




## Two New Spiny Species of *Dianthus* (Caryophyllaceae) from Turkey

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### ABSTRACT

Some *Dianthus* L. (Caryophyllaceae) specimens which have spiny epicalyx scales and spiny leaves have been collected from the Hanönü (Kastamonu) and the Mount Murat (Kütahya/Uşak). As a result of the investigations, these specimens have been decided to be new species for the scientific world and they have been named as *Dianthus varankii* Hamzaoglu & Koç and *Dianthus halisdemirii* Hamzaoglu & Koç. The descriptions, ecological preferences, distribution information and conservation status of the new species was given. Also, they were compared with closely related species, *D. aculeatus* Hamzaoglu, based on its general morphology and seed micromorphology. The relationships between new species and their close relatives were determined based on the ITS sequences.

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### Araştırma Makalesi

## Türkiye'den İki Yeni Dikenli *Dianthus* Türü (Caryophyllaceae)

### ÖZET

Hanönü (Kastamonu) ve Murat Dağı'ndan (Kütahya/Uşak) epikaliks pulları ve yaprakları dikenli olan bazı *Dianthus* L. (Caryophyllaceae) örnekleri toplandı. Yapılan incelemeler sonucu bu örneklerin bilim dünyası için yeni oldukları anlaşıldı ve bunlar *Dianthus varankii* Hamzaoglu & Koç ve *Dianthus halisdemirii* Hamzaoglu & Koç olarak adlandırıldı. Yeni türlerin betimlemeleri, ekolojik tercihleri, yayılış bilgileri ve mevcut koruma durumları verildi. Ayrıca, yakın akraba oldukları *D. aculeatus* Hamzaoglu ile genel morfoloji ve tohum mikromorfolojisi bakımından karşılaştırıldılar. Yeni türler ile yakın akrabaları arasındaki moleküler benzerlikler ITS sekans analizine bağlı olarak belirlendi.

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### INTRODUCTION

The genus *Dianthus* L. has approximately 600 species that mostly grows in Asia and Europe; there are 78 species in Turkey (Dequan and Turland, 2001; Gemici and Leblebici, 1995; Vural, 2008; Yılmaz et al., 2011; İlçim et al., 2013; Hamzaoglu et al., 2015; 2017). *Dianthus* is the second largest genus after *Silene* L. in Caryophyllaceae in Turkey (Reeve, 1967). The genus has been divided into five sections in "Flora Orientalis". The most imported characters of section *Dentati* Boiss. are having a barbate and dentate petal limbs (Reeve, 1967; Boissier, 1867). On the other hand, some of the taxa of section *Dentati* have spiny epicalyx leaves. Recently new species had been added into this spiny group (Yılmaz et al., 2011; Hamzaoglu et al., 2014). In addition, studies have shown that seed surface patterns in genus *Dianthus* are sufficiently distinctive (Hamzaoglu et al., 2014; 2015; Hamzaoglu and Koç, 2015).

The development of PCR technology has increased our knowledge about the phylogeny of the Caryophyllaceae (White et al., 1990; Rettig et al., 1992; Gardes and Bruns, 1993). In recent years, p16-p25 primer pairs (surrounding ITS region) have been frequently used for molecular phylogenetic studies in Caryophyllaceae (Popp and Oxelman, 2001; Popp et al., 2005).

The aim of this study was to contribute to *Dianthus* taxonomy by describe and adding two new species and determine the genetic relationships among other spiny species of *Dianthus* collected from Turkey.

### MATERIAL and METHODS

#### Plant Materials

*Dianthus* specimens which were identified as new species were collected from Hanönü (Kastamonu) and Murat Mountain (Kütahya/Uşak) in Turkey. Besides, the specimens included the materials needed for the seed micromorphology studies. The specimens

collected were comprehensively evaluated by the use of the literature along with the specimens present in GAZI, ANK, HUB, KNYA, E, K and P herbaria (Reeve, 1967; Bojnanský & Fargašová, 2007; Yılmaz et al., 2011; Hamzaoğlu et al., 2014). Pictures were taken with a CANON EOS60D digital camera. The seed surface micromorphology was visualized using the FEI Quanta 450 FEG (in 2017) and LEO 440 (in 2013) scanning electron microscopes. The vegetative characteristics were measured with a ruler with 0.5 mm accuracy and the floral characteristics were determined using an ocular micro-meter.

### DNA Extraction

All of the molecular experiments were performed in 'Plant Molecular Biology Laboratory of Ankara University' by İlker BÜYÜK. Leaf material of six *Dianthus* (Caryophyllaceae) specimens was dried in silica gel to preserve the tissue in the field for DNA extraction.

The genomic DNA was extracted according to the modified CTAB method as described by Aras et al. (2003). NanoDrop Lite Spectrophotometer (Thermo Scientific, USA) was used to check DNA quality and quantity and also the integrity of each DNA was checked by running a 1% agarose gel containing ethidium bromide and were visualized using an ultraviolet transilluminator (Syngene Chemi Genius 2 Bio Imaging System, UK).

