

## *Dianthus ucarii* (Caryophyllaceae): a new species from the northwest of Turkey

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**Abstract:** *Dianthus ucarii* Hamzaoglu & Koç (Caryophyllaceae) is described as a new species to science based on material collected in Kırklareli Province in northwestern Turkey. The new taxon belongs to sect. *Carthusiani* (Boiss.) F.N.Williams and it is closely related to *Dianthus pinifolius* Sm., an Eastern Mediterranean element. A description, illustration, and identification key for the new species are given. In addition, *D. ucarii* and *D. pinifolius* are compared in terms of general morphology and molecular phylogeny.

**Key words:** *Carthusiani*, *Dianthus*, morphology, new taxon, phylogeny, Thrace, Turkey

### 1. Introduction

The genus *Dianthus* L. is located within the tribe Caryophylleae of the family Caryophyllaceae (Bittrich, 1993). It is the second largest genus of Caryophyllaceae following the genus *Silene* L. in terms of numbers of taxa. The genus has approximately 600 species that mostly grow in Asia and Europe (Dequan and Turland, 2001).

The first comprehensive work on the taxa of the genus *Dianthus* from Turkey was *Flora Orientalis* (Boissier, 1867). Forty-eight of the 89 *Dianthus* species reported in this work are related to the flora of Turkey. In *Flora Orientalis*, the genus was divided into five sections: *Verruculosi* (Boiss.) Nersesian, *Leiopetali* Boiss., *Fimbriati* (Boiss.) F.N.Williams, *Dentati* Boiss., and *Carthusiani* (Boiss.) F.N.Williams. A more exhaustive study on the *Dianthus* species concerning the Turkish flora was carried out by Reeve (1967), who recognized 67 species. This number was later increased to 77, with subsequent taxonomic studies including the ones carried out by Gemici and Leblebici (1995), Vural (2008), Yılmaz et al. (2011), İlçim et al. (2013), and Hamzaoglu and Koç (2015). In these studies, several *Dianthus* taxa were defined including *Dianthus akdaghensis* Gemici & Leblebici; *D. aytachii* C.Vural; *D. goekayi* Kaynak, Yılmaz & Daşkın; *D. vanensis* Behçet & İlçim; and *D. burdurensis* Hamzaoglu & Koç. Among them, *Dianthus akdaghensis*, which belongs to the section *Carthusiani*, was the subject of the current study.

No study has addressed the taxonomy of the genus *Dianthus* using molecular approaches. Recent studies

generally focused on the order Caryophyllales, the family Caryophyllaceae, or the tribes and genera belonging to this family (Rettig et al., 1992; Downie and Palmer, 1994; Harbaugh et al., 2010; Greenberg and Donoghue, 2011). Since only one or two species belonging to the genus *Dianthus* were used in these studies, it is difficult to make comments about the whole genus in general. However, the p16–p25 primer couple that is being used recently can be said to be distinctive for the infrageneric taxa (Popp and Oxelman, 2001). Therefore, in this study, the p16–p25 primer couple was used to determine the phylogenetic relationships of the new species and its close relatives.

### 2. Materials and methods

During the work on the taxonomy of the genus *Dianthus* in Turkey, some specimens of *Dianthus* were collected from Kırklareli Province in Thrace, in northwestern Turkey. The collected specimens were compared with other related *Dianthus* specimens deposited in herbaria including GAZI, ISTE, and EGE. In addition, the collected specimens were evaluated according to the relevant literature (Boissier, 1867; Reeve, 1967). These comparisons demonstrated that our *Dianthus* specimens could represent a species from the section *Carthusiani* that is new to science. Digital images of the new species were prepared using a CANON EOS 60D digital camera. Vegetative and floral characteristics were measured with a ruler with 0.5 mm accuracy and an ocular micrometer. Measurements were performed three times for 16 samples.

