

Karyotype analysis of some taxa of *Dianthus* section *Verruculosi* (Caryophyllaceae, Sileneae)

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Abstract: The purpose of this study was to determine chromosome numbers and karyological features of six *Dianthus* taxa belonging to section *Verruculosi* from Anatolia, Turkey. The chromosome numbers were determined as $2n = 30$ in all taxa. Karyological measurements were made in detail for the first time for all taxa. Chromosome counts were given for the first time for all taxa except *D. cyri* and *D. strictus*. A new chromosome number was determined for *D. strictus*. The intrachromosomal and interchromosomal karyotype asymmetries were estimated with M_{CA} and CV_{CL} values.

Key words: *Dianthus*, *Verruculosi*, karyotype, asymmetry index

1. Introduction

Dianthus L. is a large genus of the family Caryophyllaceae, with more than 300 species widespread around the world (Reeve, 1967; Bittrich, 1993; İlçim et al., 2013). The genus contains the following five sections in Turkey: *Verruculosi* Boiss., *Leiopetali* Boiss., *Fimbriati* Boiss., *Dentati* Boiss., and *Carthusiani* Boiss., (Reeve, 1967).

Section *Verruculosi*, characterized by the verruculose calyx (Boissier, 1867; Reeve, 1967), includes nine taxa in Turkey. The lifecycle (annual or perennial) is a distinctive character among taxa of section *Verruculosi*. *Dianthus cyri* Fisch. & C.A.Mey., *D. aydogdui* Menemen & Hamzaoglu, *D. tripunctatus* Sm., and *D. multiflorus* Deniz & Aykurt are annual, whereas *D. strictus* Sm. and *D. polycladus* Boiss. are perennial. *D. aydogdui* and *D. multiflorus* are narrow endemic species in Turkey. Other species are more widely distributed (Reeve, 1967).

The diploid chromosome number is $2n = 30$ in many taxa of the genus *Dianthus* (Darlington and Wylie, 1956; Deniz et al., 2016; Şahin et al., 2016); however, there are different chromosome numbers such as $2n = 45$, 60, 90, 120, and 180 (Balao et al., 2009; Jafari and Behroozian, 2010). Previous chromosome counts of taxa belonging to the section *Verruculosi* (Darlington and Wylie, 1956; Runemark, 1996; Deniz et al., 2016) reported $2n = 30$ for *Dianthus tripunctatus*, *D. multiflorus*, and *D. cyri* and $2n = 60$ for *D. strictus* (Table 1). In this study, it is intended to detect the karyotype analyses of other taxa.

2. Materials and methods

The seeds were collected from the following localities in Turkey (Figure 1):

(1) *D. cyri* Fisch. & C.A.Mey. – Iğdır: Tuzluca, Turabi village, 340 m, fields, 4.7.2013, Hamzaoglu 6826, Aksoy & Koç.

(2) *D. strictus* Sm. var. *strictus* – Erzurum: between Aşkale and Tercan, Tepebaşı Pass, 2050 m, mountain steppes, 24.8.2012, Hamzaoglu 6630 & Koç.

(3) *D. strictus* Sm. var. *subenervis* (Boiss.) Reeve – Mersin: Aydıncık, around Köşk Stream, 6 m, rocks, 9.6.2012, Hamzaoglu 6319 & Aksoy.

(4) *D. strictus* Sm. var. *axilliflorus* (Fenzl) Reeve – Locality 1. Şanlıurfa: Şanlıurfa to Gaziantep, c. 20. km, 805 m, stony slopes, 9.7.2012, Hamzaoglu 6436, Aksoy & Koç.

(5) *D. strictus* var. *axilliflorus* – Locality 2. Şanlıurfa: between Hilvan and Siverek, 575 m, stony slopes, 10.7.2012, Hamzaoglu 6437, Aksoy & Koç.

(6) *D. strictus* var. *axilliflorus* – Locality 3. Van: Muradiye, near Muradiye Waterfall, 1805 m, stony slopes, 27.8.2012, Hamzaoglu 6662 & Koç.

(7) *D. strictus* Sm. var. *gracilior* (Boiss.) Reeve – Gaziantep: Osmaniye output, Sof Mountain, 970 m, calcareous rocks, 9.7.2012, Hamzaoglu 6433, Aksoy & Koç.

