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New Chromosomal Data and Karyological Relationships in *Geranium*: Basic Number Alterations, Dysploidy, Polyploidy, and Karyotype Asymmetry

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HIGHLIGHTS

- Karyological relationships are useful to infer processes of evolution and speciation.
- This paper reports chromosomal data of 38 taxa. First report (9) and new count (5).
- Dysploidy, polyploidy, and karyotype asymmetry are important parameters.
- Dysploidy and polyploidy variations are main factors in karyotype evolution of genus.

Abstract: Chromosomal data and karyological relationships provides valuable contributions to understanding speciation and karyotypic phylogeny. Because of the large number of species, wide distribution, morphological differences and chromosomal variations, *Geranium* is an important genus for determining the relationship between chromosomal alterations and karyotypic phylogeny. In the present study, the chromosomal data of 38 taxa are provided, nine of which are given for the first time (*G. eginense*, *G. gracile*, *G. ibericum* subsp. *jubatum*, *G. lasiopus*, *G. libani*, *G. libanoticum*, *G. petri-davisii*, *G. ponticum*, *G. psilostemon*), five present new chromosome numbers (*G. asphodeloides*, *G. ibericum* subsp. *ibericum*, *G. molle* subsp. *molle*, *G. pretense*, *G. rotundifolium*), and 24 agree with previous reports. Eleven different diploid numbers (2*n* = 18, 20, 22, 26, 28, 30, 32, 46, 48, 64, and 84) are detected. In basic numbers, infraspecific variations are encountered. The comprehensive variations of basic numbers and the relatively low rate of polyploid species showed in the present study promote the evolutionary significance of karyotype alterations by dysploidy mechanism. Regarding karyological relationships, *G. sanguineum* forms a monophyletic group by quite different karyological features, which are different basic number, diploid number, and karyotype sample and high ploidy level. Other clad consists of two subclades with a medium strong monophyletic group. In regression analyses, there are significant positive correlations between THL and 2*n*/ploidy levels. Asymmetry indices (CV_{CL} and M_{CA}) show weak positive correlations mainly caused by polyploidy. The most asymmetrical karyotypes are *G. molle* subsp. *bruitium* in intrachromosomal asymmetry and *G. asphodeloides* in interchromosomal asymmetry.

Keywords: *Geranium*; chromosome alterations; ploidy levels; symmetrical karyotype.

INTRODUCTION

Geraniaceae is widely distributed around the world and is generally localized in subtropical and temperate regions with five genera: *Erodium* L'Hér., *California* Aldasoro & C.Navarro & P.Vargas & L.Sáez & Aedo, *Pelargonium* L'Hér., *Monsonia* L., and *Geranium* L. *Geranium* is represented by almost 400 species and is localized in temperate areas and tropical elevations in many regions of the world except deserts, polar regions and tropical plains [1]. In currently accepted classification, genus is divided into three subgenera: subgen. *Geranium*, subgen. *Robertium* (Picard) Rouy, and subgen. *Erodioidea* (Picard) Yeo, [2]. In the following years, while the subgenera included in this classification are accepted, the sectional classification is reevaluated and new subsections were added as well [1,3-9]. As its systematic situation is always in dispute, *Geranium* are excellent systems to use for determining diversification and speciation.

Chromosomal and karyological data contribution the properties determining interspecific relationships and karyotype evolution. The primary chromosomal data are diploid number (2*n*), basic number (*x*), and chromosome lengths. These properties could be replaced numerically through aneuploidy and polyploidy, as well as through structural arrangements containing inversion, deletion, and translocation (which can change chromosome number by dysploidy). All of these form interspecific and intraspecific variations, alter centromere position and chromosome morphology, and affect karyotype asymmetries as intrachromosomal asymmetry and interchromosomal asymmetry [10-17]. *Geranium* seems to be an important model for understanding karyotype evolution due to global distribution, chromosome number variations (basic and diploid), and various ploidy levels.