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## 2.1. DNA isolation

Fresh leaf material was collected, stored in zip-lock bags containing silica gel, and brought to the laboratory for molecular phylogenetic analysis. Genomic DNA was isolated from 50–150 mg of leaf materials according to Lefort and Douglas (1999). The quality and quantity of the extracted DNAs were measured with a NanoDrop Lite spectrophotometer (Thermo Scientific, USA). Stock DNA was kept at  $-86^{\circ}\text{C}$ .

## 2.2. PCR amplification and sequencing

The ITS region of the rDNA gene cluster was amplified with the p16 and p25 primers (Popp and Oxelman, 2001). PCR amplifications for sequence analysis were performed as described in our previous study (Hamzaoğlu et al., 2015). Amplification products were analyzed by electrophoresis in 1.8% agarose gel containing ethidium bromide, and product sizes were determined by nucleotide size markers (100-bp ladder; Fermentas, Vilnius, Lithuania) on gels. The PCR products were sequenced with a BigDye cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) using an ABI 3130XL genetic analyzer (Applied Biosystems).

## 2.3. Sequence analysis

*Silene acaulis* (L.) Jacq. was selected as an outgroup species because of satisfying two characteristics required for being the best outgroup. These characteristics are: 1) not being a member of the ingroup, and 2) being related to the ingroup (close enough for meaningful comparisons to the ingroup) (Yang and Rannala, 2012). Sequences for the outgroup *Silene acaulis* (L.) Jacq. were obtained from GenBank to compare with the obtained sequences (Table 1). The alignment of the ITS sequences was generated using the MUSCLE algorithm of MEGA6 software with default settings (Edgar, 2004; Tamura et al., 2013). The ends of the

alignments were trimmed to make all the sequences in the final dataset equal in length. The evolutionary history and molecular phylogenetic analysis were inferred using the maximum likelihood (ML) method based on the Tamura–Nei model via MEGA6 software (Tamura et al., 2013). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances (pairwise distances) were computed using the maximum composite likelihood model in units of number of base substitutions per site (Tamura et al., 2004). The analysis involved eleven nucleotide sequences.

## 3. Results

*Dianthus ucarii* Hamzaoğlu & Koç sp. nov. (Sect. *Carthusiani*) (Figure 1)