### PCR Amplification and Sequencing

Sequence comparison of the ITS is frequently used in phylogenetic reconstructions at the intraspecific taxonomic level (Chase et al. 2000; White et al., 1990; Hamzaoğlu et al., 2017). On this basis, ITS sequences of six *Dianthus* specimens were analyzed in the current study. The ITS region was amplified using a primer set P16 (5'-TCACTGAACCTTATCATTAGAGGA-3') and P25 (5'-GGGTAGTCCCCTGACCTG-3') (Popp and

Oxelman, 2001; Popp et al., 2005). The PCR amplification was performed in 50 µl volume containing 10 µl of 10 × buffer, 1 unit Taq DNA polymerase (Promega, Madison, Wisconsin), 200 µM dNTPs, 2 mM MgCl<sub>2</sub>, and 10 pmol of both primers, p16 and p25. PCR amplification was performed in a Biometra TProfessional Standard (Biometra, GmbH, Germany) as follows: 94°C for 2 min, 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and a final extension step of 8 min at 72°C. The amplification products were analyzed by electrophoresis in 1.2% agarose gel containing ethidium bromide, and the product sizes were determined on gels by nucleotide size marker (100 bp ladder; Fermentas, Vilnius, Lithuania).

The PCR products were sequenced in both directions (once using the forward primer, and once using the reverse primer) with a BigDye cycle sequencing kit (Applied Biosystems, Foster City, California) using an ABI 3130XL genetic analyzer Applied Biosystems, Foster City, California). The derived ITS sequences identity was confirmed by appropriate program Blast (NCBI) to search the DNA sequence databases for high similarity with other *Dianthus* species (<http://www.ncbi.nlm.nih.gov/BLAST>). The quality check of the nucleotide peaks and the collapsing the forward and reverse Sanger reads into consensus sequence was done through Geneious version R9 (<http://www.geneious.com>, Kearse et al., 2012).

### Sequence Alignment and Phylogenetic Analysis

The amplified fragments were sequenced and the outgroup *Petrorhagia saxifraga* (L.) Link. was obtained from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) to compare with *Dianthus* sequences (Table 1). All sequences were first aligned using CLC Main Workbench ver. 8.0 (<https://www.qiagenbioinformatics.com/>) and the alignment was checked using the MEGA7 software (Kumar et al., 2016).

Table 1. GenBank accession numbers of the ITS sequences from six spiny *Dianthus* sect. *Dentati* specimens which were obtained in this study.

Collector number	Species	GenBank accession no.
Hamzaoğlu 6589	<i>Dianthus erinaceus</i> Boiss. var. <i>erinaceus</i>	KY364854
Hamzaoğlu 6590	<i>D. erinaceus</i> Boiss. var. <i>alpinus</i> Boiss.	KY364855
Hamzaoğlu 6596	<i>D. goekayi</i> Kaynak, Yılmaz & Daşkın	KY364856
Hamzaoğlu 6744	<i>D. aculeatus</i> Hamzaoğlu	KY364857
Hamzaoğlu 7241	<i>D. varankii</i> Hamzaoğlu & Koç	KY364858
Koç 2127	<i>D. halisdemirii</i> Hamzaoğlu & Koç	KY364859
Outgroup	<i>Petrorhagia saxifraga</i> (L.) Link	KX184019.1

The pairwise sequence divergence between species in ITS was determined by Kimura-2 parameter model (Kimura, 1980). The base composition analysis was performed using MEGA7 and the phylogenetic tree (bootstrap replicates=1000) was constructed using the

Maximum likelihood (ML) method (Felsenstein, 1985; Tamura et al., 2011; Kumar et al., 2016).

## RESULTS and DISCUSSION

*Dianthus varankii* Hamzaoğlu & Koç, **sp. nov.**

**Type:** Turkey, A5 Kastamonu, Hanönü, Küreçayı village turnout, *Pinus brutia* Ten. and *Quercus pubescens* Willd. openings, 480 m, 13.08.2016,

*Hamzaoğlu 7241 & Koç* (holo. GAZI, iso. GAZI, ANK) (Figure 1).



Figure 1. *Dianthus varankii* sp. nov. (A) habit and habitat, (B) spiny leaves, (C) petals, (D) calyx and epicalyx scales.

**Diagnosis:** *Dianthus varankii* is related to *D. aculeatus*. It differs from this species mainly by stem 10–26 cm long (not 2–6 cm); calyx 24–30 mm long (not 18–26 mm); petal 30–37 mm long (not 22–26 mm); claw 22–27 mm long (not 15–18 mm) and collar as wide as claw (not c. 1/2 as wide as claw).

