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New Chromosomal Data of *Dianthus* Section *Leiopetali* (Caryophyllaceae, Sileneae)

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ABSTRACT: In section *Leiopetali*, the diploid chromosome numbers were reported from only six of total 25 taxa. There is no karyological data relating to other 19 taxa. In this study, it is intended to determine the diploid chromosome numbers of taxa of section *Leiopetali*. The diploid number of 13 species were detected, four of which were recorded for the first time and nine numbers compatible with the previous reports. Twelve species were diploid with $2n = 2x = 30$. *D. leptopetalus* was diploid and polyploid, which reveals only one polyploidy level of tetraploidy ($2n = 4x = 60$). Polyploidy might have played a role in the karyotype evolution of the genus. One of the most important reasons for this situation is that although polyploidy is observed in the genus *Dianthus*, the dysploidy mechanism that causes a change in the basic chromosome number has not been found until now. The basic number is $x = 15$ according to the all chromosomal reports. In conclusion, this study reports new data into the karyological characteristics of section *Leiopetali* (genus *Dianthus*) that can be useful for interpreting or understanding relationships among sections.

Keywords: *Dianthus*, *Leiopetali*, cytotaxonomy, polyploidy

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INTRODUCTION

Dianthus L., contains more than 300 species, is one of the largest genus of family Caryophyllaceae. The species spread in the Mediterranean region of Europe and Asia (Şahin et al., 2016; Altay et al., 2017; Hamzaoğlu et al., 2021). However, a few species are distributed America and Africa (Madhani et al., 2018).

"Flora Orientalis" was the first study that has detailed information related to Turkish *Dianthus* species (Boissier, 1867). In this study, it was mentioned from 89 *Dianthus* taxa and 48 of them were related to the Flora of Turkey. In addition, the genus was divided into five groups: *Carthusiani* Boiss., *Dentati* Boiss., *Fimbriati* Boiss., *Leiopetali* Boiss., and *Verruculosi* Boiss. Afterwards, genus *Dianthus* was divided into 3 subgenera, which were *Carthusianastrum* F.N.Williams, *Caryophyllastrum* F.N.Williams, and *Proliferastrum* F.N.Williams, into many subsections and sections including groups given by Boissier (Williams, 1893). "Flora of Turkey and the East Aegean Islands" was the first work mentioned from only Turkish taxa and had first the group key (Reeve, 1967). In this work, genus *Dianthus* was divided into 5 sections: *Carthusiani*, *Dentati*, *Fimbriati*, *Leiopetali*, and *Verruculosi*.

The taxa of section *Leiopetali* are distinguished from other taxa with short leaf sheaths (not longer than 3 × stem diameter), entire, subentire or dentate petal margins (not fimbriate), glabrous petal surfaces (not barbulate), and not verruculose calyx (Boissier, 1867; Reeve, 1967). The section *Leiopetali* includes 25 species in the Flora of Turkey (Reeve, 1967). *Leiopetali* is one of the sections containing the highest number of taxa of the genus *Dianthus* in Turkey (Reeve, 1967). The current literature has been used in writing the valid names and taxonomic status of the section *Leiopetali* species of Turkish *Dianthus* (Hamzaoğlu, 2021).

The chromosome counts (basic, x and diploid, $2n$) are the most major parameters related to the genome of taxa. These parameters can be obtained cheaply and easily in a short time and these features are their important advantages. Therefore, chromosome data of plant organisms have been recorded worldwide since the nineteenth century (Stace, 2000). Karyological analysis is important part of cytotaxonomic works in plants. Especially, it is widely used in supporting taxonomic data (Kashmenskaya and Polyakov, 2008). In genus *Dianthus*, the basic chromosome number is $x = 15$ (Carolin, 1957) and diploid number of many species is $2n = 30$ (Darlington and Wylie, 1956; Sünter, 1979; Iyer, 1991; Başak and Güler, 2000; Şahin et al., 2016; Altay et al., 2017).