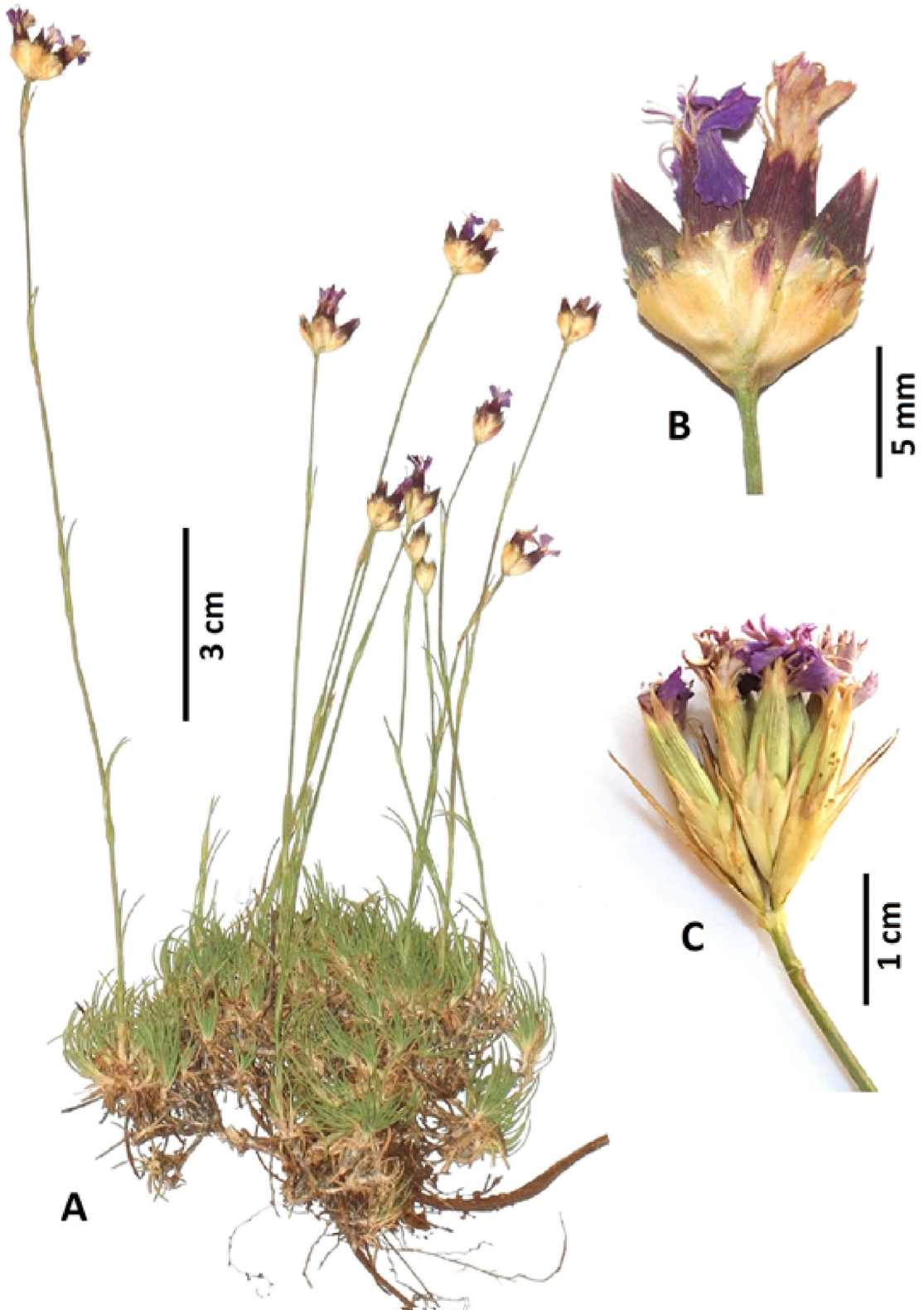
**Type.** Turkey. Kırklareli: Vize to Kıyıköy, ca. 5–6 km, 430 m, 15.6.2013, *Quercus* forest clearings, Hamzaoğlu 6719, Aksoy & Koç (holotype: GAZI; isotypes: GAZI, ANK).

**Diagnosis.** *Dianthus ucarii* is similar to *D. pinifolius* Sm. (subsp. *pinifolius*) in terms of its general habit. However, it mainly differs from *D. pinifolius* because it has stems 4–6-noded (not 7–9-noded); outer epicalyx scales with 0.4–0.5 mm scarios margins, apex obtuse-truncate to emarginate except arista, arista 1/4 as long as scale (not with 0.2–0.3 scarios margins, apex acute to obtuse except arista, arista 1/3–2/5 as long as scale); calyx teeth broadly triangular, 2–3 × 1.5–2 mm, glabrous (not narrowly triangular-lanceolate, 4–5 × 1–1.5 mm, puberulous); petals 11–15 mm long, limb ebarbellate (not 16–20 mm, barbellate).

**Description.** Perennial, few-stemmed herbs. Stems erect or ascending, 10–40 cm tall, 4–6-noded, glabrous

**Table 1.** GenBank accession numbers of *Dianthus* section *Carthusiani* species that were sequenced in the current study.