**Description:** Perennial, cushion-form, spinose and pruinose herbs. Stems erect, 10–26 cm tall, branching from middle to upper node or rarely unbranched, 3–7-noded, puberulous, dead leaves persistent at base. Leaves linear-subulate, canaliculate, thick, usually puberulous, margins scabrous, spinose at apex; sterile shoots leaves subequal to cauline leaves; cauline 20–32 × 0.5–1 mm, separated from stem, equal or shorter than internodes, rigid, 3-veined, sheaths slightly longer than wide; upper leaves similar but smaller, nodes sometimes purplish. Inflorescence (1–)2–4(–7)-flowered; pedicels 5–25 mm, puberulous, greenish. Epicalyx scales 8–10(–12), cartilaginous, spinescent, greenish to purplish, ± separated from calyx, veinless below, indistinctly 7–11-veined above, with scarios (0.3–0.5 mm) margins, apex obtuse to truncate except arista; outer linear to linear-lanceolate, 4–8 × 1–1.5 mm, puberulous, up to 1/4 as long as calyx, arista c. 3/5 as long as scale; inner oblanceolate, 12–18 × 2.2–3.6 mm, puberulous, c. 1/2 as long as calyx, arista 1/4–1/3 as long as scale. Calyx cylindrical-lanceolate, 24–30 ×

2–3.5 mm, ± veinless below, distinctly 35–40-veined above, puberulous, purplish; teeth narrowly oblong-lanceolate, 7–10 × 1–1.5 mm, 7–9-veined, with ciliate and scarios margins, apex acute and long mucronate. Petals 30–37 mm long; limb broadly cuneate, 8–10 × 7–9 mm, c. 1/4 as long as petal, claw exerted from calyx, spotted, barbulate, pinkish purple on both surfaces, 7–11-toothed at apex, teeth narrowly triangular, up to 1/5 as long as limb; claw 22–27 × 1–1.2 mm, collar as wide as claw. Capsule not exerted from calyx.

**Seed morphology:** Seeds oblong, 3.2–4.5 × 0.9–1.3 mm, blackish, ungranular; dorsal surface convex, with irregular rectangular cells, tuberculate, with 3–6 teeth on each margin, teeth V-undulate, apparent; ventral surface convex, with regular rectangular cells, tuberculate, with 6–8 teeth on each margin, teeth S-undulate, apparent; apex beaked.

**Etymology:** Prof. Dr. İlhan VARANK, Yıldız Technical University, Faculty of Education, attended to street demonstrations with the aim of protesting the coup attempt held on 15th of July 2016 in Turkey and was martyred by the coup plotters. The species *Dianthus varankii* described in this study was dedicated in memory of our martyr.

**Ecological preferences:** Hanönü is a place located in the Gökırmak valley, northeast of Kastamonu. Gökırmak



valley has a typical Mediterranean microclimate in the Euro-Siberian phytogeographic region. The rare forests which consist of *Pinus brutia* Ten., *Quercus pubescens* Willd., *Carpinus betulus* L. and *Juniperus oxycedrus* L. subsp. *oxycedrus* species are dominant on the bottom of the valley. The open areas are observed in this habitat which prefers an altitude ranging from

400 to 700 meters. These open areas are proper areas for the growing of *Dianthus varankii*.

***Dianthus halisdemirii* Hamzaoğlu & Koç, sp. nov.**

**Type:** Turkey, B2 Kütahya, Gediz, Murat Mountain, road of ski resort, towards summit, serpentine slopes, 2050 m, 02.08.2015, *Hamzaoğlu & Koç* 2127 (holo. GAZI, iso. GAZI, ANK) (Figure 2).