(8) *D. polycladus* Boiss. – Hatay: Belen, around Kıcı village, 710 m, forest clearings, rocks, 9.7.2012, Hamzaoglu 6426, Aksoy & Koç.

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Table 1. The chromosome numbers of *Dianthus* section *Verruculosi*.

Taxa	Chromosome number (2n)
<i>D. cyri</i>	30
<i>D. multiflorus</i>	30
<i>D. aydogdui</i>	Unknown
<i>D. tripunctatus</i>	30
<i>D. strictus</i>	60
<i>D. strictus</i> var. <i>strictus</i>	Unknown
<i>D. strictus</i> var. <i>subenervis</i>	Unknown
<i>D. strictus</i> var. <i>axilliflorus</i>	Unknown
<i>D. strictus</i> var. <i>gracilior</i>	Unknown
<i>D. polycladus</i>	Unknown

After germination at room temperature, the following cytogenetic procedures were used on root tips, respectively: 1) pretreatment with α -monobromonaphthalene for 16 h, 2) fixation with Carnoy's fixative (3:1, alcohol:acetic acid), 3) hydrolyzing with HCl (1 N) for 12 min at 60 °C, 4) staining with 2% acetoorcein for 2 h, 5) squashing with 45% acetic acid, 6) permanent preparation with DPX (Martin et al., 2013; Yüce et al., 2016).

The metaphase plates were photographed with an Olympus DP72 camera and measurements of

chromosomes were made with KaryoType software (Altınordu et al., 2016). Chromosome classifications were made by nomenclature following Levan et al. (1964) and chromosome total haploid length followed Peruzzi et al. (2009). Karyotype asymmetries were estimated by M_{CA} (mean centromeric asymmetry) (Peruzzi and Eroğlu, 2013) and CV_{CL} (variation coefficient of chromosome length) (Paszko, 2006). Finally, a scatter diagram between M_{CA} and CV_{CL} was drawn.

3. Results

3.1. *Dianthus cyri*

The chromosome number of *Dianthus cyri* is $2n = 30$ (Figure 2A). The chromosome lengths range between 0.72 and 1.56 μm . Total haploid length and mean haploid length are 15.89 and 1.06 μm , respectively (Table 2). The karyotype formula consists of $8m + 4sm + 18st$ pairs. The values of the intrachromosomal and interchromosomal karyotype asymmetry indexes are 38.36 and 22.22 for M_{CA} and CV_{CL} , respectively.

3.2. *Dianthus strictus* var. *strictus*

The chromosome number of *Dianthus strictus* var. *strictus* is $2n = 30$ (Figure 2B). The chromosome lengths are between 0.81 and 1.60 μm . Total haploid length and mean haploid length are 17.92 and 1.19 μm , respectively (Table 2). The karyotype formula consists of $12m + 6sm + 12st$ pairs. The values of the intrachromosomal and interchromosomal karyotype asymmetry indexes are 30.40 and 23.19 for M_{CA} and CV_{CL} , respectively.



Figure 1. Distribution of *Dianthus* section *Verruculosi*: (■) *D. cyri*; (▲) *D. strictus* var. *strictus*; (●) *D. strictus* var. *subenervis*; (◆1) *D. strictus* var. *axilliflorus* (locality 1); (◆2) *D. strictus* var. *axilliflorus* (locality 2); (◆3) *D. strictus* var. *axilliflorus* (locality 3); (▼) *D. strictus* var. *gracilior*; (♣) *D. polycladus*.

