



New chromosomal data of the genus *Dianthus* section *Dentati* (*Caryophyllaceae*, *Sileneae*)

Esra MARTİN¹, Halil Erhan EROĞLU^{2*}, Ergin HAMZAOĞLU³, Murat KOÇ⁴, Ashhan KELKİTOĞLU⁵, Emre ÖZTÜRK⁶, Esra TANHAŞ⁷, Merve MAŞA⁸, Havva BOZKURT⁹, Fatma Nagehan YAVAŞ¹⁰

^{1,5-10}Necmettin Erbakan University, Science Faculty, Department of Biotechnology, Konya, Türkiye

²Yozgat Bozok University, Science and Arts Faculty, Department of Biology, Yozgat, Türkiye

³Gazi University, Gazi Education Faculty, Department of Elementary Education, Ankara, Türkiye

⁴Yıldırım Beyazıt University, Public Health Institute, Department of Traditional, Complementary and Integrative Medicine, Ankara, Türkiye

*herhan.eroglu@bozok.edu.tr, ¹esramartin@gmail.com, ³erginhamzaoglu@yahoo.com, ⁴muratkoc@ybu.edu.tr,

⁵aslhankelkitoglu@yahoo.com, ⁶emreozturk33@yahoo.com, ⁷ekarakas@konya.edu.tr, ⁸mervemasa33@yahoo.com,

⁹hawa_bzkrt@hotmail.com, ¹⁰nagehanyavas05@gmail.com

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Dianthus cinsi *Dentati* seksiyonunun (*Caryophyllaceae*, *Sileneae*) yeni kromozomal verileri

Abstract: In section *Dentati*, the chromosome numbers were reported from only eight of 30 taxa. There is no chromosomal record relating to the other 22 taxa. In the present study, it is intended to detect the chromosome numbers of section *Dentati* taxa. Chromosome numbers of 15 taxa were obtained, 13 of which were reported for the first time and two numbers agreed with the previous reports. 14 taxa were diploid with $2x = 30$ and only one taxon was polyploid with $4x = 60$. Polyploidy might have played a role in the karyotype evolution of the genus. Although polyploidy was seen, there was no dysploidy causing to change in the basic chromosome number. According to all chromosomal data on *Dianthus*, the basic chromosome number was only one ($x = 15$). In conclusion, this study presents new data into the karyological characteristics of section *Dentati* (genus *Dianthus*) that may be useful for understanding or interpreting relationships among the sections.

Key words: Chromosome, cytotaxonomy, *Caryophyllaceae*, *Dianthus*, *Dentati*

Özet: *Dentati* seksiyonunda, toplam 30 taksonun sadece sekizinin kromozom sayıları rapor edilmiştir. Diğer 22 taksona ait kromozomal kayıt bulunmamaktadır. Bu çalışmada, *Dentati* seksiyonu taksonlarının kromozom sayılarının tespit edilmesi amaçlanmıştır. On üçü ilk kez rapor edilen, iki tanesi de önceki raporlarla uyumlu olan 15 taksonun kromozom sayısı elde edilmiştir. 14 takson $2x = 30$ ile diploid ve sadece bir takson $4x = 60$ ile poliploiddir. Poliploidi, cinsin karyotip evriminde rol oynamış olabilir. Poliploidi görülmesine rağmen, temel kromozom sayısını değiştirmeye neden olan disploidi yoktur. *Dianthus*'taki tüm kromozomal verilere göre, temel kromozom sayısı yalnızca bir tanedir ($x = 15$). Sonuç olarak, bu çalışma, *Dentati* (cins *Dianthus*) seksiyonunun karyolojik özelliklerine, seksiyonlar arasındaki ilişkileri anlamak veya yorumlamak için faydalı olabilecek yeni veriler sunmaktadır.

Anahtar Kelimeler: *Caryophyllaceae*, *Dianthus*, *Dentati*, kromozom, sitotaksonomi

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1. Introduction

Dianthus L., is a large genus of subfamily *Sileneae* DC. of family *Caryophyllaceae*, is composed of more than 300 species, and its species are widely distributed in the Asia and Mediterranean region of Europe (Şahin et al., 2016; Altay et al., 2017; Hamzaoğlu et al., 2021). However, Africa and North America have a few species of *Dianthus* (Madhani et al., 2018).