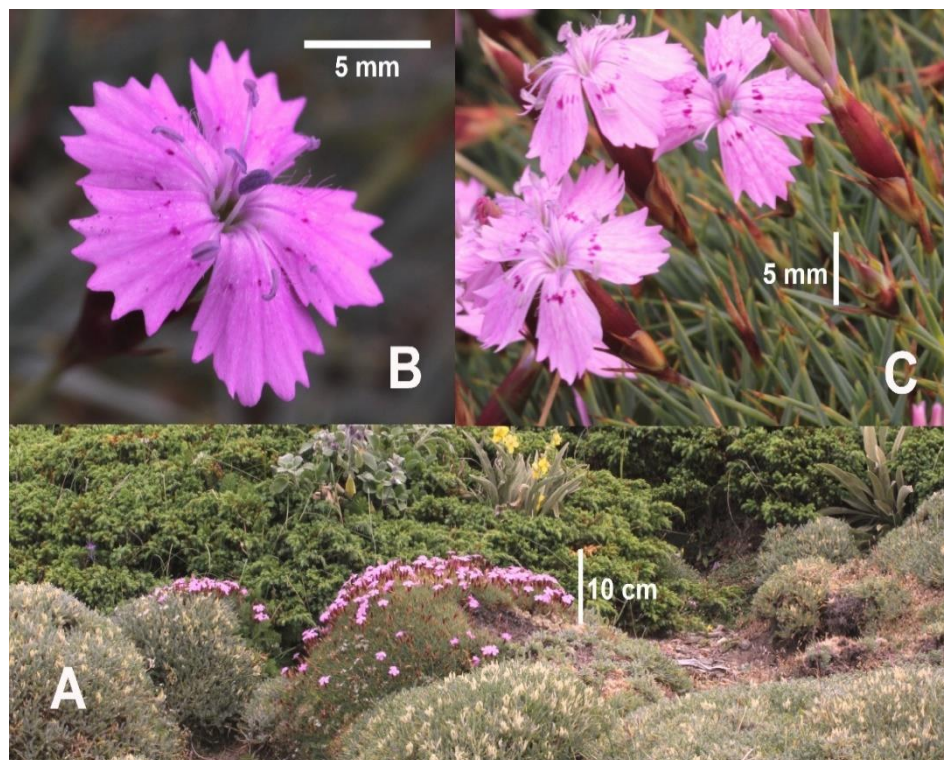


Figure 2. *Dianthus halisdemirii* sp. nov. (A) habit and habitat, (B) petals, (C) calyx, epicalyx scales and spiny leaves.

**Diagnosis:** *Dianthus halisdemirii* is related to *D. aculeatus*. It differs from its by cauline leaves (sheaths 2–3 times as long as wide; not equal or slightly longer); calyx 11–14 mm long and distinctly 35–40-veined (not 18–26 mm long and distinctly 45–50-veined); petals 15–18 mm long (not 22–26 mm long) and petal limbs cuneate, 4–6 × 3.5–5.5 mm (not broadly cuneate, 7–8 × 6.5–7.5 mm).

**Description:** Perennial, cushion-form, spinose and pruinose herbs. Stems erect, 3–9(–12) cm tall, unbranched or only branching from upper node, 2–4-noded, glabrous; dead leaves persistent at base. Leaves linear-subulate, canaliculate, thick, glabrous, margins scabrous, spinose at apex; sterile shoots leaves longer than cauline leaves; cauline 10–15 × 0.5–0.8 mm, ± separated from stem, shorter than internodes, rigid, 3–5-veined, sheaths 2–3 times as long as wide; upper similar but smaller, nodes sometimes purplish. Inflorescence 1–2(–3)-flowered with strict inflorescence; pedicels 2–20 mm, glabrous, greenish. Epicalyx scales 4–6, cartilaginous, spinose, straw-colored, greenish or purplish, separated from calyx, veinless below, indistinctly 7–11-veined above, with

scarious (0.4–0.6 mm) margins, apex acute-obtuse to truncate except arista; outer linear-lanceolate, 8–11 × 2–3.5 mm, glabrous, up to 2/3 as long as calyx, arista c. 1/3 as long as scale; inner oblanceolate to obovate, 9–12 × 3.5–4.4 mm, glabrous, c. 2/3 as long as calyx, arista c. 1/3 as long as scale. Calyx ovate-lanceolate, 11–14 × 3–4 mm, ± veinless below, distinctly 35–40-veined above, glabrous, greenish or purplish; teeth narrowly triangular, 3.5–4.5 × 1–1.5 mm, 7–9-veined, with ciliate and scarious margins, apex acuminate and long mucronate. Petals 15–18 mm long; limb cuneate, 4–6 × 3.5–5.5 mm, c. 1/3 as long as petal, c. 3/4 exerted from calyx, spotted, barbulate, pinkish purple above, greenish below, 5–9-toothed from middle to apex, teeth triangular, up to 1/5 as long as limb; claw 11–12 × 1.2–1.5 mm, collar c. 1/2 as wide as claw. Capsule not exerted from calyx.

**Seed morphology:** Seeds elliptic, 1.6–2.6 × 0.8–1.2 mm, blackish, ungranular; dorsal surface convex, with regular square cells, tuberculate, with 3–5 teeth on each margin, teeth V-undulate, apparent; ventral surface convex, with regular rectangular cells, tuberculate, with 5–9 teeth on each margin, teeth V-

undulate, apparent; apex beaked.

**Etymology:** Command Sergeant Major Ömer HALİSDEMİR was martyred by the coup plotters who intended to capture The Special Forces Command during the coup attempt held on 15th of July 2016 in Turkey. The species *Dianthus halisdemirii* described in this study was dedicated in memory of our martyr.

**Ecological preferences:** *Dianthus halisdemirii* is a local endemic species restricted to the Mount Murat, west Anatolia. The *Dianthus halisdemirii* prefers open areas in forest, on rocky sides of subalpine and alpine zones. The peak of the Mount Murat is Kartaltepe which has an altitude of 2309 meters. The forests which consist of *Pinus brutia*, *Pinus nigra* J.F.Arnold, *Pinus sylvestris* L., *Quercus ithaburensis* Decne subsp.

*macrolepis* (Kotschy) Hedge & Yalt., *Q. cerris* L., *Juniperus excelsa* M.Bieb., *Fagus orientalis* Lipsky and *Carpinus betulus* L. between 900 and 2000 meters altitude. The *Juniperus communis* L. var. *saxatilis* Pall. and the *Acantholimon* Boiss spp., *Astragalus* L. spp. are prevalent in the subalpine and alpine zone, respectively.