Collector number	Species	GenBank accession number
Hamzaoğlu 6326	<i>D. lydus</i> Boiss.	KU705518
Hamzaoğlu 6762	<i>D. akdaghensis</i> Gemici & Leblebici	KU705519
Koç 1914	<i>D. carthusianorum</i> L.	KU705520
Hamzaoğlu 6617	<i>D. carmelitarum</i> Reut. ex Boiss.	KU705521
Hamzaoğlu 6338	<i>D. giganteus</i> d'Urv. subsp. <i>giganteus</i>	KU705522
Hamzaoğlu 7085	<i>D. pinifolius</i> Sm. subsp. <i>pinifolius</i>	KU705523
Hamzaoğlu 6603	<i>D. cibrarius</i> Clem.	KU705524
Hamzaoğlu 6413	<i>D. cruentus</i> Griseb. (syn. <i>D. calocephalus</i> Boiss.)	KU705525
Hamzaoğlu 6719	<i>D. ucarii</i> Hamzaoğlu & Koç	KU705527
Outgroup	<i>Silene acaulis</i> (L.) Jacq.	U30975.1



**Figure 1.** *Dianthus ucarii*: A) habit; B) inflorescence (holotype, Hamzaoğlu 6719). *D. pinifolius* subsp. *pinifolius*: C) inflorescence (Hamzaoğlu 7085).

or sometimes puberulous below. Leaves filiform, canaliculated in cross-section, usually curved, apex acuminate; sterile shoot leaves similar to cauline leaves but usually shorter, glabrous or puberulous; cauline leaves ca. 0.5 mm wide, spreading to subadpressed, shorter than internodes, distinctly 1-3-veined, sheaths 3-4 times as long as wide. Inflorescence capitate, (2-)3-7(-11)-flowered. Bracts cartilaginous-scarious; outer linear to narrowly lanceolate, shorter than calyx; inner oblong-oblongeolate, straw-colored, brownish or partially purplish, veinless below, indistinctly 5-7-veined above, glabrous, 3/5-2/3 or as long as calyx, 5-10 × 2.5-4 mm, with scarious (0.3-0.5 mm) and sometimes undulate margins, apex obtuse-truncate to emarginate except arista, arista 1/5-1/3 as long as bract, adpressed to calyx. Epicalyx scales 4(-6); outer cartilaginous-scarious, whitish below, straw-colored, brownish or purplish above, ±distinctly 3-5-veined at apex, glabrous, 3/5-3/4 as long as calyx, oblanceolate or obovate, 5-8 × 2.2-4 mm, with 0.4-0.5 mm scarious and sometimes undulate margins, apex obtuse-truncate to emarginate except arista, arista 1/4 as long as scale, adpressed to calyx; inner similar to outer. Enlarged portions of bracts and epicalyx scales enclosing 1/2-3/5 of calyx. Calyx lanceolate, 9-15 × 2.5-4 mm, distinctly 40-45-veined above, glabrous, purplish or sometimes greenish; teeth broadly triangular, 2-3 × 1.5-2 mm, glabrous, 7-9-veined, apex acuminate. Petals 11-15 mm long; limb broadly cuneate-obovate, 4-5 × 3.5-4.5 mm, ca. 1/3 as long as petal, ±completely exerted from calyx, unspotted, ebarbellate, pinkish or reddish-purple, 3-6-toothed or sometimes entire, teeth irregular, very broadly triangular, up to 1/10 as long as limb; claw 7-10 × 0.7-1 mm, collar ±as wide as claw. Capsule shorter than calyx. Seeds elliptical, 2.6-3.2 × 1.1-2 mm.

**Distribution.** Turkey consists of two peninsulas. One of them, Thrace, is a historical and geographical area in southeastern Europe. This area is centered on the current borders of Bulgaria, Turkey, and Greece. The portion of the area in Turkey is also called "Rumeli". *Dianthus ucarii*, defined here as a new species, is endemic to the Thracian part of Turkey.

**Habitat.** *Dianthus ucarii* grows in *Quercus* L. forest clearings, grassy and stony places at altitudes of 300-500 m. It has been observed flowering in June and fruiting in August.

