkey has reached 93.

2. Materials and methods

An interesting specimen of the genus *Dianthus* was collected from stream beds in Erzincan province in 2020 and checked from related taxonomic literature such as Hamzaoğlu (2012, 2020), Hamzaoğlu et al. (2020, 2021), Reeve (1967), Shishkin (1995), Post (1932), Rechinger (1964) and Tutin (1964). Moreover, this new species of *Dianthus* was checked within GAZI, ANK, and HUB herbaria (Thiers, 2018). Both preliminary conservation assessments were made using the IUCN (2017) guidelines, also the characters given in the updated key to fimbriate *Dianthus* occurring in Turkey were mainly based on studied herbaria materials.

Pollen samples obtained from the herbarium materials and pollen grains of *D. berkayii* and the closed taxa *D. crinitus* Sm. were studied by light microscope (LM) and scanning electron microscope (SEM). For LM, pollen grains were first treated with 70% alcohol to remove oily substances and then embedded in glycerine jelly stained with basic fuchsin following the method of Wodehouse (1935). The pollen length (A), the pollen width (B), porus length (plg) and width (plt), exine thickness (Ex), pore number and the distance between the two pores were measured from at least 30 fully developed grains per sample under a Leica ICC50 HD microscope (1000×). Results are provided as minimum, maximum, and mean ± standard deviations. For SEM, pollen grains were transferred directly to stubs with double-sided adhesive tape and micrographs were obtained using Jeol-6060 SEM at Gazi University and at an accelerating voltage of 15 kV. In addition, A/B ratio, pollen shape, and exine ornamentation were determined. The terminology used is mainly that of Faegri and Iversen (1992) and Punt et al. (2007). Shape classification follows Erdtman (1969), based on A/B ratios.

After all these applications, *D. berkayii* specimens were conserved in Prof. Dr. Tuna Ekim Herbarium at Gazi University Faculty of Science (GAZI).

The leaves of the samples [GAZI: ZA 3194 (*D. crinitus*), Hamzaoğlu 6803 (*D. orientalis* Adams), and AK11147 (*D. berkayii*)] were used to extract total genomic DNA via a plant DNA extraction kit (MACHEREY NAGEL). For amplifying ITS region the primers pairs of the Hsiao et al. (1995) were used. PCR amplifications were done in a total volume of 25 µL composed of 4 µL 5 × Hot FirePol Blend PCR Mix (Solis Biodyne) (15mM MgCl2), 0.5 µL each primer pairs, 1.5 µL template DNA, and 18.5 µŞ water. A thermocycler (MultiGENE, Cleaver Scientific Ltd) was used for amplifying the regions with the followings: 5 min at 95 °C for initial denaturation, followed by 30 cycles of 30 seconds at 95 °C for template denaturation, 30 s for annealing, and 90 s at 72 °C for extension and 10 min at 72 °C for final extensions. All products were checked at 2% agarose gel in electrophoresis. Then all products were sent to BM Labosis Company (Ankara) for purifications and sequencing. After sequencing, data were controlled with Finch Tv software Version 1.4.0-manufactured by Geospiza Research Team (Patterson et al., 2004). MEGA (Molecular Evolutionary Genetics Analysis) 7.0.9 software (Kumar et al., 2016) was used with the MUSCLE (Multiple Sequence

Comparison by Log Expectation) tool (Edgar, 2004) for aligning the sequences. Moreover, for constructing the phylogenetic tree MEGA test program was used to find the best substitution model. According to the calculations, the maximum likelihood method (ML) based on Jukes Cantor (JC) model with bootstrap test analysis (1000 replicates) was used to construct phylogenetic trees with uniform sites. Also, to evaluate clear genetic relationships among species, the BEAST v 2.5.1 (Bayesian Evolutionary Analysis by Sampling Trees) package program was additionally used with the same substitution model (JC) with uniform rates for data partitions, with a Yule tree prior and a randomly generated starting tree. Then, phylogenetic complex trees were summarized and combined with Tree Annotator program with a posterior probability limit of 1(Drummond et al., 2012; Bouckaert et al., 2019). The phylogenetic trees which were drawn with both MEGA and BEAST programs were compared and combined. While constructing phylogenetic trees at MEGA and BEAST programs, to understand the best phylogenetic position of newly discovered species regard to other genus members, the sequences of some previously studied taxa of *Dianthus* genus and other species from Caryophylleae family were obtained from NCBI databank as outgroups (Table 1).

Samples from different herbaria which were examined for make a comparison were given under the "examined specimen" section.

Table 1. Accession numbers of the sequences of ITS gene regions of the species from NCBI data bank which were used to construct phylogenetic tree.