*Dianthus varankii* and *D. halisdemirii* are distinct from *D. aculeatus*, especially in terms of floral characters such as calyx and petals length. In *D. aculeatus*, the petal length is shorter than *D. varankii* and the calyx length longer than *D. halisdemirii*. There are also some differences in terms of stem length and width/length ratios of cauline leaves sheaths among these species (Figure 3, Table 2).



Figure 3. Flowers: A. *Dianthus varankii* (Hamzaoğlu 7241 & Koç), B. *D. halisdemirii* (Hamzaoğlu & Koç 2127), C. *D. aculeatus* (Hamzaoğlu 6744).

Table 2. Comparison of the diagnostic characteristics of *Dianthus varankii*, *D. halisdemirii* and *D. aculeatus*.

Characters	<i>D. varankii</i>	<i>D. halisdemirii</i>	<i>D. aculeatus</i>
Stems	10–26 cm tall	3–9(–12) cm tall	2–6 cm tall
Cauline leaves	sheaths slightly longer than wide	sheaths 2–3 times as long as wide	sheaths equal or slightly longer than wide
Calyx	24–30 mm long, distinctly 35–40-veined	11–14 mm long, distinctly 35–40-veined	18–26 mm long, distinctly 45–50-veined
Petals	30–37 mm long	15–18 mm long	22–26 mm long
Petal limbs	broadly cuneate, 8–10 × 7–9 mm	cuneate, 4–6 × 3.5–5.5 mm	broadly cuneate, 7–8 × 6.5–7.5 mm
Petal claws	22–27 mm long, collar as wide as claw	11–12 mm long, collar c. 1/2 as wide as claw	15–18 mm long, collar c. 1/2 as wide as claw

The seed surfaces of *Dianthus varankii* and *D. halisdemirii* are different than the seeds of *D.*

*aculeatus* as regard to the cell edges of dorsal surfaces (teeth V-undulate, not S-undulate) and the shape

ventral surfaces (convex, not flat). In addition, the *D. varankii* seeds differ in shape and size, and the *D. halisdemirii* seeds differ in cell shape of dorsal surfaces and cell edges of ventral surfaces (Figure 4, Table 3).

ITS-rDNA was successfully amplified for all six spiny species of section *Dentati* and ITS sequencing reactions for *Dentati* species yielded alignable 708 nucleotides long. Within these species, 553 were conservative and 53 were variable. When the base compositions were analysed, range values of 23.8–24.4, 25.1–25.6, 22.5–22.8, and 27.6–27.9 were observed for bases T, C, A, and G, respectively. Pairwise distances of aligned ITS sequences of all sect. *Dentati* species were calculated in this study. Accordingly, pairwise distances ranged from 0.001 (*D. aculeatus* and *D. goekayi*) to 0.088 (*D. erinaceus* var. *erinaceus* and outgroup *Petrorhagia saxifraga*). The pairwise distance between *Dianthus varankii* and *D. halisdemirii* and its closely related species *Dianthus aculeatus* is 0.005 and 0.007, respectively. These results supported the morphological data.

The ML tree showed that *Petrorhagia saxifraga* used as an outgroup was branched very far away from the *Dentati* species. In addition, *D. halisdemirii* and *D. varankii* were branched far away from the other *Dentati* species interestingly. All other *Dianthus* species (*D. erinaceus* var. *erinaceus*, *D. erinaceus* var.

*alpinus* Boiss., *D. goekayi* and *D. aculeatus*) were grouped together within a different clade (Figure 5).

The spiny species belonging to the section *Dentati* of genus *Dianthus* from Turkey including *D. halisdemirii* grow in the Mediterranean phytogeographic region (Reeve, 1967; Hamzaoglu et al., 2014). Whereas *Dianthus varankii* grows in the Euro-Siberian phytogeographic region of Turkey (Figure 6). According to the current data *Dianthus varankii* grows in Hanönü (Kastamonu) district, which have an area of approximately 150 km<sup>2</sup>. It has a discontinuous distribution due to sparse *Pinus brutia* and *Quercus pubescens* forests, settlement, and farming areas. The habitat of this species is may be under danger due to being destroyed and/or decreased in the future. *Dianthus halisdemirii* grows in the Mount Murat, between Gediz (Kütahya) and Banaz (Uşak) districts, which have an area of approximately 70 km<sup>2</sup>. No threats are present for this species as of today. The possibility of becoming a prey of wild or pet animals is extremely low because of being spiny species. But, land-use changes could have a detrimental impact in the future. Even so, since a thorough assessment has not been undertaken for this new species, the conservation status of Not Evaluated (NE) has been assigned to this species as per IUCN Red List categories and criteria (2017).

Table 3. Comparison of the diagnostic seed characters of *Dianthus varankii*, *D. halisdemirii* and *D. aculeatus*.