“Flora Orientalis” is the first work that contains comprehensive information about the Turkish *Dianthus* taxa (Boissier, 1867). Forty-eight of the 89 *Dianthus* species mentioned in this work are related to the flora of Türkiye. Also here, the genus *Dianthus* is grouped as *Verruculosi* Boiss., *Leiopetali* Boiss., *Fimbriati* Boiss., *Dentati* Boiss., and *Carthusiani* Boiss. under 5 groups. On the other hand, Williams (1893) divided the *Dianthus* into 3 subgenera as *Carthusianastrum* F.N.Williams, *Caryophyllastrum* F.N.Williams, *Proliferastrum*

F.N.Williams, and into many sections and subsections together with those given by Boissier. The first study focusing only on Turkish taxa was done by Reeve (1967). In this revision published in “Flora of Türkiye and the East Aegean Islands”, first the group key was given and the genus was divided into 5 sections as *Verruculosi*, *Leiopetali*, *Fimbriati*, *Dentati* and *Carthusiani*.

The taxa of section *Dentati* are distinguished from other taxa with short-leaf sheaths (not longer than $3 \times$ stem diameter), dentate petal margins (not entire or fimbriate), barbellate petal surfaces (not glabrous), and not verruculose calyx (Boissier, 1867; Reeve, 1967). *Dentati* is the section containing the highest number of taxa of the genus *Dianthus* in Türkiye and includes 20 species in the Flora of Türkiye (Reeve, 1967). Some species of the section are common in Türkiye and Greece. The current literature has been taken into account in writing the taxonomic status and valid names of the section *Dentati* taxa of Turkish *Dianthus*

(Hamzaoğlu et al., 2014, 2015; Hamzaoğlu and Koç, 2018, 2019, 2020; Hamzaoğlu, 2021).

The basic and diploid chromosome numbers are the most basic characters concerning the genome of species and they are methodologically the easiest to apply. Therefore, chromosomal data for plant organisms have been reported worldwide for long years (Stace, 2000). Karyotype analysis is a basic part of cytotaxonomic studies of organisms. Therefore, it can play a significant role, particularly in taxonomically complicated species or genera (Kashmenskaya and Polyakov, 2008). In genus *Dianthus*, the basic chromosome number is $x = 15$ (Carolin, 1957) and the diploid chromosome number of many taxa is $2n = 30$ (Darlington and Wylie, 1956; Sünter, 1979; Magulaev, 1982; Zhang, 1992; Runemark, 1996; Martin et al., 2009; Şahin et al., 2016; Altay et al., 2017; Ahıskalı et al., 2020).

In section *Dentati*, the chromosome numbers were reported from only eight of 30 taxa. Six taxa are only diploid with $2n = 2x = 30$. *D. zonatus* Fenzl is polyploid, which reveals only one polyploidy level of hexaploidy ($2n = 6x = 90$). *D.*

barbatus L. is diploid ($2n = 2x = 30$) and polyploid ($2n = 4x = 60$) (Darlington and Wylie, 1956; Sünter, 1979; Magulaev, 1982; Zhang, 1992; Runemark, 1996; Martin et al., 2009; Ahıskalı et al., 2020). There is no chromosomal record relating to the other 22 taxa. In the present study, it is intended to detect the chromosome numbers of other taxa. Thus, significant contributions will be made to the cytotaxonomy of section *Dentati*.

2. Material and method

2.1. Plant samples

Fifteen *Dianthus* taxa were collected from their natural habitats across Türkiye. Exsiccates were deposited at the herbarium of the Gazi University (GAZI) in Ankara. Table 1 presents the *Dentati* taxa, authors, distribution regions, collection information, and collector numbers.