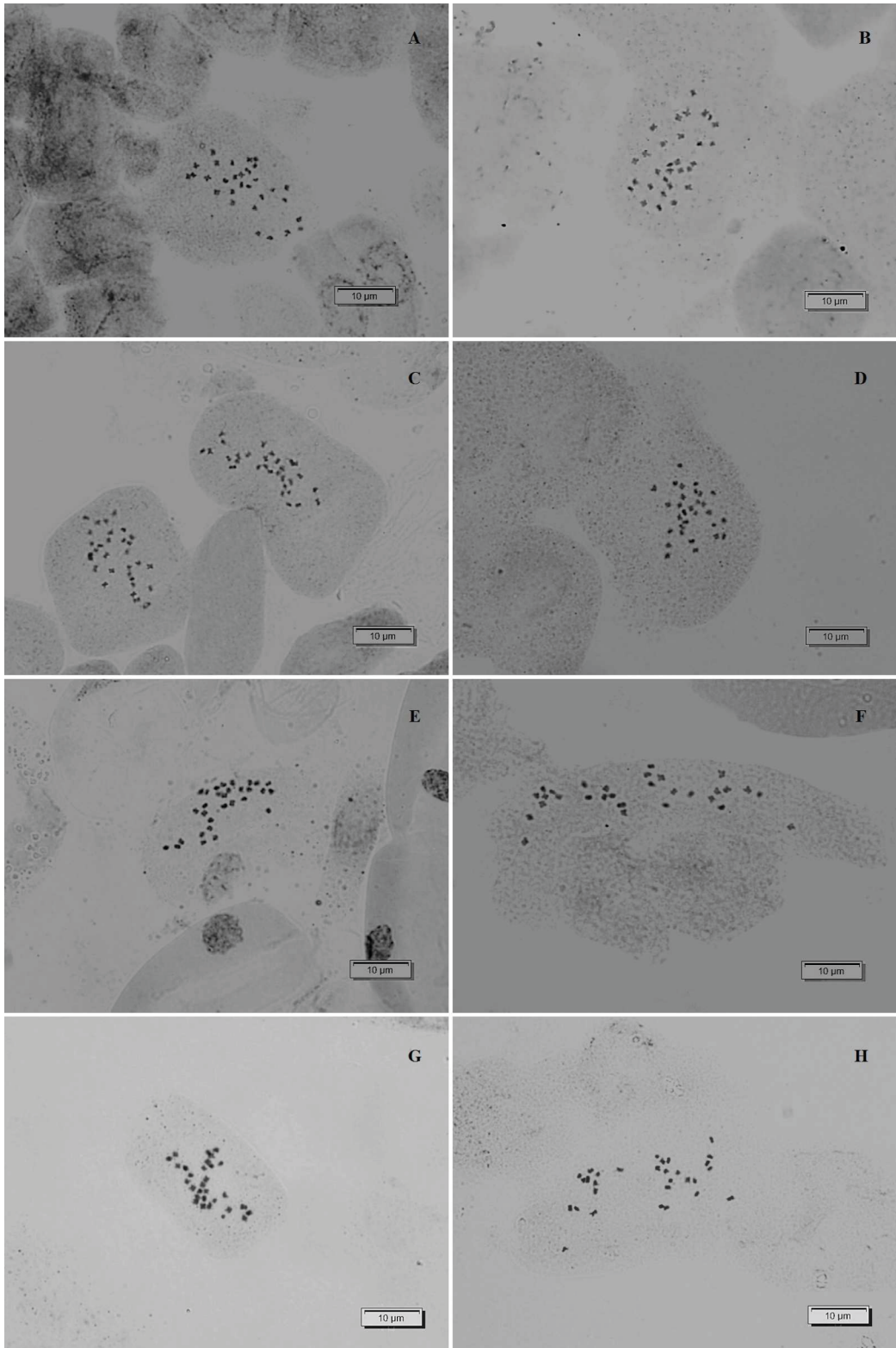


Figure 2. Somatic metaphase chromosomes of *Dianthus* section *Verruculosi*: A) *D. cyri*; B) *D. strictus* var. *strictus*; C) *D. strictus* var. *subenervis*; D) *D. strictus* var. *axilliflorus* (locality 1); E) *D. strictus* var. *axilliflorus* (locality 2); F) *D. strictus* var. *axilliflorus* (locality 3); G) *D. strictus* var. *gracilior*; H) *D. polycladus*.

Table 2. The measurement data of chromosomes of taxa of *Dianthus* section *Verruculosi*. SC, shortest chromosome length; LC, longest chromosome length; THL, total haploid chromosome length; MHL, mean haploid chromosome length; CN, chromosome number; KF, karyotype formula. * Locality 1, ** locality 2, *** locality 3.