Seed Characters	<i>Dianthus varankii</i>	<i>Dianthus halisdemirii</i>	<i>Dianthus aculeatus</i>
Shape	oblong	elliptic	elliptic
Size	3.2–4.5 × 0.9–1.3 mm	1.6–2.6 × 0.8–1.2 mm	1.8–2.5 × 1.2–1.5 mm
Cell shape of dorsal surface	with irregular rectangular	with regular square	with regular rectangular
Cell edges of dorsal surface	teeth V-undulate	teeth V-undulate	teeth S-undulate
Ventral surface	convex	convex	flat
Cell edges of ventral surface	teeth S-undulate, apparent	teeth V-undulate, apparent	teeth S-undulate, not apparent



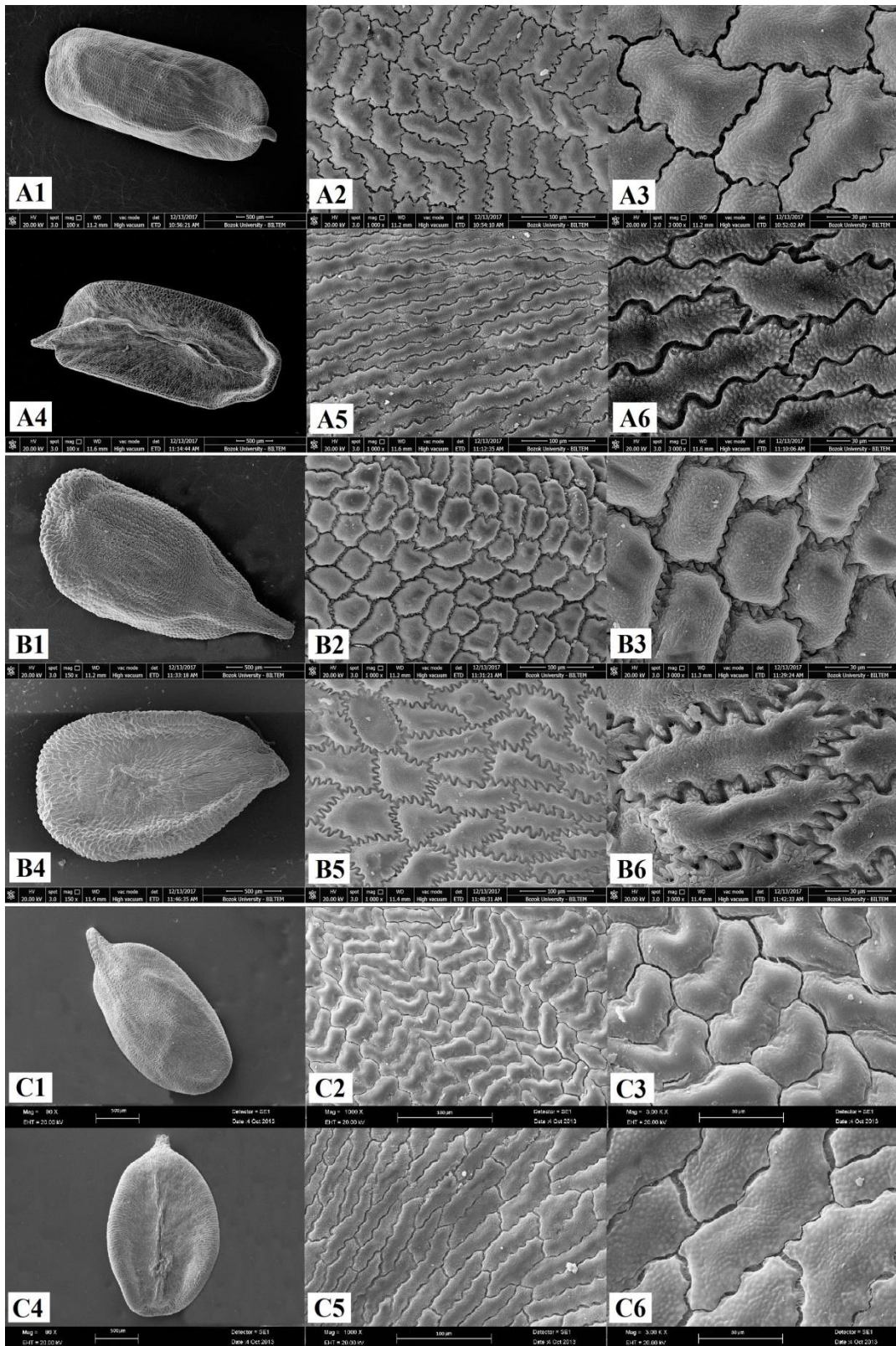


Figure 4. SEM photographs of the seed coat: A. *Dianthus varankii*, B. *D. halisdemirii*, C. *D. aculeatus*; 1-3: dorsal surface, 4-6: ventral surface; Scale bars. 1 and 4: 500 µm, 2 and 5: 100 µm, 3 and 6: 30 µm.