In section *Leiopetali*, the chromosome numbers were reported from 11 of total 25 taxa. *D. andronakii* Woronow ex Schischk., *D. arpadianus* Ade & Bornm., *D. ingoldbyi* Turrill, *D. lactiflorus* Fenzl, *D. leucophaeus* Sm., *D. micranthus* Boiss. & Heldr., *D. robustus* Boiss. & Kotschy and *D. zederbaueri* Vierh. are only diploid with $2n = 2x = 30$. Three taxa are diploid and polyploid, which reveal two different polyploidy levels of tetraploidy ($2n = 4x = 60$) and hexaploidy ($2n = 6x = 90$) (Darlington and Wylie, 1956; Sünter, 1979; Iyer, 1991; Başak and Güler, 2000). There is no chromosomal record related to other 14 taxa. In this study, it is intended to detect the chromosome numbers of other taxa. Thus, significant contributions will be made to cytotaxonomy of section *Leiopetali*. In addition, there are three critically endangered (CR) taxa (*D. ingoldbyi*, *D. robustus*, and *D. zederbaueri*) studied, which is quite important for the continuation of them.

MATERIALS and METHODS

Plant Samples

Thirteen species of section *Leiopetali* were collected from the distribution areas in Turkey (Table 1). Plant samples were deposited at the herbarium of the Gazi University (GAZI) in Ankara.

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

Taxa (alphabetically)	Distribution regions and collection information
D. andronakii Woronow ex Schischk.	Turkey. Artvin: Ardanuç, on road of Sakarya village, 1165 m a.s.l., 3 July 2013, calcerous rocks, Hamzaoğlu **6807** & Koç (GAZI).
D. arpadianus Ade & Bornm.	Turkey. Balıkesir: Edremit, Kazdağı National Park, on road of Sarıkız Hill, 1300 m a.s.l., 25 June 2012, forest clearings and rocks, Hamzaoğlu **6352**, Aksoy & Koç (GAZI).
D. cretaceus Adams	Turkey. Ardahan: Posof, Kolköy, on road of Arsiyan Mountain, 2270 m a.s.l., 26 August 2012, grassy slopes, Hamzaoğlu **6644** & Koç (GAZI).
D. ingoldbyi Turrill	Turkey. Çanakkale: Bozcaada, south of Sulubahçe village, Ayazma beach, 10 m a.s.l., 6 August 2012, coastal cliffs, in Phrygana, Hamzaoğlu **6591**, Aksoy & Koç (GAZI).
D. lactiflorus Fenzl	Turkey. Niğde: Ulukışla, Bolkar Mountain, Maden village, South of Meydan Yaylası, 2400 m a.s.l., 26 July 2012, grassy slopes, Hamzaoğlu **6523**, Aksoy & Koç (GAZI).
D. leptopetalus Willd.	Turkey. Edirne: Between Havsa and Edirne, 95 m a.s.l., 26 June 2012, grassy slopes, Hamzaoğlu **6371**, Aksoy & Koç (GAZI).
D. liboschitzianus Ser. ex DC.	Turkey. Erzincan: Between Erzincan and Refahiye, around Karadağ R/L station, 2820 m a.s.l., 24 August 2012, stony slopes, Hamzaoğlu **6619** & Koç (GAZI).
D. micranthus Boiss. & Heldr.	Turkey. Karaman: Ermenek, between Başyayla and Taşkent, 7-9. km, 1810 m a.s.l., 21 June 2006, calcareous rocky places, Hamzaoğlu **4015**, Aksoy & Budak (GAZI).
D. multicaulis Boiss. & A.Huet	Turkey. Ardahan: Posof, Kolköy, on road Arsiyan Mountain, 2290 m a.s.l., 26 August 2012, meadows, grassy slopes, Hamzaoğlu **6647**, Aksoy & Budak (GAZI).
D. pallens Sibth. & Sm. var. *oxylepis* Boiss.	Turkey. Karaman: Between Karaman and Seyithasan, 1200 m a.s.l., *Juniperus* sp. clearings, 18 June 2013, Hamzaoğlu **6771**, Aksoy & Koç (GAZI).
D. robustus Boiss. & Kotschy	Turkey. Muş: Varto, above Sağlıcak village, Sırınk place, 2100 m a.s.l., 25 July 2013, grassy and stony slopes, Hamzaoğlu **6933**, Aksoy & Koç (GAZI).
D. siphonocalyx Blakelock	Turkey. Şanlıurfa: Gaziantep-Şanlıurfa motorway, about 20 km from Şanlıurfa, 805 m a.s.l., 9 July 2012, stony slopes, Hamzaoğlu **6434**, Aksoy & Koç (GAZI).
D. zederbaueri Vierh.	Endemic. Turkey. Kayseri: Hacılar, Erciyes Mountain, around Ski Resort, Perilikartın place, 2155 m a.s.l., tuffaceous slopes, igneous cliffs, 7 July 2012, Hamzaoğlu **6411** & Koç (GAZI).