Taxa	SC (μm)	LC (μm)	THL (μm)	MHL (μm)	CN and KF
<i>D. cyri</i>	0.72	1.56	15.89	1.06	$2n = 2x = 30$ $8m + 4sm + 18st$
<i>D. strictus</i> var. <i>strictus</i>	0.81	1.60	17.92	1.19	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. strictus</i> var. <i>subenervis</i>	0.74	1.54	16.36	1.09	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. strictus</i> var. <i>axilliflorus</i> *	0.82	1.49	16.52	1.10	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. strictus</i> var. <i>axilliflorus</i> **	1.03	1.47	18.28	1.22	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. strictus</i> var. <i>axilliflorus</i> ***	0.87	1.65	17.45	1.16	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. strictus</i> var. <i>gracilior</i>	0.93	1.49	17.25	1.15	$2n = 2x = 30$ $12m + 6sm + 12st$
<i>D. polycladus</i>	0.87	1.52	18.07	1.20	$2n = 2x = 30$ $12m + 8sm + 10st$

3.3. *Dianthus strictus* var. *subenervis*

The chromosome number of *Dianthus strictus* var. *subenervis* is $2n = 30$ (Figure 2C). The chromosome lengths are between 0.74 and 1.54 μm . Total haploid length and mean haploid length are 16.36 and 1.09 μm , respectively (Table 2). The karyotype formula consists of $12m + 6sm + 12st$ pairs. The values of the intrachromosomal and interchromosomal karyotype asymmetry indexes are 32.79 and 21.42 for M_{CA} and CV_{CL} , respectively.

3.4. *Dianthus strictus* var. *axilliflorus*

The chromosome number and karyotype formula of *Dianthus strictus* var. *axilliflorus* is $2n = 30 = 12m + 6sm + 12st$ (Figures 2D–2F). The chromosome lengths are between 0.82 and 1.65 μm . Total haploid lengths of localities 1, 2, and 3 are 16.52, 18.28, and 17.45 μm , respectively. Mean haploid lengths of localities 1, 2, and 3 are 1.10, 1.22, and 1.16 μm , respectively (Table 2). M_{CA} and CV_{CL} values are 31.45 and 17.98 for locality 1, 35.20 and 10.48 for locality 2, and 30.25 and 18.56 for locality 3, respectively.

3.5. *Dianthus strictus* var. *gracilior*

The chromosome number of *Dianthus strictus* var. *gracilior* is $2n = 30$ (Figure 2G). The chromosome lengths are between 0.93 and 1.49 μm . Total haploid length and mean haploid length are 17.25 and 1.15 μm , respectively (Table 2). The karyotype formula consists of $12m + 6sm + 12st$ pairs. The values of the intrachromosomal and interchromosomal karyotype asymmetry indexes are 31.67 and 13.22 for M_{CA} and CV_{CL} , respectively.

3.6. *Dianthus polycladus*

The chromosome number of *Dianthus polycladus* is $2n = 30$ (Figure 2H). The chromosome lengths are between 0.87 and 1.52 μm . Total haploid length and mean haploid length are 18.07 and 1.20 μm , respectively (Table 2). The karyotype formula consists of $12m + 8sm + 10st$ pairs. The values of the intrachromosomal and interchromosomal karyotype asymmetry indexes are 28.86 and 17.26 for M_{CA} and CV_{CL} , respectively.

4. Discussion

The number, size, and asymmetry of chromosomes are important parameters to elucidate the phylogenetic relationships of species (Eroğlu et al., 2013). The karyotypes of *Dianthus cyri*, *D. strictus* var. *strictus*, *D. strictus* var. *subenervis*, *D. strictus* var. *axilliflorus*, *D. strictus* var. *gracilior*, and *D. polycladus* are $2n = 30$ with small chromosomes between 0.72 and 1.65 μm (Table 2). The ideograms are given in Figures 3A–3H.

In this study, the chromosome number of *D. cyri* was verified and the chromosomal measurements of *D. cyri* were reported for the first time. It was formerly reported that the chromosome number of *D. strictus* was $2n = 60$ (Darlington and Wylie, 1956), but in this study, the chromosome numbers of four varieties of *D. strictus* were $2n = 30$. The following topics can be considered: polyploidy, or the reporting of different chromosome numbers of the same species. According to almost all reports of section *Verruculosi*, the basic diploid number is $2n = 30$.

