Dianthus berkayii (Caryophyllaceae), a new species from Turkey

ZEKİ AYTAÇ
ALİ KANDEMİR
TUĞBA ERTUĞRUL
AHTER FİŞNE
MEVLÜDE ALEV ATEŞ

Follow this and additional works at: https://journals.tubitak.gov.tr/botany

Part of the Botany Commons

Recommended Citation
AYTAÇ, ZEKİ; KANDEMİR, ALİ; ERTUĞRUL, TUĞBA; FİŞNE, AHTER; and ATEŞ, MEVLÜDE ALEV (2022) "Dianthus berkayii (Caryophyllaceae), a new species from Turkey," Turkish Journal of Botany: Vol. 46: No. 3, Article 5. https://doi.org/10.55730/1300-008X.2686
Available at: https://journals.tubitak.gov.tr/botany/vol46/iss3/5

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Botany by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.
Dianthus berkayii (Caryophyllaceae), a new species from Turkey

Zeki AYTAÇ1, Ali KANDEMİR2, Tuğba ERTUĞRUL1, â, Ahter FIŞNE1, Mevlüde Alev ATEŞ3

1Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey
2Department of Biology, Faculty of Science and Art, Erzincan University, Turkey
3Department of Agricultural Biotechnology, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkey

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 scarios 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 revisited 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, 2015¹). 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 than 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

* Correspondence: tugbaekici@gazi.edu.tr

This work is licensed under a Creative Commons Attribution 4.0 International License.
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. Yildiz (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 Karaisalıoğlu 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 µL 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 FinchTv 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 Caryophyllae 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.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>NCBI accession numbers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dianthus yilmazii</td>
<td>MK431019.1</td>
<td>Hamzaoglu et al., 2021</td>
</tr>
<tr>
<td>Dianthus stramineus</td>
<td>MK431016.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus sessiliflorus</td>
<td>MK431017.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus erythrocoleus</td>
<td>MK431018.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus versicolor x Dianthus superbus</td>
<td>MK112559.1</td>
<td>Skaptsov, 2019¹ (direct submission)</td>
</tr>
<tr>
<td>Dianthus pontederae</td>
<td>KC293982.1</td>
<td>Somogyi et al., 2012²</td>
</tr>
<tr>
<td>Dianthus lumnitzeri</td>
<td>KC293976.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus giganteus subsp. giganteus</td>
<td>KU705522.1</td>
<td>Hamzaoglu et al., 2017</td>
</tr>
<tr>
<td>Dianthus amurensis</td>
<td>AY594318.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus sylvestris</td>
<td>AY594317.1</td>
<td>Begerow, 2016⁶ (direct submission)</td>
</tr>
<tr>
<td>Dianthus carthusianorum</td>
<td>AY594316.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus monspessulanus</td>
<td>AY594314.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus superbus</td>
<td>AY594315.1</td>
<td></td>
</tr>
<tr>
<td>Dianthus longicalyx</td>
<td>KF954520.1</td>
<td>Park, 2010</td>
</tr>
<tr>
<td>Acanthophyllum acerosum</td>
<td>KF924680.1</td>
<td>Pirani et al., 2014</td>
</tr>
<tr>
<td>Cerastium arvense</td>
<td>KF737533.1</td>
<td>Dillenberger &amp; Kadereit, 2014</td>
</tr>
</tbody>
</table>

¹Skaptsov 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


⁶Begerow 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. Erţuğrul 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_.

264
3.6. Ecology


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

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

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).

3.7. 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. nassirreddini (Stepf) Rech.f. and D. vanensis, because of its fimbriate petal limbs (Ilçim 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. aytchii* 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 Karaismağloğlu et al. (2019) and Mete et al. (2021). According to Karaismağloğlu 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. ylimazi* 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. seguerci* Vill. subsp. *glaber* Célak. 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. longalycins* (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. crythrocoleus, D. ylimazi, 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*.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>D. berkayii</em></th>
<th><em>D. crinitus</em></th>
<th><em>D. vanensis</em></th>
<th><em>D. orientalis</em></th>
<th><em>D. libanotis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>Curved divaricate</td>
<td>Straight</td>
<td>Curved divaricate</td>
<td>straight</td>
<td>straight</td>
</tr>
<tr>
<td>Flower numbers</td>
<td>1 (–2–3)</td>
<td>1 (–2)</td>
<td>1 (–2)</td>
<td>1 (–2)</td>
<td>2–4</td>
</tr>
<tr>
<td>Pedisel length (mm)</td>
<td>4–25</td>
<td>over 20</td>
<td>over 20</td>
<td>5–10</td>
<td>2–4</td>
</tr>
<tr>
<td>Bracts / calyx raito</td>
<td>1/3</td>
<td>1/3–1/2</td>
<td>2/3–4/5</td>
<td>1/8–1/3</td>
<td>1/2</td>
</tr>
<tr>
<td>Calyx (long mm)</td>
<td>22–30</td>
<td>(25–)30(–35)</td>
<td>30–32</td>
<td>20–23(–25)</td>
<td>35–40</td>
</tr>
<tr>
<td>Calyx teeth length (mm)</td>
<td>5–6</td>
<td>8–11</td>
<td>7–10</td>
<td>6–9 (–11)</td>
<td>10–11</td>
</tr>
<tr>
<td>Petal limb</td>
<td>ebarbulate</td>
<td>ebarbulate</td>
<td>barbulate</td>
<td>barbulate</td>
<td>barbulate</td>
</tr>
</tbody>
</table>
Key to *Dianthus berkayii* and related taxa:
1. Petals ebarbulate ................................................................. 2
2. Leaves straight; calyx teeth 8–11 mm long .......................... 2
3. Petals barbulate ................................................................... 3
4. Calyx at most 25 mm long ........................................... *D. orientalis*
5. Calyx 30–40 mm long 
6. Leaves curved-divaricate; bracts 4–6 .......... *D. vanensis*
7. Leaves straight; bracts (4–) 6–8 ............... *D. libanotis*

Acknowledgments
The authors are grateful to the curators of ANK and HUB for access to *Dianthus* materials for this study and Dr. Ergin Hamzaoğlu for his comments on new species and Samet H. Genç for IUCN comments.


>D. vanensis

References


CBOL Plant Working Group (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. Proceedings of the National Academy of Sciences, USA 108: 19641-19646. doi: 10.1073/pnas.1104551108