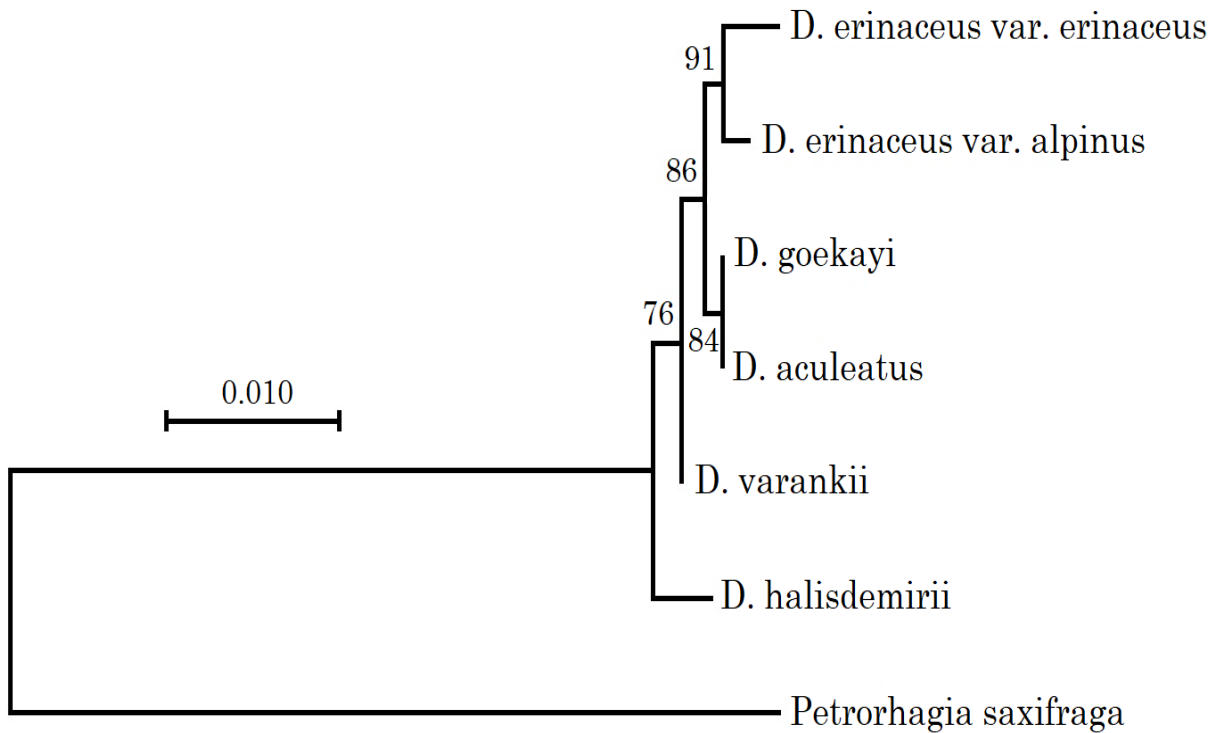


Figure 5. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-1105.5009) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

The specimen Davis 36814 (Herbarium E) which was collected from the Mount Murat, was evaluated as *Dianthus erinaceus* var. *alpinus* in the Flora of Turkey (Reeve, 1967). The specimen Davis 36871 which was collected from the same area with the specimen Davis 36814 was introduced as paratypes of *D. aculeatus* in another study (Hamzaoğlu et al., 2014). However, this assessment was made using the pictures obtained from the Edinburgh online herbarium instead of collected

real specimens. Finally, new specimens from the Mount Murat were collected and investigated morphologically in 2015. Based on the morphological and molecular data, the specimens of the Mount Murat were identified as new species and named as *D. halisdemirii* (Tables 2 and 3, Figures 3, 4 and 5). The distinctive characteristics of the species compared to the other morphological closely related species were given in the diagnostic key below.