**Conservation status.** It is known in several localities, and the sum of these areas is smaller than 20,000 km<sup>2</sup> (Criterion B1). Because of overgrazing, the habitat of this species is under threat, and destruction of the species is leading to the reduction in the number of plants (Criterion A). The number of mature individuals in each subpopulation is less than 1000 (Criterion C2). Therefore, it should be considered vulnerable (VU) according to the IUCN Red List Criteria (IUCN, 2014).

**Etymology.** The species is named in honor of our good and valued friend Hacı Mustafa Uçar (Ankara, Turkey), who has participated in many field studies with us.

**Additional specimens for *Dianthus ucarii* (paratypes).** Turkey. Kırklareli: between Armutveren and Karadere (mountain road), around Karlıktepe, 500 m, 15.6.2013, *Hamzaoğlu* 6725, *Aksoy & Koç* (GAZI); Poyralı to Kırklareli, 305 m, grassy and stony places, 15.6.2013, *Hamzaoğlu* 6734 & *Koç* (GAZI).

**Specimens examined: *Dianthus pinifolius* Sm. subsp. *pinifolius*** - Turkey. İstanbul: Lieux sees, pres du sommet de Kaiche-dagh, non loin Erenkeuy [Kayış Mountain, near Erenköy], 13 Jul 1902, *Aznavour* s.n. (P, digital image); *ibid.*, 5.7.1950, *Baytop & Berk* 3524 (ISTE); Büyükçekmece, Kırac district, 145 m, 16.7.2014, *Hamzaoğlu* 7085 & *Koç* (GAZI); Çanakkale: Gökçeada, Peygamber Hill, ca. 330 m, 18.6.1976, *Seçmen* s.n. (EGE-20022); *ibid.*, Tepeköy, 30.5.2015, *Hamzaoğlu & Koç* 1938 (GAZI); Balıkesir: Bandırma, Edincik village road, around wind power plant, 295 m, 17.7.2014, *Hamzaoğlu* 7087 & *Koç* (GAZI); Tekirdağ: Ganos [Işıklar] Mountain, 750 m, 14.7.1968, *Baytop* s.n. (ISTE-13539). ***Dianthus lydus* Boiss.** - Turkey. Manisa: Spil Mountain National Park, 275 m, 11.6.2012, *Hamzaoğlu* 6326 & *Aksoy* (GAZI). ***Dianthus akdaghensis* Gemici & Leblebici** - Turkey. Antalya: between Elmalı and Seki, Akdağ, Yılmazlı Yaylası road, 1700 m, 17.6.2013, *Hamzaoğlu* 6762, *Aksoy & Koç* (GAZI). ***Dianthus carthusianorum* L.** - Turkey. Tekirdağ: Muratlı, around Arzulu village, 110 m, 29.5.2015, *Hamzaoğlu & Koç* 1914 (GAZI). ***Dianthus carmelitarum* Reut. ex Boiss.** - Turkey. Giresun: between Şebinkarahisar and Dereli, below Tamdere village, 1600 m, 23.8.2012, *Hamzaoğlu* 6617, *Aksoy & Koç* (GAZI). ***Dianthus giganteus* d'Urv. subsp. *giganteus*** - Turkey. Bilecik: between Pazaryeri and Bilecik, 615 m, 24.6.2012, *Hamzaoğlu* 6338, *Aksoy & Koç* (GAZI). ***Dianthus cibrarius* Clem.** - Turkey. Bursa: İnegöl, Hilmiye village, between Oylat Cave and Hacıkara village, 650 m, 9.8.2012, *Hamzaoğlu* 6603, *Aksoy & Koç* (GAZI). ***Dianthus cruentus* Griseb.** - Turkey. Kayseri: Melikgazi, Yılanlı Mountain, Kazım Karabekir neighborhood, ruins, 1240 m, 7.7.2012, *Hamzaoğlu* 6413 & *Aksoy* (GAZI).