Taxa	NCBI accession numbers	References
Dianthus yilmazii	MK431019.1	Hamzaoğlu et al., 2021
Dianthus stramineus	MK431016.1	
Dianthus sessiliflorus	MK431017.1	
Dianthus erythrocoleus	MK431018.1	
Dianthus versicolor x Dianthus superbus	MK112559.1	Skaptsov, 2019[1] (direct submission)
Dianthus pontederae	KC293982.1	Somogyi et al., 2012[2]
Dianthus lumnitzeri	KC293976.1	
Dianthus giganteus subsp. *giganteus*	KU705522.1	Hamzaoğlu et al., 2017
Dianthus amurensis	AY594318.1	Begerow, 2016[3] (direct submission)
Dianthus sylvestris	AY594317.1	
Dianthus carthusianorum	AY594316.1	
Dianthus monspessulanus	AY594314.1	
Dianthus superbus	AY594315.1	
Dianthus longicalyx	KF954520.1	Park, 2010
Acanthophyllum acerosum	KF924680.1	Pirani et al., 2014
Cerastium arvense	KF737533.1	Dillenberger & Kadereit, 2014

[1]Skaptsov M V (2019) *Dianthus versicolor x Dianthus superbus* voucher ALTB 1100006621 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence–Direct submission-NCBI databank GenBank: MK112559.1

[2]Somogyi G (2014). A *Dianthus* sect. Plumaria (Opiz) Asch. et Graebn. közép-európai fajainak komplex molekuláris taxonómiai értékelése= Taxonomical evaluation of Central-European *Dianthus* species of sect. Plumaria (Opiz) Asch. et Graebn based on molecular evidences (Doctoral dissertation, Budapesti Corvinus Egyetem).

[3]Begerow D (2016). *Dianthus carthusianorum* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. – Direct submission- NCBI databank GenBank: AY594316.1
Begerow D (2016). *Dianthus monspessulanus* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. – Direct submission- NCBI databank GenBank: AY594314.1
Begerow D (2016). *Dianthus superbus* 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. – Direct submission- NCBI databank GenBank: AY594315.1

3. Results

Dianthus berkayii Kandemir, Aytaç & T. Ertuğrul **sp. nov.** (Figure 1)

Type: Turkey, B7 Erzincan: On Sivas road, Yalnızbağ, Hürrem Palangası (Erzincan Binali Yıldırım University Campus), stream beds, dunes, 1200 m, 10.06.2020, Kandemir 11147 (holotype GAZI, isotypes: ANK, HUB).

Paratype: Turkey, B7 Erzincan: On Sivas road, Yalnızbağ, Hürrem Palangası road junction, sandy-pebbled areas, 1285 m, 06.06.2021, Kandemir 11314 (GAZI).

3.1. Diagnosis

D. berkayii is related with *D. crinitus,* but it has curved divaricate leaves (not straight) and stems with 3–4 (–5) internodes (not 5–8). It is also similar with *D. vanensis*, but petals are ebarbullate and long fimbriate (not ebarbullate and short fimbriate).

3.2. Description

Perennial, with woody rootstock. Flowering stem ascending-erect, 9–15 cm long, unbranched except inflorescence, 1(–3) flowered, with 3–4 (–5) internodes, pruinose. Lower leaves crowded at base, 10–15 × 1 mm, curved divaricate, linear, acute, glabrous, shorter to equal internodes; middle and upper cauline leaves 2–4(–5) pairs, 10–20 × 1 (–2) mm, curved divaricate, linear, acute, glabrous, usually shorter than internodes, rarely equal; sheet 1–3 mm, membranous. Inflorescence 1–2(–3) flowered, pedicel 4–25 mm. Bracts 6, lower two of them equal to longer the others, linear, slightly broadened at