In genus *Geranium*, cytogenetic studies represent the variations of chromosome numbers from $2n = 14$ in *G. phaeum* L. to $2n = 128$ in *G. robertianum* L. and *G. palmatum* Cav., including intraspecific variations [18-20]. According to the chromosome count database (CCDB, http://ccdb.tau.ac.il), the chromosome numbers are reported from 91 species [18,20-38]. Fifty-two species are diploid; however, they exhibit five different basic numbers: $x = 11$ ($2n = 22$), $x = 12$ ($2n = 24$), $x = 13$ ($2n = 26$), $x = 14$ ($2n = 28$), and $x = 23$ ($2n = 46$). Twenty-three species are polyploid and reveal different polyploidy levels: triploidy ($2n = 3x = 30, 39, 42$), tetraploidy ($2n = 4x = 32, 36, 48, 52, 56, 68$), pentaploidy ($2n = 5x = 50$), and heptaploidy ($2n = 7x = 84$). *G. magellanicum* Hook., *G. palmatum*, *G. potentilloides* L'Hér. ex DC., and *G. robertianum* indicate quite high polyploidy ($2n = 8x = 112, 128$). Sixteen species are both diploid and polyploid [39]. *Geranium* seems to have basic numbers ranging from as low as 7 up to 23 and more common $x = 13$ and 14. Probably some species show dysploidy, which is an alteration of basic chromosome number generally [16,40]. *Geranium* taxa are well characterized in point of all these chromosomal data. In some species, e.g. *G. columbinum* L. and *G. dissectum* L., there is a large cytotaxonomic database [20-21,25,27,29,35]. However, there are still serious shortcomings in the detailed karyotype data of genus *Geranium* and this prevents a more comprehensive definition of major chromosome rearrangements in terms of karyotype evolution.

Seven sections, *Batrachioidea* W.D.J Koch, *Divaricata* Rouy, *Dissecta* Yeo, *Lucida* R. Knuth, *Ruberta* Dumort., *Unguiculata* (Boiss.) Reiche, and *Tuberosa* (Boiss.) Reiche are distributed in Mediterranean region and western Asia [1,3,7].

Sect. *Batrachioidea* and sect. *Divaricata* occurs six taxa, which are between Macaronesia and Himalayas, Turkey, Caucasus, Iran, and north Africa [3]. In sect. *Divaricata*, diploid numbers are $2n = 20, 28$ in *G. albanum* and $2n = 26, 28$ in *G. divaricatum* Ehrh. and basic number is probably $x = 14$ [20-21,25,29,35]. In sect. *Batrachioidea*, the basic number is $x = 13$ by $2n = 26, 52$ in diploid and polyploid species [18,25,31,35]. Aedo and coauthors [3] reported that annual species, with various basic numbers, probably evolved independently. In this context, the basic number ($x = 13$) could be seen as a derived character by dysploidy.

Sect. *Dissecta* occurs four taxa, which are Lebanon, Turkey, between Sicily and Caucasus, and a species distributed worldwide; it is probably indigenous to the Eurasian [7]. The only one chromosome number is reported in *G. dissectum* ($2n = 22$) and *G. sintenisii* Freyn ($2n = 26$) [20,26,35]. By contrast, *G. asphodeloides* Burm. has different chromosome numbers with $2n = 24, 28, 30$, which are probably shaped by dysploidy [20,31,37].

Sect. *Lucida*, *Ruberta* and *Unguiculata* occur 12 taxa, which are Macaronesia, Turkey, southern Spain, Morocco, southern France, and western Asia [9]. The only one chromosome number is reported in *G. glaberrimum* Boiss. & Heldr. (2*n* = 30), *G. cataractarum* Coss. (2n = 36), *G. dalmaticum* (Beck) Rech. (2*n* = 46), and *G. maderense* Yeo (2*n* = 68) [26,29,41]. By contrast, *G. lucidum* L. has different chromosome numbers with 2*n* = 20 and 2*n* = 40 to 42 (probably dysploidy) [20-21,24,29,35]. In addition, other species show the high ploidy levels by 2*n* = 64, 68, 92, 112, and 128 [20-21,24,31-32,35].