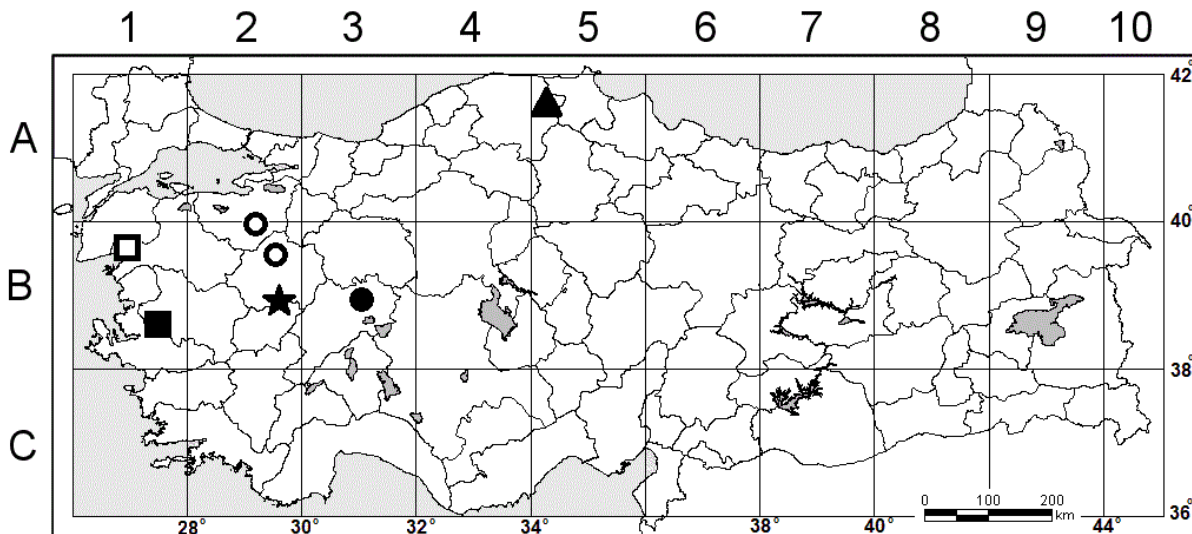


Figure 6. Distribution map of *Dianthus varankii* (▲), *D. halisdemirii* (★), *D. aculeatus* (●), *D. goekayi* (○), *D. erinaceus* var. *erinaceus* (■) and *D. erinaceus* var. *alpinus* (◻) in Turkey.



**Key to closely related *Dianthus* species**

1. Inner epicalyx scales c. 1/2 as long as calyx; petals 22-37 mm long ..... **2**  
– Inner epicalyx scales more than 1/2 as long as calyx; petals 14-21 mm long ..... **3**
2. Stems 10–26 cm tall; epicalyx scales indistinctly 7–11-veined; calyx distinctly 35–40-veined; petals 30–37 mm long, claws 22–27 mm long .. ***D. varankii***  
– Stems 2–6 cm tall; epicalyx scales indistinctly 3–5-veined; calyx distinctly 45–50-veined; petals 22–26 mm long, claws 15–18 mm long ..... ***D. aculeatus***
3. Cauline leaves adpressed to stem; petal limbs 3-3.2 mm wide ..... ***D. goekayi***  
– Cauline leaves separated from stem; petal limbs 3.5-5.5 mm wide ..... **4**
4. Epicalyx scales 4–6 pieces, indistinctly 7–11-veined ..... ***D. halisdemirii***  
– Epicalyx scales 8–10(–12) pieces, distinctly 1–3-veined ..... **5**
5. Cauline leaves 12-20 mm long; calyx 16-20 mm long; petals 18-21 mm long .... ***D. erinaceus* var. *erinaceus***  
– Cauline leaves 7-9 mm long; calyx 12-15 mm long; petals 14-16 mm long ..... ***D. erinaceus* var. *alpinus***

**Additional Specimens Seen**

***Dianthus aculeatus***: Turkey, Afyonkarahisar: Between Bayat and İscehisar, Köroğlu Pass, 1500 m, rocky igneous slopes with tuff gravels and shrub openings, 16.06.2013, *Hamzaoğlu* 6744, *Aksoy & Koç* (holotype, GAZI; isotypes, GAZI, ANK, HUB); Bayat, between Asartepe and İnpazarcık, c. 1300 m, rocky slopes with schist, 03.07.1975, *M. Vural* 874 (paratype, KNYA); İscehisar, NW of Karakaya village, 1250–1500 m, slopes with tuff gravels, 27.06.2002, *Z. Aytaç* 8413 (paratype, GAZI); ***Dianthus goekayi***: Turkey, Bursa: Between Soğukpınar and Karaislah villages, 860 m, *Quercus* forest openings, serpentine stony slopes, 08.08.2012, *Hamzaoğlu* 6596, *Aksoy & Koç* (GAZI); Kütahya: Tavşanlı, between Derbent and Elmaağacı villages, 960 m *Quercus* forest openings, serpentine stony slopes, 09.08.2012, *Hamzaoğlu* 6605, *Aksoy & Koç* (GAZI); ***Dianthus erinaceus* var. *erinaceus***: Turkey, Manisa: National Park of Spil Dağı, Atalanı, around forest watchtower, 1475 m, rocks, 05.08.2012, *Hamzaoğlu* 6589, *Aksoy & Koç* (GAZI); In mt. Sypilos supra Magnesiam, ?? .07.1842, *Boissier* s.n. (E, E00301868-photo; K, K000725492-photo, K000725493-photo, K000725495-photo); Sommel du Mont Sipyle, au-dessus de Magnesie, 11.08.1854, *Balansa* s.n. (P, P04948451-photo); Spil Mountain, S of Atalanı, 1200–1300 m, rocks, 08.08.1983, *H. Duman* 1083 (GAZI); ***Dianthus erinaceus* var. *alpinus***: Turkey, Balıkesir: Edremit, Zeytinli village, National Park of Kaz Dağı, Sarıkız road, 1675 m, rocks, 06.08.2012, *Hamzaoğlu* 6590, *Aksoy & Koç* (GAZI); *ibid.*,

calcareous rocks in summit, ?? .1968, *Quezel* et al. s.n. (ANK); *ibid.*, 07.07.1965, *Pamukçuoğlu* s.n. (HUB, 03827); *ibid.*, 25.07.1968, *Pamukçuoğlu & Quezel* s.n. (HUB, 03826).

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