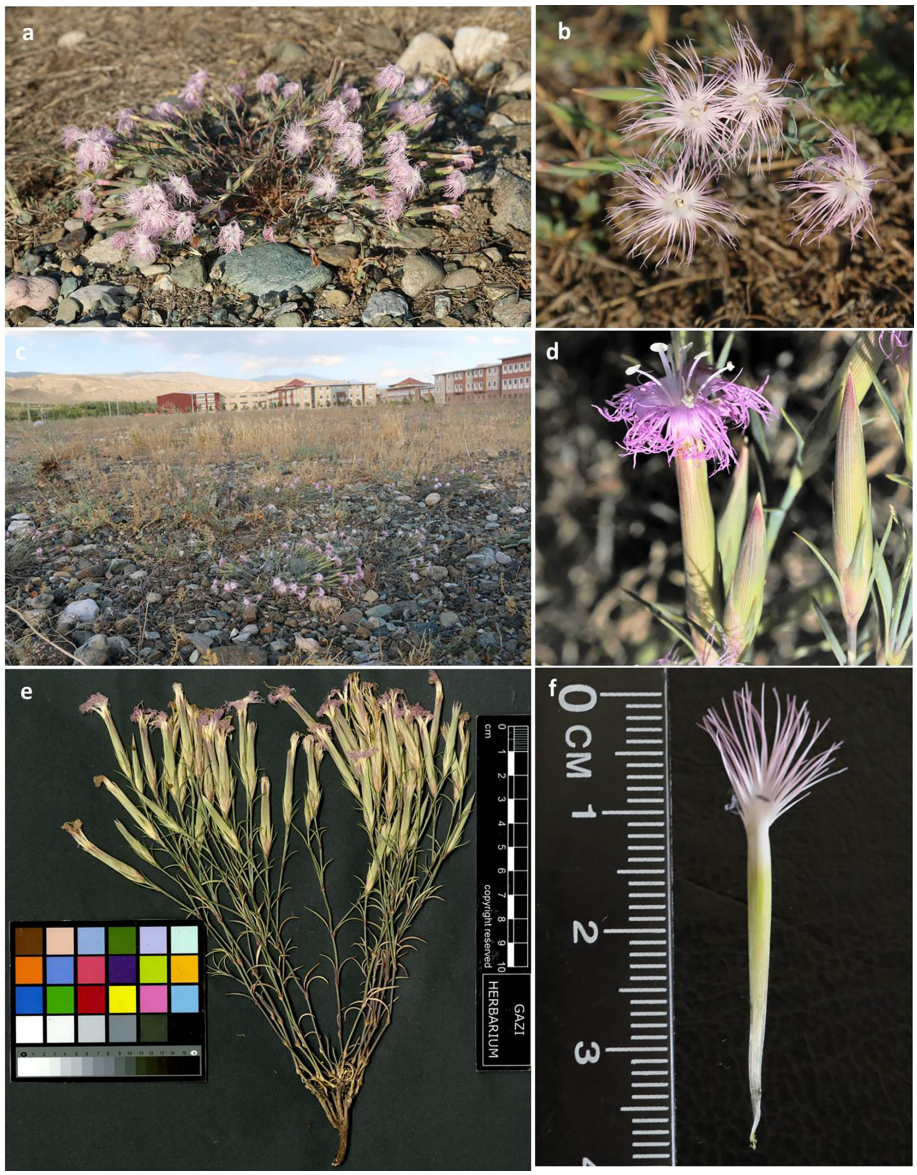

Figure 1. *Dianthus berkayii* a, b, d, e- habitus, c- habitat, f- petal.

the base, grassy, cartilaginous, 9–12 mm long; middle and upper bracts same length and grassy and cartilaginous with scarious margin, acuminate-aristate apex, 8–10 mm long, 1/3 calyx length. Calyx 22–30 mm long, cylindrical, glabrous, theet 5–6 mm long, lanceolate, mucronate, with a narrow scarious margin. Petals 35–40 mm long, lilac to purplish, limb 10–13 × 5 mm, ebarbulate, fimbriate, and fimbria more than 2/3 of limb (7–8 mm). Stamens included in corolla. Styles longer than petals, papillose, yellowish. Immature fruits 7–10 × 2–2.5 mm.
Flowering time: June-July.

3.3. Pollen morphology

The pollen grains of *D. berkayii* and *D. crinitus* are radially symmetrical, apolar, and their pollen shapes are spheroidal. Aperture type of pollen grains is pantoporate. The pores are circular, with distinct margine and operculate. The distance between two pori is from 14.44 ± 1.22 µm (*D. crinitus*) to 16.11 ± 1.73 µm (*D. berkayii*). There is only a minor difference in pollen size and exine thickness between the pollen of the two taxa. However, the pollen length (A) is 48.7 ± 1.31 µm, width is 48.9 ± 1.49 µm in *D. berkayii*, pollen length (A) is 45.28 ± 1.93 µm, width is 45.71 ± 2.48 µm in *D. crinitus*. Exine thickness is 2.17 ± 0.31 in *D. berkayii* and 4.00 ± 0.53 in *D. crinitus.* Exine sculpturing (ornamentation) is examined with SEM, which shows that pollen surface ornamentation is microechinate-punctate in both taxa (Figure 2). Detailed pollen morphological characters of *D. berkayii* and *D. crinitus* taxa are given in Table 2.

3.4. Etymology

The species name is taken from the names of the children of the first two authors.

3.5. Phylogeny

In the study, ITS regions (ITS1 + 5.8S + ITS2) were amplified totally as 600 bp in length. After aligning the sequences in MEGA program, 570 bp of them were used for analysis (22 bp of these bases were variable among studied *Dianthus* species). Moreover, overall mean genetic divergence was calculated as 0.005, meaning that studied species of *Dianthus* genus were genetically close to each other. According to the phylogenetic tree, *D. amurensis* hort. ex Jacques created a different clade alone with a bootstrap value of 100 and a posterior probability value of 1. Even if the second main clade was composed of the rest of the studied species, *D. slyvestris* Boiss. was formed a single clade. The rest of the samples were divided into 2 main clades. *D. crinitus, D. erythrocoleus* Boiss., *D. yilmazii* Hamzaoğlu & Koç, *D. orientalis, D. longicalyx* Miq., *D. superbus,* and newly named species *D. berkayii* were formed one of the main clades. Furthermore, *D. berkayii* was separated with a branch from the group of *D. crinitus, D. erythrocoleus, D. yilmazii, D. orientalis* which means that *D. berkayii* was genetically different than these species and this clue was also supported with morphological evidence. Moreover, in the other main clade, there were different subclades which were composed of the rest of the studied species (Figure 3).