In sect. *Tuberosa*, subsect. *Mediterranea* occurs ten taxa, which are spread in the Caucasus, Turkey, Iran, northwestern Africa, and western Europe [1]. The only one chromosome number is reported in *G. bohemicum* L., *G. gymnocaulon* DC. (2*n* = 28), and *G. ibericum* Cav. (2*n* = 56) [18,20,22,35]. By contrast, *G. lanuginosum* Lam. has different chromosome numbers with 2*n* = 42 and 2*n* = 48 [21,29]. van Loon [29] reported that chromosome number is probably shaped by dysploidy. In addition, *G. platypetalum* Fisch. & C. A. Mey. has tetraploids and pentaploids [18]. It was considered that the basic number is *x* = 14 in subsect. *Mediterranea* [29]. Subsect. *Tuberosa* occurs seven taxa, two of them are recorded as 2*n* = 28 [27-28]. The basic number looks the same as for subsect. *Mediterranea*.

In the present study, it is aimed to provide a detailed survey of chromosomal variations and to contribute to the cytotaxonomy, karyotype evolution and karyotypic phylogeny of *Geranium*. In summary: (i) we determined chromosome numbers and made karyotype analyses of almost all Turkish *Geranium*; (ii) we examined to ploidy levels and possible aneuploid forms to explain the variations in monoploid diploid sets; (iii) we showed the karyotype asymmetries through the latest parameters for the first time; (iv) we established a dendrogram with combined data to determine the interspecific relationships; and (v) we made the regression analysis of chromosomal data versus karyomorphological data.

MATERIAL AND METHODS

Plant material

Distribution map is generated by Google Maps (Figure 1). Thirty-eight *Geranium* taxa were collected from their natural habitats across Turkey. Exsicates were deposited at the herbarium of the Department of Biological Sciences, at the Middle East Technical University (METU) in Ankara. The distribution regions, collection information and chromosomal status are given in Table 1.

Figure 1. Distribution map of the studied species in Turkey. (1) *G. asphodeloides*; (2) *G. bohemicum*; (3) *G. collinum*; (4) *G. columbinum*, *G. lucidum*; (5) *G. dissectum*; (6) *G. divaricatum*; (7) *G. eginense*; (8) *G. glaberrimum*; (9) *G. gracile*, *G. sanguineum*, *G. sintenisii*; (10) *G. gymnocaulon*; (11) *G. ibericum*; (12) *G. lanuginosum*, *G. macrorrhizum*; (13) *G. lasiopus*, *G. purpureum*, *G. robertianum*; (14) *G. libani*; (15) *G. libanoticum*; (16) *G. macrostylum*, *G. tuberosum*; (17) *G. molle*; (18) *G. palustre*, *G. pretense*; (19) *G. petri-davisii*; (20) *G. platypetalum*; (21) *G. ponticum*; (22) *G. psilostemon*; (23) *G. pusillum*; (24) *G. pyrenaicum*; (25) *G. rotundifolium*; (26) *G. sibiricum*; (27) *G. subcaulescens*; (28) *G. sylvaticum*.

Table 2. The karyological parameters and formulae used for chromosome characterization.