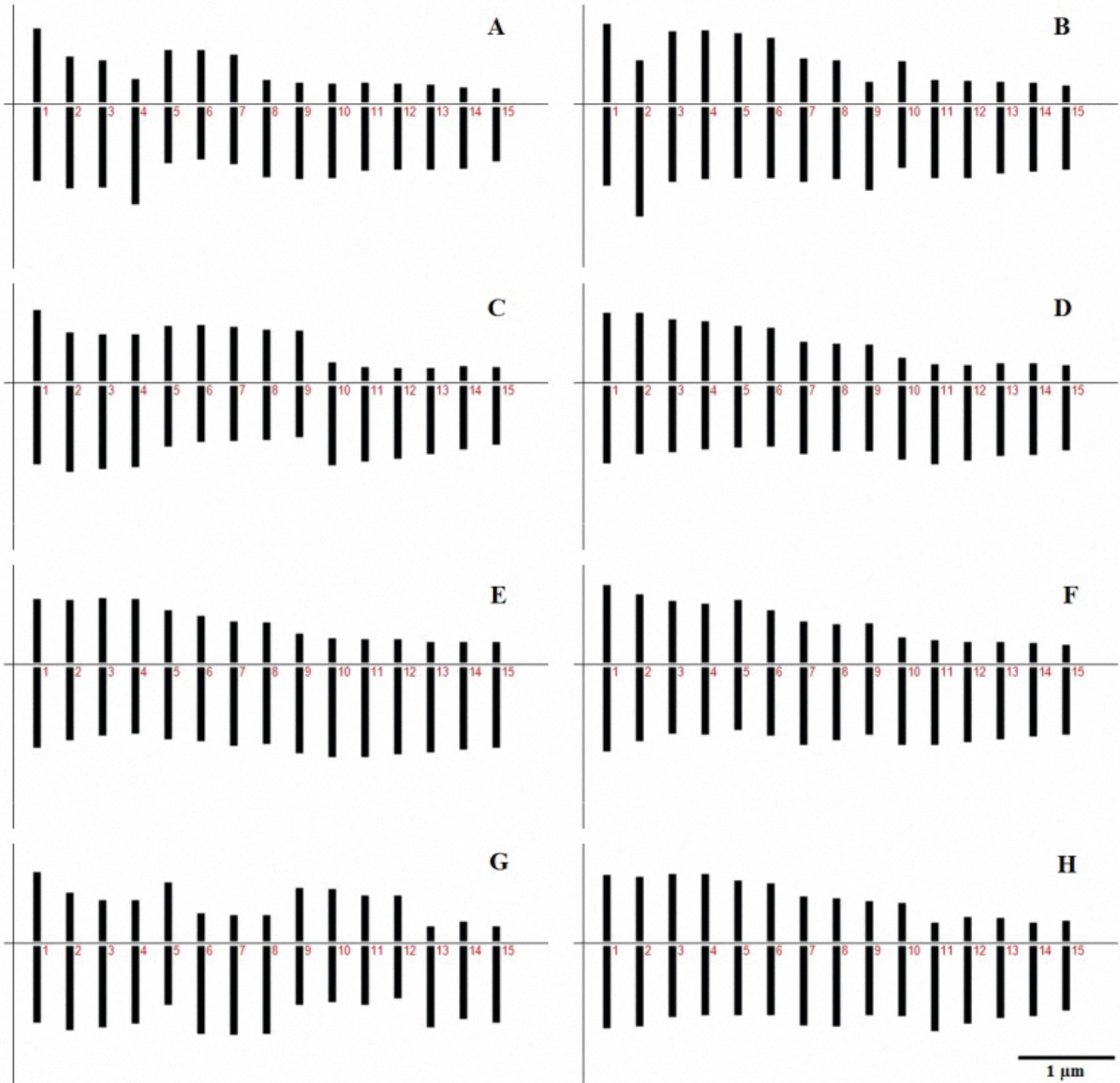


Figure 3. Ideograms of *Dianthus* section *Verruculosi*: A) *D. cyri*; B) *D. strictus* var. *strictus*; C) *D. strictus* var. *subenervis*; D) *D. strictus* var. *axilliflorus* (locality 1); E) *D. strictus* var. *axilliflorus* (locality 2); F) *D. strictus* var. *axilliflorus* (locality 3); G) *D. strictus* var. *gracilior*; H) *D. polycladus*.