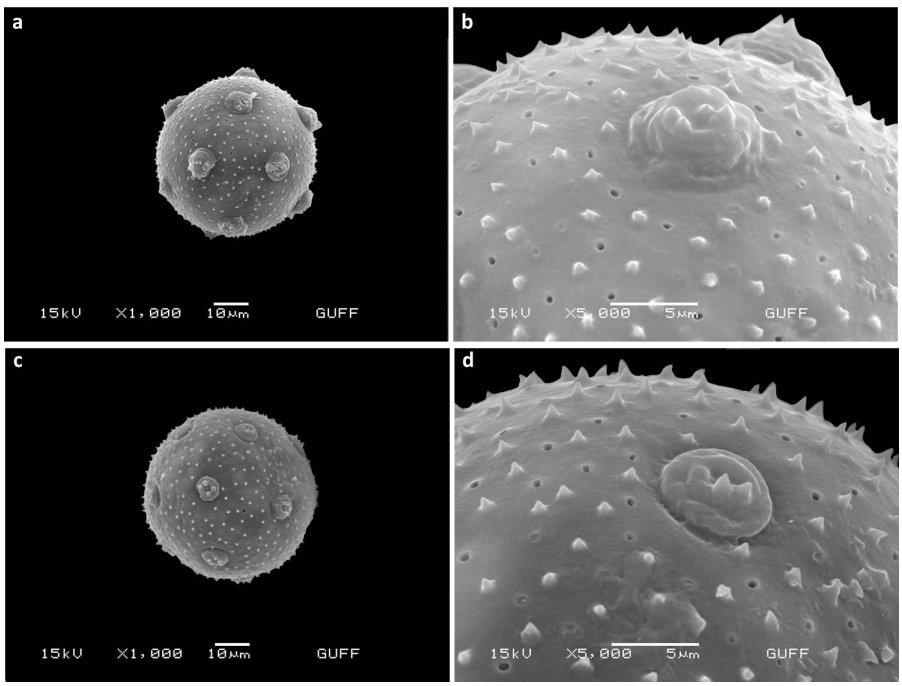

Figure 2. SEM microphotographs of pollen grain in *Dianthus* species examined: a, b- *Dianthus berkayii*, c, d- *D. crinitus.*

Table 2. Palynological characteristics of examined *D. berkayii* and *D. crinitus*. (values in µm; minimum, maximum, mean ± standard deviation)

Species/characters		*D. berkayii*	*D. crinitus*
A (µm)	Min.	46	43.18
	Max.	51	47.72
	Mean	48.7 ± 1.31	45.28 ± 1.93
B (µm)	Min.	46	43.63
	Max.	52	50
	Mean	48.9 ± 1.49	45.71 ± 2.48
Pollen shape		spheroidal	spheroidal
Aperture type		pantoporate	pantoporate
Ornamentation		microechinate-punctate	microechinate-punctate
Pore (Pl)	Pore length (Plg)	7.06 ± 0.73	6.70 ± 0.96
	Pore width (Plt)	7.83 ± 0.83	7.15 ± 0.85
Exine thickness		2.17 ± 0.31	4.00 ± 0.53
Interporal distance		16.11 ± 1.73	14.44 ± 1.22
Pore number		18.73 ± 1.52	17.07 ± 1.59