Preparation and observation

All chromosome preparations were made by squash technique. Root-tip meristems were obtained from seeds by germinating on wet filter paper in Petri dishes at room temperature. Firstly, root tips were pretreated for 16 h in α-monobromonaphthalene at 4°C, fixed in 3:1 absolute alcohol/glacial acetic acid, hydrolyzed with 1 N HCl for 9 min at room temperature, stained with 2% aceto-orcein for 3 h at room temperature, and squashed in a drop of 45% acetic acid. The permanent slides were made by mounting in Depex (Eroğlu and Budak, 2020; Eroğlu et al., 2021). The chromosomes were counted by Software Image Analyses (Bs200ProP) loaded on a personal computer. At least 10 metaphase microphotographs used to detect chromosome number.

RESULTS and DISCUSSION

Chromosome Number

Figure 1 represents metaphase plates selected from the most prominent chromosome images of section *Leiopetali*. The chromosome numbers of four species were reported for the first time: *D. cretaceus*, *D. liboschitzianus*, *D. multicaulis*, and *D. siphonocalyx* ($2n = 30$). The chromosome numbers of nine taxa were same of previous reports, which were *D. andronakii*, *D. arpadianus*, *D. ingoldbyi*, *D. lactiflorus*, *D. leptopetalus*, *D. micranthus*, *D. pallens* var. *oxylepis*, *D. robustus*, and *D. zederbaueri* (Darlington and Wylie, 1956; Petrova, 1975; Sünter, 1979; Başak and Güler, 2000; Martin et al., 2017) (Table 2). The detailed chromosomal measurements were not obtained due to small

chromosomes with indistinct centromere region. Two different diploid numbers such as 2*n* = 30 and 2*n* = 60 were determined and 2*n* = 30 was the most reported diploid number in the genus *Dianthus*. *D. leptopetalus* was the only taxon with two different numbers by 2*n* = 30 and 60.