Species	**Distribution regions and collection information**	**Chromosomal status**
G. asphodeloides	Trabzon, Hayrat, Küçük Mesoraş, 2055 m, 06.08.2015, A. Kahraman 2185 (B)	Studied
G. bohemicum	Bolu, between Bolu and Yedigöller, 1433 m, 14.07.2015, A. Kahraman 2157	Studied
G. collinum Stephan ex Willd.	Ardahan, Haçuvan village (Hasköy), 1500 m, 04.08.2015, A. Kahraman 2170	Studied
G. columbinum	Karabük, between Karabük and Bartın, 667 m, 12.07.2015, A. Kahraman 2141	Studied
G. dissectum	Antalya, between Akseki and İbradı, 955 m, 11.05.2014, A. Kahraman 1774	Studied
G. divaricatum	Sivas, between Akdağmadeni and Şarkışla, Çamlıca village, 1386 m, 24.06.2015, A. Kahraman 2109	Studied
G. eginense Hausskn. & Sint. ex R.Knuth	Erzincan, Kemaliye, Karanlık Canyon road, 890 m, 15.06.2016, A. Kahraman 2387(B)	Unstudied
G. glaberrimum	Antalya, Gündoğmuş, Gelesandra plateau, 1473 m, 13.05.2014, A. Kahraman 1789	Studied
G. gracile Ledeb. ex Nordm.	Gümüşhane, towards to Zigana gateway, 1696 m, 17.07.2014, A. Kahraman 1879	Unstudied
G. gymnocaulon	Artvin, Şavşat, 2210 m, 18.07.2016, A. Kahraman 2412	
G. ibericum	Trabzon, between Hayrat and Sarmaşık village (Büyük Mesoraş), 1815 m, 19.07.2014, A. Kahraman 1900	Unstudied
G. lanuginosum	Balıkesir, Erdek, between Ormanlı and Ballı, 70-100 m, 19.05.2016, A. Kahraman 2361	Studied
G. lasiopus Boiss. & Heldr.	Antalya, Akseki, Güzelsu, 1190 m, 14.05.2014, A. Kahraman 1791	Unstudied
G. libani P.H.Davis	Hatay, Belen, Güzelyayla, 980-1050 m, 30.05.2015, A. Kahraman 2076	Unstudied
G. libanoticum Schenk	Antalya: between Derebucak-İbradı, 1343 m, 23.04.2016, A. Kahraman 2311	Unstudied
G. lucidum	Karabük, between Karabük and Bartın, 667 m, 12.07.2015, A. Kahraman 2144	Studied
G. macrorrhizum L.	Balıkesir, Erdek, between Ormanlı and Ballı, 70-100 m, 19.05.2016, A. Kahraman 2362	Studied
G. macrostylum Boiss.	Antalya, Elmalı, Uzungeriş hill, 2116 m, 20.06.2014, A. Kahraman 1837	Studied
G. molle L.	Manisa, between Kula and Salihli, 129 m, 06.04.2014, A. Kahraman 1701	Studied
G. palustre L.	Kars, between Sarıkamış and Karaurgan, 2139 m, 04.08.2015, A. Kahraman 2174	Studied
G. petri-davisii Aedo	Kahramanmaraş, Höbek mountain, 2107 m, 01.07.2015, A. Kahraman 2130	Unstudied
G. platypetalum	Artvin, between Şavşat and Ardahan, 2275 m, 03.08.2015, A. Kahraman 2167	Studied
G. ponticum (P.H.Davis & J.Roberts) Aedo	Gümüşhane, Zigana mountain, from Kepenek hill to Yayla village (Alas), 2322 m, 17.07.2014, A. Kahraman 1886	Unstudied
G. pratense L.	Kars, between Sarıkamış and Karaurgan, 2139 m, 04.08.2015, A. Kahraman 2175	Studied
G. psilostemon Ledeb.	Gümüşhane, between Kayaiçi village (Toroslu) and Yağmurdere, 1613 m, 18.07.2014, A. Kahraman 1891	Unstudied
G. purpureum Vill.	Antalya, between Manavgat and Akseki, 303 m, 11.05.2014, A. Kahraman 1770	Studied
G. pusillum Burm.f.	Burdur, road of Fethiye-Denizli, between Çavdır and Acıpayam, 1030 m, 04.05.2015, A. Kahraman 2060	Studied
G. pyrenaicum Burm.f.	Karabük, Gölören village, 1058 m, 11.07.2015, A. Kahraman 2137	Studied
G. robertianum	Antalya, between Manavgat and Akseki, 534 m, 11.05.2014, A. Kahraman 1772	Studied
G. rotundifolium L.	Muğla, Ortaca, 0 m, 03.05.2015, A. Kahraman 2058	Studied