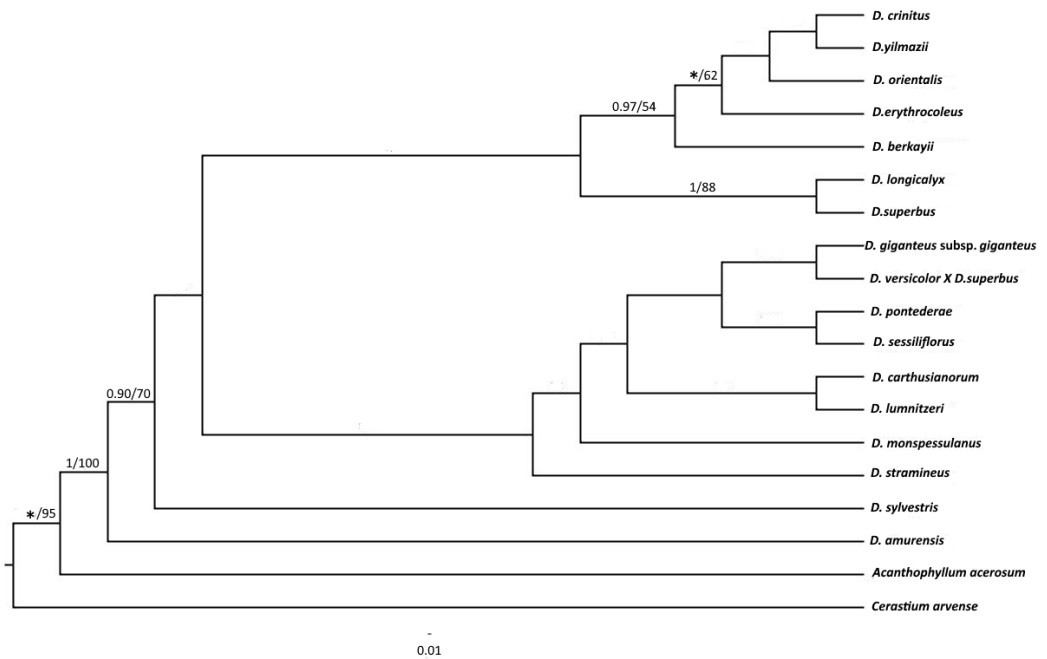

Figure 3. Bayesian consensus tree with JC model of ITS gene region of *Dianthus* species (Fig Tree v 1.4.4). The results of ML analysis with subsequent optimization (bootstrap values with 1000 replicates) and posterior probabilities (PP) values of over 1 were given over the branches and separated with slashes (the bootstrap values lower than 50 and posterior probability values lower than 0.90 were not shown).

3.6. Ecology

Dianthus berkayii grows on the steppe of Erzincan province at an altitude of 1200–1300 m. Area vegetation is formed by herbaceous plants including *Gundelia tournefortii* L., *Jurinea brevicaulis* Boiss., *Artemisia santonicum* L., *Cnicus benedictus* L., *Scorzonera pseudolanata* Grossh., *Lactuca viminea* (L.) J. Presl & C.Presl, *Rindera lanata* (Lam.) Bunge, *Euphorbia orientalis* L., *Gagea reticulata* (Pall.) Schult. & Schult.f., *Gypsophila pallida* Stapf, *Paronychia* sp., *Convolvulus calvertii* Boiss., *Astragalus onobrychis* L.,

Erodium oxyrhinchum M. Bieb., *Erodium cicutarium* (L.) L'Hér., *Hypericum scabrum* L., *Glaucium corniculatum* (L.) Rudolph, *Acantholimon* sp., *Atraphaxis grandiflora* Willd., *Stipa* sp.

3.7. Distribution, ecology and suggested conservation status

The new species is known from only type locality, but the collection date and altitude are different. It is a local endemic and Irano-Turanian element. It also grows in sandy soils in stream beds. Moreover, the type locality is at the Erzincan University campus area where it is still under construction and still trying to complete its development. The species is distributed in a narrow area and the number of individuals was detected about 250. The extent of occurrence (EOO) and area of occupancy (AOO) are less than 4 km². AOO and habitat quality are continuing to decline, for these ArcGIS and spatial analysis programs were used for these. It is considered "critically endangered" CR B1ab (i, ii, iii, v) + 2ab (i, ii, iii, v) because under constructions are present in this area (IUCN, 2017) (Figure 4).

3.8. Proposed Turkish name for the new species

This new species was called "*Cankaranfil*" in Turkish, according to the guidelines of Menemen et al. (2016).

4. Discussion and conclusion

D. berkayii belongs to section *Fimbriati* Boiss., which is different from other sections with its fragmented petals. According to Rechinger (1988), this section contains 28 species in Flora Iranica and 8 species in the Flora of Turkey. There are 9 taxa belonging to this section in the revision study which was conducted by Hamzaoğlu et al. (2015). In that study the specimens were cross-checked with the keys that were provided by Reeve (1967) and the *Dianthus* data in the literature, including both *Flora Iranica* (Rechinger, 1988) and the *Flora of Syria, Palestine, and Sinai* (Post, 1932). Therefore, we concluded that the closest species to our material seem to be *Dianthus libanotis* Labill., *D. crinitus* subsp. *crinitus*, *D. orientalis* subsp. *nassireddini* (Stapf) Rech.f. and *D. vanensis*, because of its fimbriate petal limbs (İlçim et al., 2013).

Although *D. berkayii* is similar to *D. crinitus* bades on its ebarbulate petals, it is distinguished by its more delicate form, shorter calyx, and long fimbriate lops of petal and curved divaricate leaves. On the other hand, the new species looks like *D. libanotis, D. orientalis*, and *D. vanensis* with its general appearance, but it is different from these taxa with its ebarbulate petals. Moreover, it is morphologically distinct from both *D. orientalis* and *D. libanotis* with its curved divaricate leaves. Among these taxa, only *D. vanensis* has curved divaricate leaves, but as mentioned above its petals are barbullate. Furthermore, its leaves are wider than *D. berkayii*. Also, even if the petal segments are much longer in *D. berkayii* than *D. vanensis*; calyx is purple in *D. vanensis* but it turns green to straw-colored in *D. berkayii*.