2.2. Preparation and observation

The cytogenetic procedure consisted of the different processes; which were germination in petri dishes at room temperature, pretreatment by α -mono-bromonaphthalene at

Table 1. The collection information of section *Dentati* (*Dianthus*) by last taxonomic status and valid names

Taxa (alphabetically)	Distribution regions and collection information
<i>D. acrochlonis</i> Stapf	Endemic. Türkiye. Antalya: Elmalı, Bey Mountain, Küçüksöğle village, above Serkizalan High Plateau, Kırkmar Gedığı place, 2240 m a.s.l., grassy plains in doline, 28 July 2012, Hamzaoğlu 6549 , Aksoy & Koç (GAZI).
<i>D. aculeatus</i> Hamzaoğlu	Endemic. Türkiye. Afyonkarahisar: Between Bayat and İncehisar, Köroğlu Pass, 1500 m a.s.l., rocky igneous slopes with tuff gravels and shrub openings, 16 June 2013, Hamzaoğlu 6744 , Aksoy & Koç (GAZI).
<i>D. armeria</i> L.	Europe (except Eastern Europe), Caucasus, Iran and Türkiye. Zonguldak: Between Ereğli and Zonguldak, c. 10 km, 265 m a.s.l., 10 August 2012, forest clearings, Hamzaoğlu 6613 , Aksoy & Koç (GAZI).
<i>D. aticii</i> Hamzaoğlu	Endemic. Türkiye. Bilecik: Bilecik highway exit towards Eskişehir, 330 m a.s.l., stony slopes and steppes, 16 June 2013, Hamzaoğlu 6743 & Koç (GAZI).
<i>D. brevicaulis</i> Fenzl	Endemic. Türkiye. Niğde: Ulukışla, Bolkar Mountain, above Maden village, south of Meydan High Plateau, 2400 m a.s.l., 26 July 2012, calcareous rocks, Hamzaoğlu 6521 , Aksoy & Koç (GAZI).
<i>D. erinaceus</i> Boiss.	Endemic. Türkiye. Manisa: Spil Mount National Park, Atalanı place, around the fire tower, 1475 m a.s.l., 5 August 2012, rocks, Hamzaoğlu 6589 , Aksoy & Koç (GAZI).
<i>D. glutinosus</i> Boiss. & Heldr.	Eastern Aegean Islands and Türkiye. İzmir: above Yamanlar village, Karagöl road, 530 m a.s.l., 11 June 2012, forest clearings, Hamzaoğlu 6329 , Aksoy & Koç (GAZI).
<i>D. goekayi</i> Kaynak, Yılmaz & Daşkın	Endemic. Türkiye. Bursa: Between Soğukpınar and Karaslah villages, 860 m a.s.l., 8 August 2012, <i>Quercus</i> sp. clearings, serpentine stony slopes, Hamzaoğlu 6596 , Aksoy & Koç (GAZI).
<i>D. hymenolepis</i> Boiss.	Iraq and Türkiye. Muş: Varto, Sağlıcak village, Değirmendere place, 1840 m a.s.l., 6 July 2013, grassy and stony slopes, Hamzaoğlu 6842 , Aksoy & Koç (GAZI).
<i>D. kastembeluensis</i> Freyn & Sint.	Endemic. Türkiye. Karabük: Between Eskipazar and Mengen, north of Çötler village, 1010 m a.s.l., <i>Quercus</i> sp. clearings, 9 September 2012, Hamzaoğlu 6679 (GAZI).
<i>D. masmenaeus</i> Boiss. var. <i>glabrescens</i> Boiss.	Endemic. Türkiye. Kars: Arpaçay, Dağköyü village turnout, 2010 m a.s.l., 23 July 2013, dry meadows, Hamzaoğlu 6909 & Koç (GAZI).
<i>D. nihatii</i> Güner	Endemic. Türkiye. Mersin: Arslanköy, Tırtar village, around Dümbelek Pass, 2185 m a.s.l., 26 July 2012, grassy plains in doline, Hamzaoğlu 6530 , Aksoy & Koç (GAZI).
<i>D. preobrazhenskii</i> Klokov	Armenia and Türkiye. Ağrı: Doğubayazıt, northeast of Örtülü village, Ağrı Mountain slopes, 2120 m a.s.l., grassy slopes between igneous rocks, 27 August 2012, Hamzaoğlu 6660 & Koç (GAZI).
<i>D. roseoluteus</i> Velen.	Bulgaria and Türkiye. Kırklareli: Between Kofçaz and Kocayazı, 685 m a.s.l., 7 July 2012, <i>Quercus</i> sp. clearings, Hamzaoğlu 6593 , Aksoy & Koç (GAZI).
<i>D. webbianus</i> Parl. ex Vis.	Endemic. Türkiye. Balıkesir: Edremit, Zeytinli village Kazdağı National Park, Sarıkız road, 1675 m a.s.l., 6 August 2012, rocks, Hamzaoğlu 6590 , Aksoy & Koç (GAZI).