Cont. Table 2

G. sanguineum L.	Gümüşhane, Zigana road, Zigana village, 1412 m, 17.07.2014, A. Kahraman 1877	Studied
G. sibiricum L.	Trabzon, Uzungöl area, 1100 m, 19.07.2016, A. Güngör & A. Kahraman 2414A	Studied
G. sintenisii	Gümüşhane, towards to Zigana gateway, 1704 m, 17.07.2014, A. Kahraman 1880	Studied
G. subcaulescens L Hér. ex DC.	Isparta, Aksu, Dedegöl mountain, Melikler plateau, 1815 m, 17.06.2014, A. Kahraman 1831	Studied
G. sylvaticum L.	Düzce, between Düzce and Sakarya, Kaynaşlı, 413 m, 08.04.2015, A. Karaman 1978	Studied
G. tuberosum L.	Antalya, Elmalı, Uzungeriş hill, 2138 m, 20.06.2014, A. Karaman 1838	Studied

Chromosome preparation

The seeds were germinated between moist Whatman papers in Petri dishes. The root tips were pretreated in α-mono-bromonaphthalene at 4°C for 16 h. Then, the roots were fixed with Carnoy's fixative (absolute alcohol:glacial acetic acid - 3:1, v:v) at 4°C for 24 h and stored in 70% ethanol at 4°C until use. The fixed roots were hydrolyzed in 1 N HCl at 60°C for 12 min, stained in 2% aceto-orcein, and squashed for observations [16,42].

Karyotype analysis

At least ten metaphase cells were investigated to determine chromosome numbers. The chromosomal measurements were made using the Software Image Analyses (Bs200ProP) loaded on a personal computer. The following parameters were used to characterize the chromosomes numerically (Table 2). Karyotype formulae were by chromosome morphology based on centromere position according to Levan and coauthors [43]. The ideograms were drawn based on chromosome arm length (arranged large to small). Some data obtained from Havva Bozkurt's master thesis were used in the article [44]. In Table 2, karyotype asymmetry was estimated by two parameters: interchromosomal asymmetry (CV_{CL}) and intrachromosomal asymmetry (M_{CA}) [45-46].

Table 2. The karyological parameters and formulae used for chromosome characterization.

Karyotypic parameters	Abbreviations and formulae
Long arm	L
Short arm	S
Total chromosome length	TL = LA + SA
Arm ratio	AR = LA / SA
Centromeric index	CI = [(SA) / (LA + SA)] × 100
Mean haploid length	MHL
Total haploid length	THL
Relative length	RL = [(LA + SA) / THL] × 100
Median chromosome Submedian chromosome Subtelocentric chromosome Terminal chromosome	m, LA / SA = 1.0 – 1.7 sm, LA / SA = 1.7 – 3.0 st, LA / SA = 3.0 – 7.0 t, LA / SA = 7.0 – ∞
Mean centromeric asymmetry (Intrachromosomal asymmetry)	$M_{CA} = [\text{mean}\ (L_T - S_T) / (L_T + S_T)] \times 100$ L_T, total length of long arms S_T, total length of short arms
Coefficient variation of chromosome length (Interchromosomal asymmetry)	$CV_{CL} = (S_{CL} / X_{CL}) \times 100$ S_{CL}, standard deviation X_{CL}, mean chromosome length

Karyological relationships and regression analysis

Karyological relationships were evaluated by following seven parameters: basic number (*x*), diploid number (2*n*), ploidy level, karyotype formula, total haploid length (THL), mean centromeric asymmetry (M_{CA}), and coefficient of variation of chromosome length (CV_{CL}). A dendrogram showing karyological relationships was drawn by bootstrap values (BV) with UPGMA software, chord coefficient. The dendrogram contains 12 *Geranium* species with detailed chromosomal data. In dendrogram, parameters are classified in the following order: THL [$10 < THL \leq 20$ (1), $20 < THL \leq 30$ (2), $30 < THL \leq 40$ (3), $40 < THL$ (4)]; M_{CA} [$10 < M_{CA} \leq 15$ (1), $15 < M_{CA} \leq 20$ (2), $20 < M_{CA} \leq 25$ (3)], and CV_{CL} [$10 < CV_{CL} \leq 20$ (1), $20 < CV_{CL} \leq 30$ (2), $30 < CV_{CL} \leq 40$ (3)].