#### Key to the species and varieties of *Dianthus* section *Carthusiani* in Turkey

- 1 Epicalyx scales narrowed gradually into an acute or acuminate apex ..... *D. giganteus*
- Epicalyx scales abruptly contracted into an aristate apex ..... 2
- 2 Enlarged portions of bracts and epicalyx scales enclosing 1/3 of calyx; epicalyx scales with membranous margin 0.6-1.1 mm wide ..... *D. cruentus*
- Enlarged portions of bracts and epicalyx scales enclosing 1/2-4/5 of calyx; epicalyx scales with membranous margin 0.1-0.5 mm wide ..... 3

- 3 Epicalyx scales 5–8 mm long; cauline leaves filiform, 0.5–1 mm wide, circular to canaliculated in cross-section ..... 4
- Epicalyx scales 9–26 mm long; cauline leaves linear, 1–3(–5.5) mm wide, flat to canaliculate in cross-section ..... 5
- 4 Apex of epicalyx scales acute to obtuse, arista 1/3–2/5 as long as scale; petal 16–20 mm long, limb 5–7 mm long and barbellate ..... *D. pinifolius*
- Apex of epicalyx scales obtuse-truncate to emarginate, arista 1/4 as long as scale; petal 11–15 mm long, limb 3.5–5 mm long and ebarbellate ..... *D. ucarii*
- 5 Sterile shoot leaves similar to cauline leaves, straight .. 6
- Sterile shoot leaves narrower than cauline leaves, usually curved ..... 7
- 6 Inner bracts with 0.3–0.5 mm wide scarious margins, arista 2/5–1/2 as long as bract; enlarged portions of bracts and epicalyx scales enclosing ca. 1/2 of calyx; calyx teeth 3–4 mm long; petal usually white spotted .. ..... *D. akdaghensis*
- Inner bracts with 0.5–1.1 mm wide scarious margins, arista 1/4–1/2 as long as bract; enlarged portions of bracts and epicalyx scales enclosing 1/2–2/3 of calyx; calyx teeth 4.5–7 mm long; petal unspotted ..... *D. carmelitarum*
- 7 Epicalyx scales indistinctly 3–5-veined at apex; calyx 3.5–4.5 mm wide; petal limbs broadly obovate or suborbicular, 8–11 × 6–10 mm, usually white spotted; seeds elliptic, 1.5–1.8 mm wide ..... *D. lydus*
- Epicalyx scales ±distinctly (5–)7–13-veined at apex; calyx 2.5–3.4 mm wide; petals limbs cuneate or obovate, 4–8 × 4–7 mm, unspotted; seeds suborbicular or ovate, 1.7–2.3 mm wide ..... 8
- 8 Stems glabrous; apex of epicalyx scales acute except arista, indistinctly 5–7-veined; calyx 13–15 mm long; petal claw 11–14 × 1.2–1.6 mm; seed suborbicular, 2.3–2.6 mm long ..... *D. carthusianorum*
- Stems puberulous; apex of epicalyx scales obtuse-truncate to emarginate except arista, ±distinctly 9–13-veined; calyx 17–22 mm long; petal claw 13–18 × 1–1.2 mm; seed ovate, 2.5–4 mm long ..... *D. cibrarius*

#### 4. Discussion

##### 4.1. Morphological comparison

*Dianthus pinifolius* Sm. is a polytypic species, and according to the *Flora Hellenica* it contains three subspecies (Strid, 1997). Among them, subsp. *lilacinus* (Boiss. & Heldr.) has distinct bracts large enough to surround the young inflorescence, different from Wettst. *D. pinifolius*. The subspecies is widespread in Albania, Macedonia, and Greece (Mora Peninsula, ~Peloponnese), and it prefers altitudes between 800 and 2000 m. In the subsp. *serbicus* West, bract and epicalyx scales are gradually narrowed

and end with a long awn. These awns are usually long enough to exceed the calyx teeth. Subsp. *serbicus* is widespread in Albania, Bulgaria, Macedonia, Romania, and Greece (Pindos Mountain). In another subspecies, subsp. *tenuicaulis* (Turrill) Strid, the inflorescence is (2-)3-5-flowered with sparse capitula (not 5-9-flowered and dense). This subspecies is widespread in northeastern Greece and south-central Bulgaria, and it prefers the fissures of rocks at altitudes between 500 and 1400 m. On the other hand, *Dianthus pinifolius* subsp. *pinifolius* grows in Macedonia, Greece, and Turkey (Thrace). The taxon prefers rocks, stony slopes, thickets, and glades at altitudes of 100–1200(–1800) m.