Karyotype asymmetry is an important parameter in karyological studies (Eroğlu, 2015). CV_{CL} and M_{CA} are the most appropriate parameters to measure the interchromosomal and intrachromosomal asymmetry (Paszko, 2006; Peruzzi and Eroğlu, 2013; Peruzzi and Altınordu, 2014). M_{CA} and CV_{CL} increase with increasing asymmetry. According to the M_{CA} and CV_{CL} values, the symmetrical and asymmetrical karyotypes are different. *D. polycladus* is the most symmetric karyotype and *D. cyri* is the most asymmetric karyotype in M_{CA} . *D. strictus* var. *axilliflorus* is the most symmetric karyotype and *D.*

strictus var. *strictus* is the most asymmetric karyotype in CV_{CL} . A very weak negative correlation was determined between M_{CA} and CV_{CL} ($r = -0.0001$) (Figure 4). The weak correlation was due to the CV_{CL} values. Peruzzi and Eroğlu (2013) reported that CV_{CL} gives reliable results together with M_{CA} .

Dianthus cyri, *D. strictus* var. *strictus*, *D. strictus* var. *subenervis*, *D. strictus* var. *axilliflorus*, *D. strictus* var. *gracilior*, and *D. polycladus* are different taxa morphologically. An identification key based on the *Dianthus* taxonomy accepted in *Flora of Turkey* is given

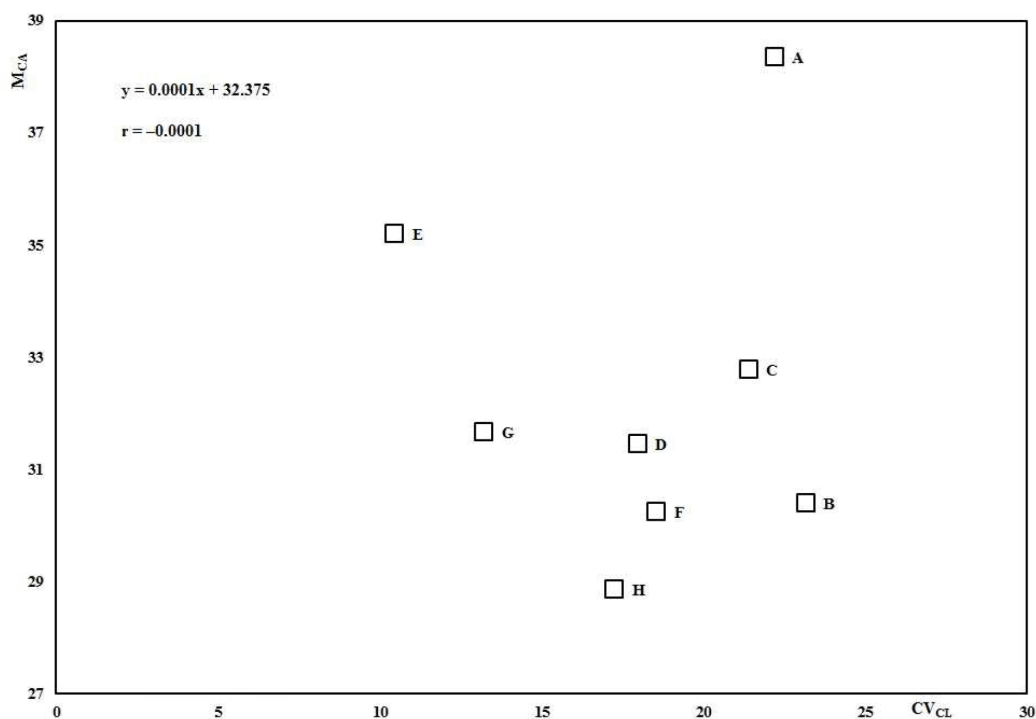


Figure 4. Scatter diagram between M_{CA} and CV_{CL} : A) *D. cyri*; B) *D. strictus* var. *strictus*; C) *D. strictus* var. *subenervis*; D) *D. strictus* var. *axilliflorus* (locality 1); E) *D. strictus* var. *axilliflorus* (locality 2); F) *D. strictus* var. *axilliflorus* (locality 3); G) *D. strictus* var. *gracilior*; H) *D. polycladus*.

below for these taxa (see specimens examined) (Reeve, 1967).