For regression analysis, linear models were calculated between three predictor variables (x, $2n$, and ploidy levels) and four dependent variables (MHL, THL, CV_{CL}, and M_{CA}) using the software Past 4.04. Then, a scatter diagram was drawn between interchromosomal asymmetry and the intrachromosomal asymmetry.

RESULTS

Chromosomal data

Figure 2 shows the metaphase chromosomes of *Geranium* taxa. Chromosome counts in 38 taxa are listed in Table 3; nine are reported here for the first time, five present new chromosome numbers, and 24 have similar number with previous reports. Eleven different diploid numbers ($2n$ = 18, 20, 22, 26, 28, 30, 32, 46, 48, 64, and 84) are detected. Chromosomes of *Geranium* are small. Mean haploid length varies from 0.83 µm in *G. ibericum* to 2.11 µm in *G. columbinum*. The smallest total haploid length is 18.43 µm in *G. lucidum*, and the highest value is 71.56 µm in *G. sanguineum*. The smallest chromosome size among the taxa detected detailed chromosomal measurements is 0.87 µm, in *G. rotundifolium*. The largest chromosome size is 3.77 µm in *G. asphodeloides* (Table 4).

Basic numbers, ploidy levels and polyploidy

In genus *Geranium*, there are generally 2 common basic numbers, which are x = 13 and most commonly x = 14. In the present study, the basic numbers are x = 13 in 11 taxa and x = 14 in 18 taxa. In addition, the other basic numbers and ploidy levels are x = 8 in *G. purpureum* and *G. robertianum* with ploidy levels of $4x$ and $8x$; x = 9 in *G. columbinum*; x = 10 in *G. lucidum* and *G. glaberrimum* with ploidy level of $3x$; x = 11 in *G. dissectum*; x = 12 in *G. lanuginosum* and *G. sanguineum* with ploidy levels of $4x$ and $7x$; and x = 23 in *G. macrorrhizum* (Table 3 and Figure 3). In basic numbers, infraspecific variations are encountered in *Geranium*.

Karyotypes and karyotype asymmetry

In Table 4, twelve taxa have metacentric and submetacentric chromosomes and only x = 7 heptaploid *G. sanguineum* has subtelocentric chromosomes; there are no telocentric chromosomes. Four different karyotype samples are detected, which are M-m-sm (in two taxa), m (in only one taxon), m-sm (in nine taxa), and m-sm-st (in only one taxon).

Intrachromosomal asymmetry (M_{CA}) varies from 14.18 (*G. petri-davisii*) to 20.76 (*G. molle* subsp. *bruitium*), which refer to symmetric karyotypes. Interchromosomal asymmetry (CV_{CL}), indicating the karyotype heterogeneity, varies from 13.93 (*G. collinum*) to 30.38 (*G. asphodeloides*).

Interspecific relationships

Figure 4 presents a dendrogram including chromosomal data of 12 *Geranium* species. The dendrogram consists of two main clades. Firstly, *G. sanguineum* is separated as a monophyletic group by quite different karyological features, which are different basic number (x = 12), diploid number ($2n$ = 84), and karyotype sample (m-sm-st) and high ploidy level (Clade I). Clade II consists of two subclades with a medium strong monophyletic group (BV = 64). Subclade 1 contains different karyotype sample (M-m-sm) and relatively more intrachromosomal asymmetry. Subclade 2 is branched by low bootstrap values and contains nine taxa by strong karyological variations.

Regression analysis

In Figure 5, linear regression models are presented by scatter plots, which refer to the independent (x, $2n$, ploidy levels) and dependent variables (MHL, THL, CV_{CL}, M_{CA}). The following parameters are listed under each plot: regression slope, standard error, r value, and test statistic. Dotted lines in E and F represent significant linear regression, and solid lines in the other plots show not significant correlations. The significant regression models (E and F) have p values < 0.0001 that are significant after adjusting the p value for multiple testing. In other regression models, p values are quite high.