4°C for 16 h, fixation by Carnoy's fixative at 4 °C for 24 h, storage in 70% ethanol at 4°C until use, hydrolysis by 1 N HCl at 60°C for 12 min, staining by 2% aceto-orcein for 2h, and preparation by squash method (Eroğlu et al., 2019). At least 10 metaphase microphotographs were used to detect chromosome numbers.

3. Results

3.1. Chromosomal data

Figure 1 shows the mitotic metaphase chromosomes selected from the clearest images of section *Dentati*. Chromosome numbers of 15 taxa were obtained, 13 of which were reported for the first time and two numbers agreed with the previous reports (Table 2). In genus *Dianthus*, because the chromosomes are very small and the centromere region is unclear, detailed chromosomal measurements were not made. Two different chromosome numbers ($2n = 30$ and 60) were found and $2n = 30$ was the most common diploid number in genus. *D. hymenolepis* was the only taxon with a different chromosome number by $2n = 60$.

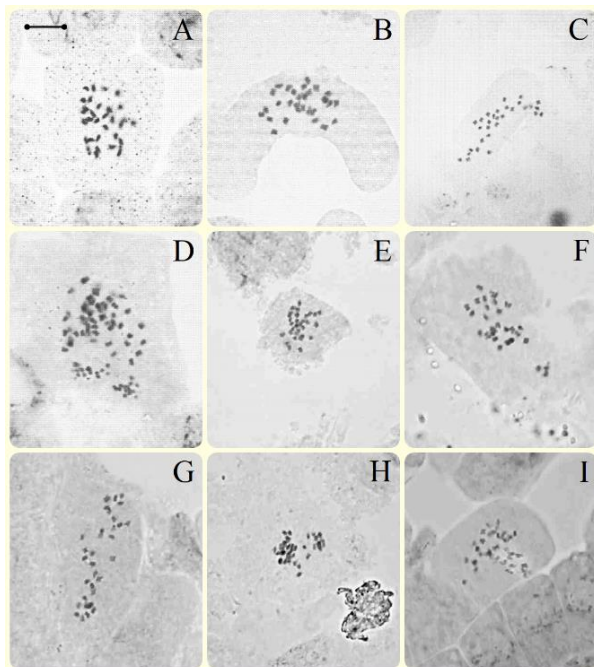


Figure 1. Mitotic metaphase chromosomes of *D. acrochlonis* (A), *D. aculeatus* (B), *D. erinaceus* (C), *D. hymenolepis* (D), *D. masmenaevs* var. *glabrescens* (E), *D. nihatii* (F), *D. preobrazhenskii* (G), *D. roseoluteus* (H), and *D. webbianus* (I). Scale bar: 10 μ m

3.2. Basic number, ploidy levels, and polyploidy

The *Dentati* was a monobasic section by $x = 15$ with ploidy levels of $2x$ and $4x$. Fourteen taxa were diploid with $2n = 2x = 30$. *D. hymenolepis* was polyploid, which reveals only one polyploidy level of tetraploidy ($2n = 4x = 60$).

4. Discussions

The chromosome numbers of 13 taxa were reported for the first time: *D. acrochlonis*, *D. aculeatus*, *D. aticii*, *D. brevicaulis*, *D. erinaceus*, *D. glutinosus*, *D. goekayi*, *D. kastembeluensis*, *D. masmenaevs* var. *glabrescens*, *D. nihatii*, *D. roseoluteus*, *D. webbianus* ($2n = 30$), and *D. hymenolepis* ($2n = 60$). The chromosome numbers of the two species were the same as previous reports, which were