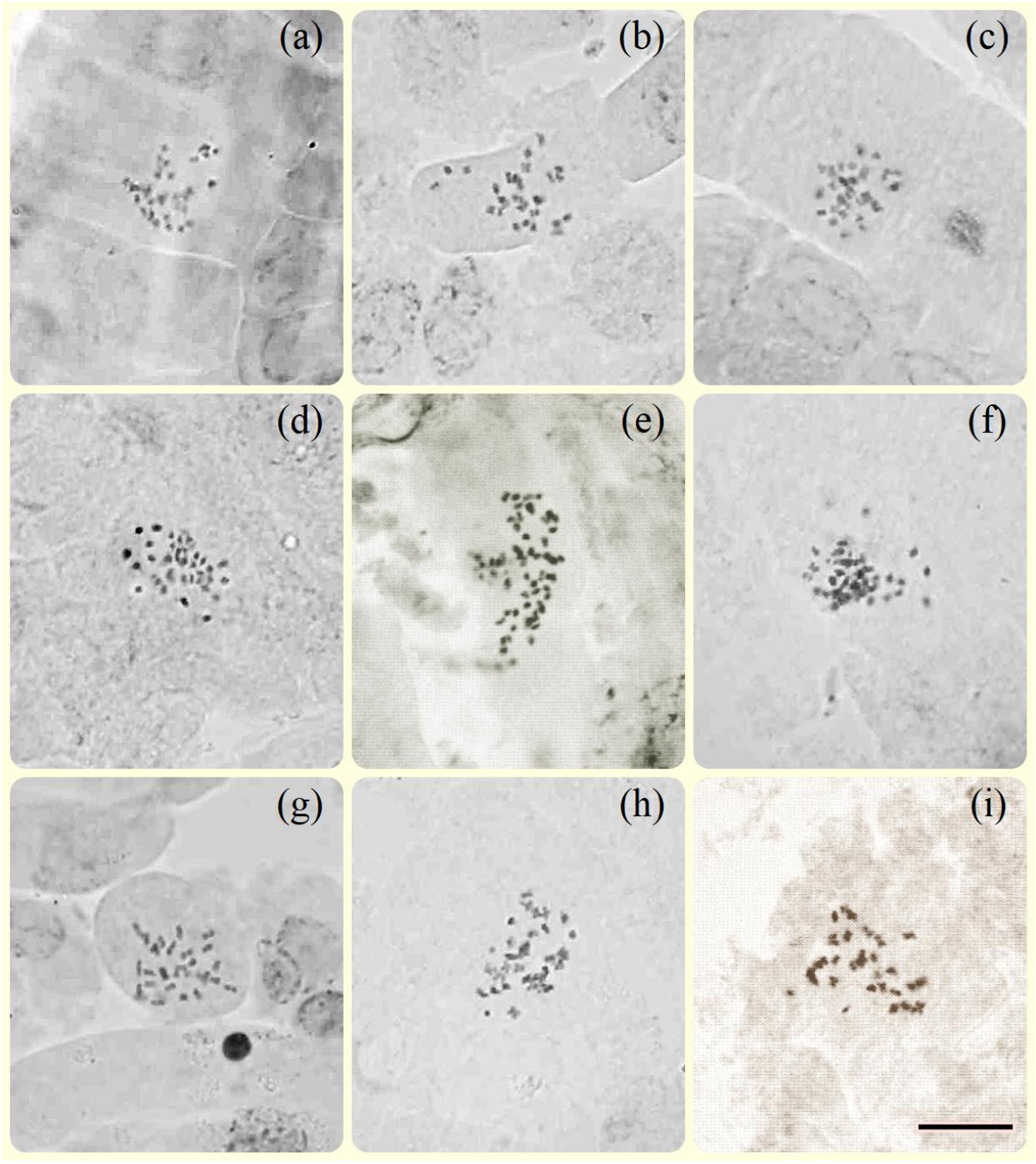

Figure.1. Mitotic metaphase chromosomes of *D. andronakii* (a), *D. cretaceus* (b), *D. ingoldbyi* (c), *D. leptopetalus* (2*n* = 30) (d), *D. leptopetalus* (2*n* = 60) (e), *D. micranthus* (f), *D. pallens* var. *oxylepis* (g), *D. robustus* (h), and *D. siphonocalyx* (i). Scale bar = 10 µm