Moreover, *D. berkayii* is similar to *D. libanotis,* with its leaves being shorter and narrower Also, its calyx is shorter

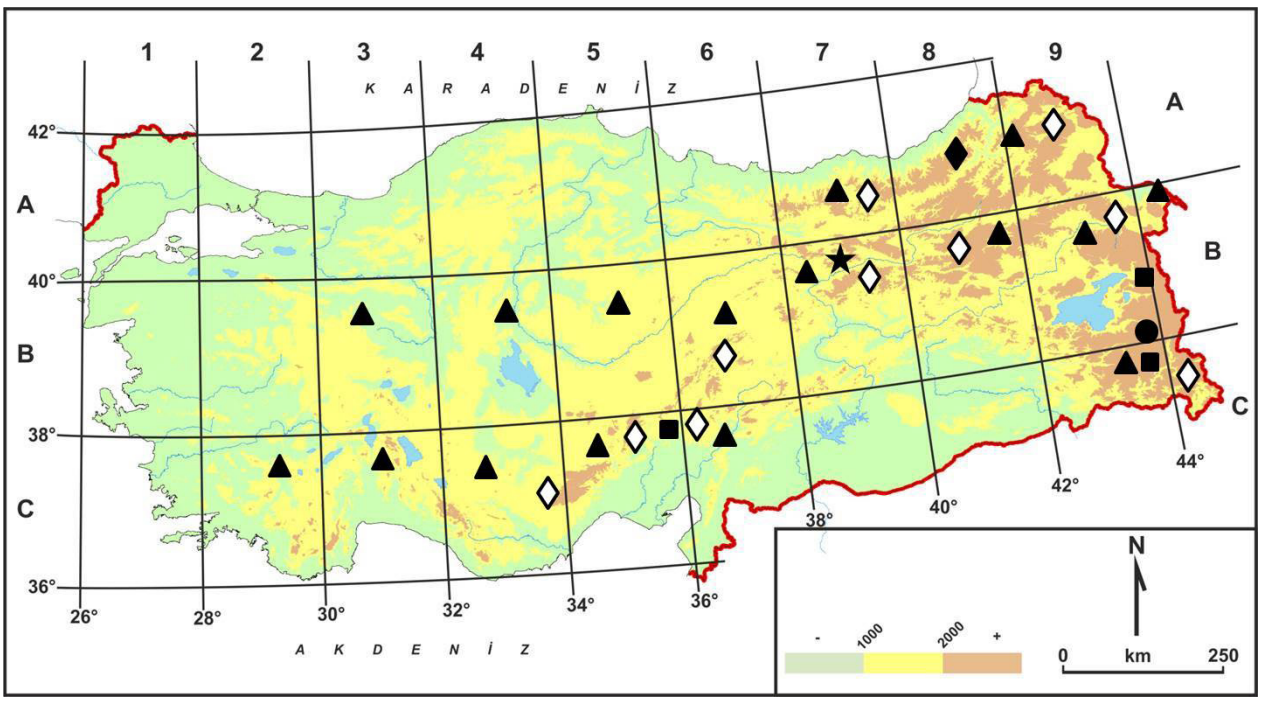

Figure 4. Distribution map of *Dianthus berkayii* (★), *D. orientalis* (◇), *D. crinitus* (▲), *D. libanotis* (■) and *D.vanensis* (●).

and its petals are ebarbulate. A more detailed comparison of the species can be seen in Table 3.

Vural (2008) used pollen size to distinguish *D. aytachii* C. Vural and *D. zederbaueri* Vierh. which are morphologically similar. In agreement with them, in our study, differences in pollen grains of the studied taxa were found especially in pollen size and exine thickness. The size of pollen grains in *D. berkayii* (A: 48.7 ± 1.31 µm, B: 48.9 ± 1.49 µm) was bigger than *D. crinitus* pollens (A: 45.28 ± 1.93 µm, B: 45.71 ± 2.48 µm). Moreover, exine thickness of *D. berkayii* (2.17 ± 0.31) is thinner than *D. crinitus* pollens (4.00 ± 0.53). Except for these characters, little differences in the measurements of palynological character have been observed in these two taxa.

Mete et al. (2021) studied the pollen morphology of 89 taxa belonging to the genus *Dianthus* via utilizing light, scanning and transmission electron microscopy. Pollen grains of the studied taxa of *Dianthus* were morphologically quite similar. In the same way, in our study, the pollen grains of the new species and the close related taxa were generally rather similar to each other in relation to the aperture type (pantoporate), pollen shape, pore diameter, interporal distance, pore number and also ornamentation (microechinate-punctate). Little differences in the measurements of palynological character were observed.