- 1 Annual *D. cyri*
- Perennial 2
- 2 Calyx 8-11 × 2-3 mm; petal 11-17 mm long, lamina oblong-cuneate *D. polycladus*
- Calyx 12-17 × 3-5.5 mm; petal 19-23 mm long, lamina cuneate-oblongate or cuneate-obovate 3
- 3 At least some flowers sessile; epicalyx segments 8-12 ..
..... *D. strictus* var. *axilliflorus*
- Flowers pedicellate; epicalyx segments 4-6
..... 4
- 4 Calyx tube enervate *D. strictus* var. *subenervis*
- Calyx tube nervosa-striate, with enervate areas 5
- 5 Calyx 4-5.5 mm wide; petal limb 7-10 mm long
..... *D. strictus* var. *strictus*
- Calyx 3-4 mm wide; petal limb up to 6 mm long
..... *D. strictus* var. *gracilior*

There are some differences among taxa in the karyotype asymmetry and karyotype formulae. They seemed to have contributed to the morphological differences. Now it certainly cannot be said that karyotype asymmetry and karyotype formulae are distinctive features for all section *Verruculosi* taxa, but there are some similarities between cytogenetic differences and

morphological differences of studied taxa. It was reported that the differences in karyotype formulae and karyotype symmetry/asymmetry may contribute to the variation of the section (Seijo and Fernandez, 2003). For example, *D. cyri* is different from other taxa because it is annual (see key), as well as in the following karyological aspects: lower number of median and submedian chromosomes, higher number of subterminal chromosomes, and most asymmetric karyotype. Later *D. polycladus* is separated by shorter calyx and petal long and lamina oblong-cuneate (see key) and, concerning the karyological features, it is different from the other taxa in the following aspects: higher number of submedian chromosomes, lower number of subterminal chromosomes, and most symmetric karyotype. *Dianthus cyri* and *D. polycladus* also show different positions in the scatter diagram between M_{CA} and CV_{CL} (Figure 4).

The chromosome numbers and karyotype formulae of *D. strictus* var. *axilliflorus* collected from three different localities are the same but there are some small differences in karyotype symmetry/asymmetry values. The sample from Van (locality 3 in Figure 1) of *D. strictus* var. *axilliflorus* grows at high altitudes and alpine regions and it also shows a more symmetrical karyotype than other plants collected from the other localities. The plant from

Şanlıurfa (locality 2 in the Figure 1) grows at the lowest altitude and shows the most asymmetric karyotype.

With this study, new chromosome data were given for six taxa of section *Verruculosi*. Thus, *Dianthus aydogdui* is the last species with an unknown chromosome number in section *Verruculosi*.

Specimens examined: *Dianthus cyri* – Turkey. A9 Iğdır: Tuzluca, Turabi village, 340 m, fields, 4.7.2013, Hamzaoglu 6826, Aksoy & Koç (GAZI); B7 Erzincan: between Erzincan and Üzümlü, 1215 m, salted grass, 14.7.2006, Hamzaoglu 4254, Budak & Aksoy (GAZI); B9 Ağrı (Karaköse): northwest of the city, around Tayyare neighborhood, 1620 m, fallow field, 4.7.1976, M.Dalcı 480 (E-photo); B10 Kars: 5–10 km east of Iğdır (Aras valley), 800 m, fallow land at edge of *Phragmites* marsh, 29.5.1966, P.H.Davis 43837 (E, E00475275-photo); *Dianthus aydogdui* – Turkey. B4 Aksaray: Tuz Gölü, between Gölyazı and Eski, 950 m, salted areas, 24.6.1999, Hamzaoglu 2444 & M.Aydoğdu (syntype, GAZI); *Dianthus strictus* var. *strictus* – Turkey. B7 Erzincan: 10 km north east of Kemah, 1200–1300 m, along dry creek, stony bushy slope, 17.7.1976, M.Dalcı 506 (E-photo); B8 Erzurum: between Aşkale and Tercan, Tepebaşı Pass, 2050 m, mountain steppes, 24.8.2012, Hamzaoglu 6630 & Koç (GAZI); B9 Ağrı: Patnos, west of Kızkapan village, 2020 m, rocky slopes, 5.7.2013, Hamzaoglu 6831, Koç & Aksoy (GAZI); C5 İçel: Gözne, at north boundary of town, roadside wasteland, serpentine, 17.7.1998, R.D.Reeves 1947, A.R.Kruckeberg, N.Adigüzel 339 (E, E00295522-photo); C6 Hatay: Belen, Atik village, 1035 m, forest, 9.7.2012, Hamzaoglu 6427, Aksoy & Koç (GAZI); C7 Diyarbakır: Siverek, towards Karacadağ ski center, 1200 m, grassy places, 10.7.2012, Hamzaoglu 6445, Koç & Aksoy (GAZI); *Dianthus strictus* var. *subenervis* – Turkey. C4 Mersin: Aydıncık, around Köşk Stream, 6 m, rocks, 9.6.2012, Hamzaoglu 6319 & Aksoy (GAZI); C5 Niğde: north of Fesleğen village, 1800 m, rocky slopes, 7.7.2012, Hamzaoglu 6418, Koç & Aksoy (GAZI); C6 Hatay: Yayladağı, Kerep border area, c. 400 m, 27.6.1944, B.Kasaplıgil 16 (ANK); *Dianthus strictus* var. *axilliflorus* – Turkey. B9 Van: Muradiye, near Muradiye Waterfall,