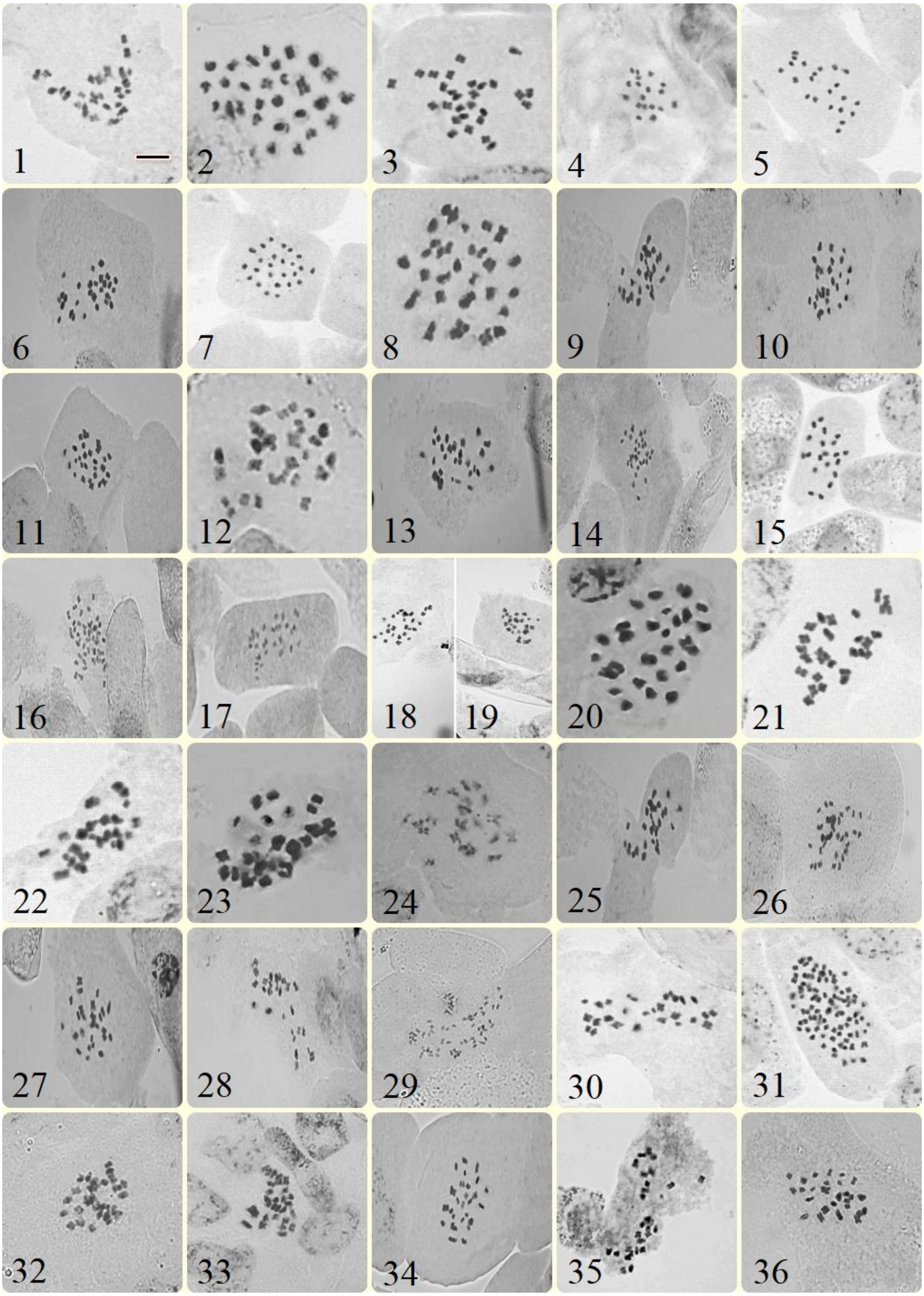

Figure 2. Metaphase chromosomes of *Geranium* species. (1) *G. asphodeloides*; (2) *G. bohemicum*; (3) *G. collinum*; (4) *G. columbinum*; (5) *G. dissectum*; (6) *G. divaricatum*; (7) *G. eginense*; (8) *G. glaberrimum*; (9) *G. gracile*; (10) *G. gymnocaulon*; (11) *G. ibericum*; (12) *G. lasiopus*; (13) *G. libani*; (14) *G. libanoticum*; (15) *G. lucidum*; (16) *G. macrorrhizum*; (17) *G. macrostylum*; (18) *G. molle* subsp. *bruitium*; (19) *G. molle* subsp. *molle*; (20) *G. palustre*; (21) *G. petri-davisii*; (22) *G. platypetalum*; (23) *G. ponticum*; (24) *G. pretense*; (25) *G. psilostemon*; (26) *G. purpureum*; (27) *G. pusillum*; (28) *G. pyrenaicum*; (29) *G. robertianum*; (30) *G. rotundifolium*; (31) *G. sanguineum*; (32) *G. sibiricum*; (33) *G. sintenisii*; (34) *G. subcaulescens*; (35) *G. sylvaticum*; (36) *G. tuberosum*. Scale bar 10 µm.

Table 3. The chromosome counts of the taxa in present and previous studies. In addition, the karyological data with basic number (*x*), ploidy levels, mean haploid length (MHL), total haploid length (THL), and asymmetry indices (CV_{CL} and M_{CA}). * probably dysploidy.

Taxa (alphabetically)	**Previous results**		**Present results**							**Note**
	2*n*	**References**	**2*n***	***x***	**Ploidy**	**MHL**	**THL**	**CV_{CL}**	**M_{CA}**	
G. asphodeloides	24, 28*	[20,31,37]	26	13	2*x*	1.25	32.49	30.38	16.25	New count
G. bohemicum	28	[20,35]	28	14	2*x*					Equal number
G. collinum	28	[38]	28	14	2*x*	0.87	24.28	13.93	18.38	Equal number
G. columbinum	18	[20-21,25, 27,29,35]	18	9	2*x*	2.11	18.99	20.98	17.92	Equal number