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

Taxa (alphabetically)	Previous results *x*: basic number 2*n* (ploidy level)	References	Present Results *x*: basic number 2*n* (ploidy level)	Observation
D. andronakii	*x* = 15 30 (diploid)	Başak and Güler, 2000 Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. arpadianus	*x* = 15 30 (diploid)	Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. cretaceus			*x* = 15 30 (diploid)	First report
D. ingoldbyi	*x* = 15 30 (diploid)	Başak and Güler, 2000 Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. lactiflorus	*x* = 15 30 (diploid)	Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. leptopetalus	*x* = 15 30 (diploid) 60 (tetraploid)	Darlington and Wylie, 1956 Sünter, 1979	*x* = 15 30 (diploid) 60 (tetraploid)	Equal count
D. liboschitzianus			*x* = 15 30 (diploid)	First report
D. micranthus	*x* = 15 30 (diploid)	Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. multicaulis			*x* = 15 30 (diploid)	First report
D. pallens var. *oxylepis*	*x* = 15 30 (diploid) 60 (tetraploid)	Petrova, 1975 Sünter, 1979	*x* = 15 30 (diploid)	Equal count
D. robustus	*x* = 15 30 (diploid)	Martin et al., 2017	*x* = 15 30 (diploid)	Equal count
D. siphonocalyx			*x* = 15 30 (diploid)	First report
D. zederbaueri	*x* = 15 30 (diploid)	Martin et al., 2017	*x* = 15 30 (diploid)	Equal count

Basic number and ploidy levels

The *Leiopetali* was a monobasic section by $x = 15$ with ploidy levels of $2x$ and $4x$. Twelve taxa were diploid with $2x = 30$ (92.30% of the taxa) and only one taxon (*D. leptopetalus*) was diploid and polyploid with $2x = 30$ and $4x = 60$ (7.70% of the taxa). Polyploidy mechanism might have played an important role in the karyotype evolution of the genus *Dianthus*. Genus *Dianthus* was a monobasic ($x = 15$) by different sections having polyploid origins, which were $3x$, $4x$, $6x$, and $8x$ (Chromosome Counts Database, CCDB, version 1.59). In section *Fimbriati*, all taxa were diploid (Şahin et al., 2016). In addition, in section *Verruculosi*, all taxa were diploid except for *D. strictus* Sm (Darlington and Wylie, 1956). However, there was no polyploidy in four varieties of *Verruculosi*, which were *D. strictus* var. *strictus*, *D. strictus* var. *axilliflorus* (Fenzl), *D. strictus* var. *gracilior* (Boiss.), and *D. strictus* var. *subenervis* (Boiss.) (Altay et al., 2017).

Polyploidy and speciation

Genus *Dianthus* has geographically limited ranges and is a taxonomically difficult genus containing many endemic species (Tutin and Walters, 1993). Geography is an important factor in the evolution of *Dianthus* species in Europe and Asia. Although the genus *Dianthus* does not show great ecological differentiation between species, it has many narrow endemic species in Europe and Asia (especially Anatolia). This provides strong evidence that geography and range are the most dominant factors in speciation (Valente et al., 2010). Therefore, the connection among interspecific relationships and karyotype evolution, which is more clearly observed in other large genera, is not clear in genus *Dianthus*. One of the most important reasons for this situation is that although polyploidy is observed

in the genus *Dianthus*, the dysploidy mechanism that causes a change in the basic chromosome number has not been found until now. The basic number is $x = 15$ according to the all chromosomal reports, which are in 125 taxa in chromosome counts database (Chromosome Counts Database, CCDB, version 1.59), in four taxa of section *Fimbriati* (Şahin et al., 2016), in nine taxa of section *Verruculosi* (Altay et al., 2017), and in 13 taxa in the present study.

CONCLUSION

In the present study, it was reported two different diploid numbers, first report for diploid numbers of four taxa and same chromosome numbers from previous reports in nine taxa. In conclusion, this study reports new data into the karyological characteristics of section *Leiopetali* (genus *Dianthus*) that can be useful for interpreting or understanding relationships among the sections. On the other hand, polyploidy seems to be an important mechanism for speciation and the contribution to *Dianthus* cytotaxonomy of results is important. In conclusion, karyological studies of all sections should be performed and these results should be supported by molecular studies.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Altay D, Eroğlu HE, Hamzaoğlu E, Koç M, 2017. Karyotype Analysis of Some Taxa of *Dianthus* section *Verruculosi* (Caryophyllaceae, Sileneae). Turkish Journal of Botany 41:367-374.