1805 m, stony slopes, 27.8.2012, Hamzaoglu 6662 & Koç (GAZI); C4 Mersin: Tarsus, Eshab-ı Kefh entry, 260 m, 26.7.2012, Hamzaoglu 6529, Aksoy & Koç (GAZI); C6 Kahramanmaraş: Göksu, Fındık village, Kandil mountain, 1600–1800 m, forest, 27.7.1981, B.Yıldız 3106 (HUB); C7 Şanlıurfa: Şanlıurfa to Gaziantep, c. 20. km, 805 m, stony slopes, 9.7.2012, Hamzaoglu 6436, Aksoy & Koç (GAZI); Şanlıurfa: between Hilvan and Siverek, 575 m, stony slopes, 10.7.2012, Hamzaoglu 6437, Aksoy & Koç (GAZI); C8 Mardin: Between Mazıdağı and Mardin, Akresta passage, 1060 m, stony slopes, 10.7.2012, Hamzaoglu 6441, Aksoy & Koç (GAZI); C9 Hakkâri: Konak village, around Koçaniş church, 1900 m, stony slopes, 23.7.2008, Budak 2313 & Koç (GAZI); *Dianthus strictus* var. *gracilior* – Turkey. B7 Tunceli: between Pertek and Çemişgezek, 30 km, c. 1250 m, 11.8.1983, N.Adigüzel 8174 & T.Ekim (ANK; GAZI); B8 Diyarbakır: Silvan, disturbed steppe, 24.6.1954, P.H.Davis 22113 (ANK); B9 Bitlis: Tatvan, Sorgun, Van Lake, volcanic slope, 1650–1700 m, 25.7.1972, H.Peşmen 3123 (HUB); C6 Gaziantep: Osmaniye output, Sof Mountain, 970 m, calcareous rocks, 9.7.2012, Hamzaoglu 6433, Aksoy & Koç (GAZI); C10 Hakkâri: north of Yüksekova, Başkale, 1790 m, K.P.Buttler 23155 & R.Bothmer (ANK); *Dianthus polycladus* – Turkey. A7 Gümüşhane: Köse-Kelkit road, north of Koşmaşat stream, in the vicinity of Havcuş, 1500 m, roadside, fields, 27.7.1950, H.Demiriz 162 & A.Huber-Morath (E, E00475304); B8 Erzurum: Aktoprak village, 29.8.1981, Özyurt & Beyazoglu s.n. (ATA-3794); C6 Hatay: Belen, around Kıcı village, 710 m, forest clearings, rocks, 9.7.2012, Hamzaoglu 6426, Aksoy & Koç (GAZI); Kahramanmaraş: Ahır mountain, Kazma Bağları, 915 m, maquis, 7.7.2007, Hamzaoglu 4762, Aksoy & Budak (GAZI); C8 Mardin: Mardin, in deserto ad Khurs, 14.6.1888, Sintenis 1018 (E, E00475303-photo).

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