*Dianthus pinifolius* subsp. *pinifolius* is distributed in the İstanbul (Europe and Asia), Çanakkale (Gökçeada), Balıkesir (Bandırma), and Tekirdağ (Işıklar Mountain) provinces of Turkey. It also grows in Greece and Macedonia (Tutin and Walters, 1993; Strid, 1997). This species grows in grassy plains, forest clearings, and stony slopes. Its altitude ranges between 140 and 750 m. It has been observed flowering from late May through late July.

The habit of *Dianthus ucarii* is similar to that of *D. pinifolius* subsp. *pinifolius* in terms of distribution, habitat preference, and flowering period. However, these two species have significant differences, especially in flower characters such as the outer epicalyx scales with 0.4–0.5 mm scarious margins (not 0.2–0.3 mm), inner epicalyx scales equal to outer (not longer), calyx glabrous (not puberulous), calyx teeth being broadly triangular and 2–3 mm long (not narrowly triangular-lanceolate and 4–5 mm), and petal 11–15 mm long (not 16–20 mm) (Table 2).

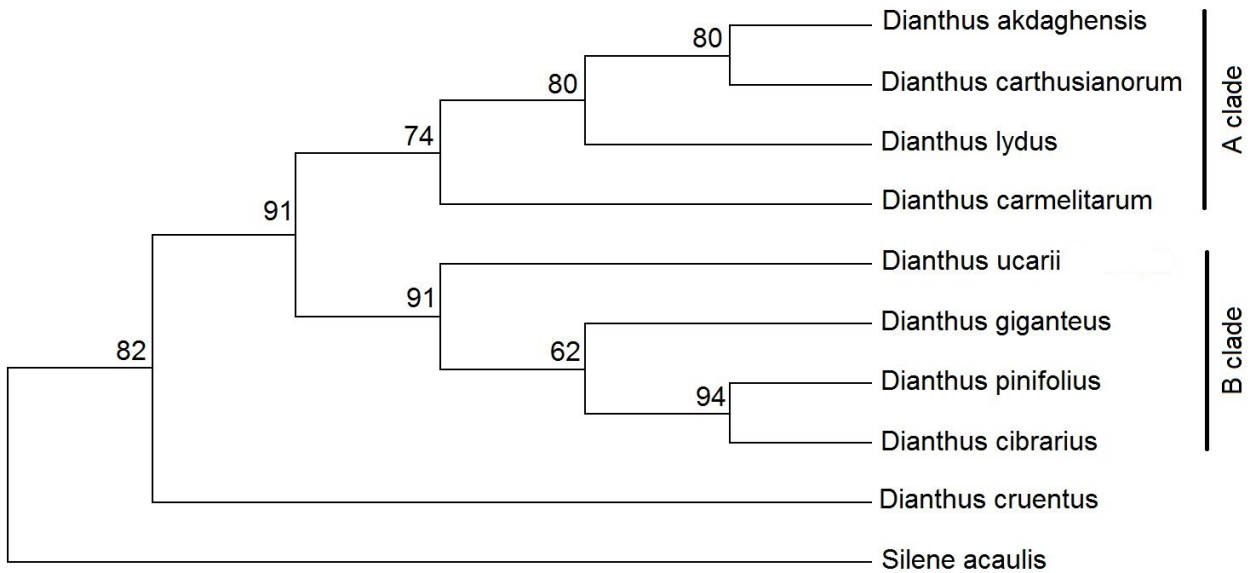
##### 4.2. Molecular phylogeny

The p16–p25 sequencing reactions for Turkish *Dianthus* sect. *Carthusiani* species yielded sequences of 562–700 nucleotides long. Within these sites, 462 were conservative, 162 were variable, and 74 were parsimony informative. When the base compositions were analyzed, range values of 24.6%, 25.2%, 22.6%, and 27.3% were observed for bases T, C, A, and G, respectively. These results are informative due to reflecting the polymorphism ratio within the current population. The average GC content of ITS regions of all examined *Dianthus* sect. *Carthusiani* specimens was 52.3%.