Another species close to *D. berkayii, D. vanensis* was studied by Karaismailoğlu et al. (2019) and Mete et al. (2021). According to Karaismailoğlu et al. (2019) who worked the pollen morphology of *D. vanensis*, which is endemic to Turkey, pollen grains of *D. vanensis* were radially symmetric, apolar, pantoporate and spheroidal, pollen lengths range from 39 to 41 µm, the pore length is 6.17 ± 0.23 µm, pore width is 4.17 ± 0.39 µm, the pores are elliptical and the number of pori is 9–12. Furthermore, Mete et al. (2021) reported that *D. vanensis* was the smallest pollen in the Fimbriati section with 32.1–34.6 (32.7) µm. As a result, *D. berkayii* had significantly larger pollen than *D. vanensis* when compared to these two studies. Except for pollen size (A: 48.7 ± 1.31 µm, B: 48.9 ± 1.49 µm), pore diameter (plg 7.06 ± 0.73 µm, plt 7.83 ± 0.83 µm) and pore number-shape (18.73 ± 1.52-circular), other morphological features were generally similar.

Due to its different morphological features within the Caryophyllaceae family, the genus *Dianthus* has always been problematic among differentiation of the genus species. Thus, molecular analysis mostly made clear view for these studies (Fior et al., 2006; Harbaugh et al., 2010; Pirani et al., 2014). For instance, Hamzaoğlu et al. (2021) studied on the genus *Dianthus* to describe new species *D. yilmazii* with both morphological characters and molecular data. Additionally, again Hamzaoğlu et al. (2017) published an article to name new species *D. ucarii* Hamzaoğlu & Koç supporting their evidence with molecular phylogenies. Moreover, Busch and Reish (2016) focused on endangered plant species of the genus named *D. segueri* Vill. subsp. *glaber* **Čelak**. whose population size was affected by its genetic variations. Furthermore, Raman and Park (2015) studied on complete chloroplast genome of medicinal plant *D. superbus* var. *longicalyncinus* (Maxim.) Will. to check out species from a molecular perspective. In the current study, the DNA barcoding method was applied to discover the new species (ITS regions), so molecular clues always helped us to check out the taxonomic situations of the genus. According to the phylogenetic tree of *Dianthus* species, the evidence from the molecular data indicated to us the understanding of character evolution in species divergence that was compatible with morphological characters. Although *D. berkayii* (AK11147) was positioned under the same group as *D. crinitus, D. erythrocoleus, D. yilmazii, D. orientalis*, it was separated with a branch. Moreover, even if *D. crinitus* was the most similar species to *D. berkayii* with its petals which were ebarbulate, they were separated from each other both morphologically and genetically.

As a result of the evaluation of morphological, palynological and molecular data, and examination of related specimens, it was decided that the specimens collected from Erzincan, is a new species to science. This species is named *Dianthus berkayii* and placed in section *Fimbriati*.

Table 3. Comparison of the diagnostic characteristics of *Dianthus berkayii, D. vanensis, D. orientalis D. crinitus* and *D. libanotis*.

Characters	*D. berkayii*	*D. crinitus*	*D. vanensis*	*D. orientalis*	*D. libanotis*
leaves	Curved divaricate	Straight	Curved divaricate	straight	straight
Flower numbers	1 (–2–3)	1	1 (–2)	1 (–2)	2–4
Pedisel length (mm)	4–25	over 20		over 20	5–10
Bracts / calyx raito	1/3	1/3–1/2	2/3–4/5	1/8–1/3	1/2
Calyx (long mm)	22–30	(25–)30(–35)	30–32	20–23(–25)	35–40
Calyx teeth length (mm)	5–6	8–11	7–10	6–9 (–11)	10–11
Petal limb	ebarbullate	ebarbullate	barbullate	barbullate	barbullate

Key to *Dianthus berkayii* and related taxa:

1. Petals ebarbulate 2
2. Leaves straight; calyx teeth 8–11 mm long …..……...
.....…….……..……..… *D. crinitus*
2. Leaves curved-divaricate; calyx teeth 5–6 mm long ..…..
……..….....…….. *D. berkayii*
1. Petals barbulate 3
3. Calyx at most 25 mm long .....…….…..... *D. orientalis*
3. Calyx 30–40 mm long
4. Leaves curved-divaricate; bracts 4–6 ..…….. *D. vanensis*
4. Leaves straight; bracts (4 –) 6–8 …… *D. libanotis*

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Additional examined specimens:

Dianthus crinitus: A4 Ankara: Çubuk, Ovacık-Saracık villages, 1250–1380 m, 03.08.1992, E. Dündar s.n. (GAZI); Ayaşbeli, forestation region, roadsides, 1100 m, 21.07.1993, Z. Aytaç 6100 (GAZI). A5 Amasya: Sarılar village, 850 m, limestone rocks, 23.07.1993, A.A. Dönmez 3762 (GAZI). A6 Sivas: Yıldızeli, around Çakmakçı pass, 1720 m, steppe, 30.07.1996, A.A. Dönmez 5343 (GAZI & HUB). A8 Erzurum: between Erzurum and İspir, 50 km, Eğerti village area, 2100–2200 m, 19.07.1990, steppe, Z. Aytaç 5161 (GAZI). B5 Nevşehir: Göreme, volcanic tuff, borders of vineyards, 1130 m, 17.06.1989, M. Vural, Ü. Kol & N. Adıgüzel 5161 (GAZI); Göreme, Topraktepe, 1000 m, 23.06.1995, M. Vural 7325 (GAZI). C2 Antalya: Elmalı-Korkuteli, 40 km, protected Quercus coccifera forest, limestone rocks, 1170 m, 30.06.1996, Z. Aytaç 7425 (GAZI); Manisa: south of Çırpıcı Dede Mountain, 1200 m, 21.06.1984, H. Duman 1808 (GAZI). C4 Konya; Ereğli, Halkapınar, around Yayıklı village, protected areas, 1250 m, 22.06.1997, Z. Aytaç 7605 (GAZI).

Dianthus orientalis: A8 Artvin: Yusufeli, Dereiçi village, stony slopes, 720 m, 20.05.1983 A. Güner 4796 (GAZI); Rize: Çamlıhemşin, between Çatak village and Vanki plateau, Picea orientalis forest, 1400–2100 m, 22.08.1981, A. Güner 4279 (GAZI); Erzurum: İspir-Çamlıkaya, İspir exit, Çoruh valley, stony steppe, 1170 m, 28.07.1991, A. Güner (GAZI); B7 Erzincan: above Üzümlü, Keşiş Mountain, 2000–2250 m, 13.08.2001, N.Adıgüzel 4126 & R.Reeves (GAZI); Erzincan: between Refahiye and İmranlı, 100 m beyond Kayınboğazı crossroad, 1900 m, serpentine areas, 15.08.2001, N. Adıgüzel 4179 & R. Reeves (GAZI); between Refahiye İmranlı, serpentine area, 1840 m, 15.08.2001, N. Adıgüzel 4168 & R. Reeves (GAZI).B9 Bitlis: Mt. Nemrut, 2000–2200 m, 20.08.1983, T.Ekim 3295 (GAZI); Bitlis: Adilcevaz, Süte plateau, mountain steppe, 2000 m, 12.08.1993, Y. Altan 5530 (GAZI); C5 Mersin: Erdemli, Limonluk, Kayacı valley, 10 m, 31.07.1995, above rocks, Z. Aytaç 7521 (GAZI); C6 Kahramanmaraş: Engizek Mountain, Gici hill, 2000 m, 22.07.1987 H. Duman 3654 (GAZI); Çağlayancerit, Öksüz Mountain, Ziyaret Tree hill, north hillside, 1200–1300 m, 06.07.1991, Z. Aytaç & H. Duman 4047 (GAZI). C10 Hakkari: Aruna crevice, ca. 2500 m, 27.07.1983, T. Ekim 7998 (GAZI).

Dianthus libanotis: B9 Van: 5 km N. of Çatak, 21.07.1954, P.H. Davis 22984; ANK!); Van: Muradiye, Muradiye waterfalls, south of the facilities at the entrance, 1805 m, 27.08.2012, rocky places, Hamzaoğlu 6661 & Koç (GAZI!); Bitlis: Tatvan exit, afforestation area, 1180 m, 31.07.2007, rocky places, Budak 2224 & Hamzaoğlu (GAZI!); C6 Kahramanmaraş: Çağlayancerit, Öksüz Dağı, Akdut place, 1100–1250 m, steppe, 4.07.1991, Z.Aytaç 3916 & H. Duman (GAZI!); C9 Hakkari: Zap gorge, beneath (2–3 km) Hakkari, 1400 m, dry S. slopes, 24.06.1966, P.H. Davis 45457 (E-foto!); Koçaniş, 2340 m, rocky slope, 13.08.1954, P.H.Davis 24332 (E-foto!); C10 Hakkâri: between Yüksekova-Dağlıca, 1830 m, 30.06.2006, steppe, Hamzaoğlu 4086 & Budak (GAZI!); 10 km from Bajirge (Esendere) to Yüksekova, 1900 m, bare shaley hillsides, 18.6.1966, P.H.Davis 45206 (E-foto!).

Dianthus vanensis: Turkey. C9 Van: Çatak, Konalga village, Tanrıverdi hamlet, around Zevviçal, steppe, 2372 m, 25.06.2010, M. Mükemre 300 (isotypes: GAZI).

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