D. armeria (Sünter, 1979) and *D. preobrazhenskii* (Borsos, 1971).

In the present study, 14 taxa were diploid with $2x = 30$ (93.33% of the taxa) and only one taxon was polyploid with $4x = 60$ (6.67% of the taxa). Polyploidy might have played a role in the karyotype evolution of the genus. *Dianthus* was a monobasic genus by different groups having polyploid origins, which were $3x$, $4x$, $6x$, and $8x$ (Chromosome Counts Database, CCDB, version 1.59). While all species were diploid in the section *Fimbriati* (Şahin et al., 2016), all taxa were diploid excluding *D. strictus* Sm. in section *Verruculosi* (Darlington and Wylie, 1956). However, there was no polyploidy in four varieties, which were *D. strictus* var. *strictus*, *D. strictus* var. *subenervis* (Boiss.), *D. strictus* var. *axilliflorus* (Fenzl), and *D. strictus* var. *gracilior* (Boiss.) (Altay et al., 2017).

Dianthus is a genus with geographically limited ranges and is a taxonomically difficult group characterized by a large number of endemic species (Tutin and Walters, 1993). Geography is an important factor in the evolution of *Dianthus* species in Eurasia. Although *Dianthus* does not have great interspecific ecological differentiation, it contains a large number of narrowly endemic species (especially Anatolia). This is strong evidence that the most dominant model in speciation is geographic (Valente et al., 2010). Therefore, as in other large genera, there is no clear relationship between interspecific relationships and karyotype evolution. In genus *Dianthus*, although polyploidy is seen, there is no dysploidy causing to change in the basic chromosome number. According to all chromosomal data on *Dianthus*, the basic chromosome number is one ($x = 15$). The basic chromosome number is $x = 15$ in 15 taxa in the present study, in 125 taxa in chromosome counts database (Chromosome Counts Database, CCDB, version 1.59), in four species of section *Fimbriati* (Şahin et al., 2016), and in nine taxa of section *Verruculosi* (Altay et al., 2017).

In the present study, it was reported two different chromosome numbers, the first report for chromosome numbers of 13 taxa and the same chromosome count from previous reports in two taxa. The other five species in the Flora of Türkiye could not be studied because they could not be germinated. These species were *D. elegans* d'Urv., *D. engleri* Hausskn. & Bornm., *D. raddeanus* Vierh., *D. muschianus* Kotschy & Boiss., and *D. barbatus*. However, the diploid numbers of *D. elegans* and *D. barbatus* were reported as $2n = 30$, 60 and $2n = 30$, respectively (Chromosome Counts Database, CCDB, version 1.59). In conclusion, this study presents new data into the karyological characteristics of section *Dentati* (genus *Dianthus*) that may be useful for understanding or interpreting relationships among the sections. In addition, polyploidy variations may probably play an important role in speciation. In this respect, the results contributed to some missing parts in *Dianthus* cytotaxonomy. However, all sections should be examined to elucidate the relationships between *Dianthus* sections and these should be supported by molecular data.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Table 2. The chromosome counts of the investigated taxa in present and previous studies

Taxa (alphabetically)	Previous x: basic number, 2n, ploidy	Present x: basic number, 2n, ploidy	Report
<i>D. acrochlonis</i>		x = 15, 30, diploid	First report
<i>D. aculeatus</i>		x = 15, 30, diploid	First report
<i>D. armeria</i>	x = 15, 30, diploid (Sünter, 1979)	x = 15, 30, diploid	Equal count
<i>D. aticii</i>		x = 15, 30, diploid	First report
<i>D. brevicaulis</i>		x = 15, 30, diploid	First report
<i>D. erinaceus</i>		x = 15, 30, diploid	First report
<i>D. glutinosus</i>		x = 15, 30, diploid	First report
<i>D. goekayi</i>		x = 15, 30, diploid	First report
<i>D. hymenolepis</i>		x = 15, 60, tetraploid	First report
<i>D.kastembeluensis</i>		x = 15, 30, diploid	First report
<i>D. masmenaeus</i> var. <i>glabrescens</i>		x = 15, 30, diploid	First report
<i>D. nihatii</i>		x = 15, 30, diploid	First report
<i>D. preobrazhenskii</i>	x = 15, 30, diploid (Borsos, 1971)	x = 15, 30, diploid	Equal count
<i>D. roseoluteus</i>		x = 15, 30, diploid	First report
<i>D. webbianus</i>		x = 15, 30, diploid	First report

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