The ML tree based on the ITS sequences ensured adequate contribution to the taxonomic evaluation of *Dianthus* sect. *Carthusiani* species in the current study. The bootstrap values were more than 60% for most of the nodes (Figure 2). Phylogenetic tree analysis of section *Carthusiani* revealed mainly two distinct clades that are referred to as A and B. The bootstrap value was quite high (91) for this node. The eight taxa in these clades were observed at sufficiently high bootstrap values (between 62 and 94).

**Table 2.** Diagnostic characters of *Dianthus ucarii* and *D. pinifolius* (subsp. *pinifolius*).

	<i>D. ucarii</i>	<i>D. pinifolius</i> (subsp. <i>pinifolius</i> )
Stem	4-6-noded	7-9-noded
Epicalyx scale	Outer scales 3/5-3/4 as long as calyx, with 0.4-0.5 mm scarios margins, arista 1/4 as long as scale; inner equal to outer	Outer scales 3/5-2/3 as long as calyx, with 0.2-0.3 mm scarios margins, arista 1/3-2/5 as long as scale; inner longer than outer, 2/3-3/4 as long as calyx
Calyx	Glabrous; teeth broadly triangular, 2-3 × 1.5-2 mm, glabrous	Puberulous; teeth narrowly triangular-lanceolate, 4-5 × 1-1.5 mm, puberulous
Petal	11-15 mm long; limb 4-5 mm long, ebarbellate, teeth up to 1/10 as long as limb; claw 7-10 × 0.7-1 mm	16-20 mm long; limb 5-7 mm long, barbellate, teeth up to 1/5 as long as limb; claw 11-13 × 1.1-1.4 mm

**Figure 2.** The ML tree showing the genetic relationships within section *Carthusiani* based on the ITS gene.

Clades A and B constituted monophyletic groups themselves, although together they constituted a paraphyletic group. *D. carmelitarum* is seen as an ancestor species of clade A, which includes *Dianthus lydus*, *D. akdaghensis*, *D. carthusianorum*, and *D. carmelitarum* according to Figure 2. The most distinct characteristic of clade A is having completely cartilaginous bracts. The bracts of *D. pinifolius* subsp. *pinifolius*, *D. giganteus* subsp. *giganteus*, and *D. cibrarius* that are found in clade B are cartilaginous-scarious. *Dianthus cruentus*, which has a high bootstrap value (82), is seen as an ancestor species of clades A and B (Figure 2).

The bracts of this species are cartilaginous-scarious. Hence, it may be thought that clade A, which contains completely cartilaginous species, and clade B, which contains cartilaginous-scarious species, originated from

*D. cruentus*. In addition, *D. cruentus* is found in the same distribution area of all the species that are found in clades A and B. Four species being endemic (*D. lydus*, *D. akdaghensis*, *D. carmelitarum*, *D. cibrarius*) of the eight species of clades A and B supports the hypothesis that these species might have originated from a widely distributed species (except SE Turkey), such as *D. cruentus*. *Dianthus ucarii*, which has both cartilaginous and scarios bracts, has formed a monophyletic branch. When they are evaluated together, it can be seen that section *Carthusiani* is revealed as a monophyletic branch (Figure 2).

Considering both the morphological and molecular differences, the examples collected in Kırklareli from three different localities have been defined as a new species for the scientific world and they are named *Dianthus ucarii*.

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