Başak N, Güler N, 2000. Karyological Investigation and Distribution of Some Rare and Endemic Plants of European Turkey. Proceeding of the 2nd Balkan Botanical Congress, 14-18 Mayıs 2000, İstanbul.

Boissier E, 1867. Flora Orientalis, Vol. 1. H. Georg., Geneva-Switzerland.

Carolin RC, 1957. Cytological and Hybridization Studies in the Genus *Dianthus*. New Phytologist 56:81-97.

Darlington CD, Wylie AP, 1956. Chromosome Atlas of Flowering Plants. George Allen and Unwin Ltd, London- United Kingdom.

Eroğlu HE, Budak Ü, 2020. Karyotype Analysis of *Paronychia anatolica* subsp. *anatolica* (Caryophyllaceae, Paronychioideae). Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi 10(4):2424-2430.

Eroğlu HE, Altay D, Budak Ü, Martin E, 2021. Karyotype analysis of Two Varieties of *Paronychia amani* (Caryophyllaceae, Paronychioideae). Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi 11(3):1809-1816.

Hamzaoğlu E, 2021. Taxonomic Contributions to the Genus *Dianthus* (Caryophyllaceae) from Turkey Name of the Article. Communications Faculty of Sciences University of Ankara Series C-Biology 30(1):85-97.

Hamzaoğlu E, Koç M, Büyük İ, 2021. *Dianthus yilmazii* (Caryophyllaceae), a New Species from Central Turkey. Kew Bulletin 76:523-530.

Iyer YK, 1991. Mediterranean Chromosome Number Reports 1 (30--37). Flora Mediterranea 1:238-240.

Kashmenskaya MN, Polyakov AV, 2008. Karyotype Analysis of Five Species of Earthworms (Oligochaeta: Lumbricidae). Comparative Cytogenetics 2(2):121-125.

Madhani H, Rabeler R, Pirani A, Oxelman B, Heubl G, Zarre S, 2018. Untangling Phylogenetic Patterns and Taxonomic Confusion In Tribe Caryophylleae (Caryophyllaceae) with Special Focus on Generic Boundaries. Taxon 67:83-112.

Martin E, Hamzaoğlu E, Koç M, Karakaş E, Yavaş FN, 2017. Karyological Studies on Section *Leiopetali* of Aromatic *Dianthus* (Caryophyllaceae) from Turkey. 1st Internatıonal Congress on Medicinal and Aromatic Plants, Konya, May 09-12, 2017, p:459.

Petrova AV, 1975. In IOPB Chromosome Number Reports XLIX. Taxon 24:501-516.

Plant Counts Database (CCDB, version 1.59), 2022. http://ccdb.tau.ac.il/ (Date of access: 02 February 2022).

Reeve H, 1967. *Dianthus* L. in Davis PH, Flora of Turkey and the East Aegean Islands, Vol. 2. Edinburgh University Press, pp. 99-131, Edinburgh-Scotland.

Stace CA, 2000. Cytology and Cytogenetics as a Fundamental Taxonomic Resource for the 20th and 21st Centuries. Taxon 49(3):451-477.

Sünter G, 1979. Morphological and Cytological Studies on the *Dianthus* Species of Istanbul Area. Review of the Faculty of Science, University of Istanbul, Series B 44:31-48.

Şahin E, Eroğlu HE, Hamzaoğlu E, Koç M, 2016. Karyotype Analysis of Four Species of *Dianthus* Section *Fimbriati* (Caryophyllaceae, Sileneae). Caryologia 69(3):267-272.

Tutin TG, Walters SM, 1993. *Dianthus* L. in: Tutin TG, Burges NA, Chater AD, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM, Webb DA, Flora Europaea, Vol. 1. Cambridge University Press, pp. 227-246, Cambridge-United Kingdom.

Valente LM, Savolainen V, Vargas P, 2010. Unparalleled Rates of Species Diversification in Europe. Proceedings of the Royal Society B 277:1489-1496.

Williams FN, 1893. A Monograph of the Genus *Dianthus*. Botanical Journal of the Linnean Society 29:346-378.