G. dissectum	22	[20,35]	22	11	2*x*	1.72	18.88	25.42	18.85	Equal number
G. divaricatum	26, 28*	[20,25,35]	28	14	2*x*	0.99	27.74			Equal number
G. eginense			26	13	2*x*					First report
G. glaberrimum	30	[26]	30	10	3*x*					Equal number
G. gracile			26	13	2*x*	0.94	24.54			First report
G. gymnocaulon	28	[22]	28	14	2*x*					Equal number
G. ibericum subsp. *ibericum*	56	[18]	28	14	2*x*	0.83	23.19			New count
G. ibericum subsp. *jubatum*			28	14	2*x*	0.92	26.68			First report
G. lanuginosum	42, 48*	[18,21]	48	12	4*x*					Equal number
G. lasiopus			26	13	2*x*					First report
G. libani			28	14	2*x*	1.00	27.94			First report
G. libanoticum			28	14	2*x*					First report
G. lucidum	20, 40, 41, 42, 43, 44*	[20,24, 26,35]	20	10	2*x*	1.84	18.43	15.57	15.62	Equal number
G. macrorrhizum	46, 92	[20,31]	46	23	2*x*					Equal number
G. macrostylum	28	[27]	28	14	2*x*					Equal number
G. molle subsp. *bruitium*	26, 52	[20]	26	13	2*x*	1.72	22.21	19.01	20.76	Equal number
G. molle subsp. *molle*	26	[24-25]	28	14	2*x*	1.71	23.92	24.65	15.26	New count
G. palustre	28, 56	[20,25,35]	28	14	2*x*					Equal number
G. petri-davisii			28	14	2*x*	2.32	32.41	21.97	14.18	First report
G. platypetalum	28, 42	[18]	28	14	2*x*	1.91	26.67	27.79	18.46	Equal number
G. ponticum			26	13	2*x*					First report
G. pratense	24, 28, 56	[36]	26	13	2*x*					New count

Cont. Table 3

G. psilostemon			26	13	2*x*	1.03	26.72			First report
G. purpureum	32, 64	[20,24,31-32,35]	32	8	4*x*	0.90	28.95			Equal number
G. pusillum	26	[20,25,35]	26	13	2*x*	1.99	25.82	25.81	19.39	Equal number
G. pyrenaicum	26, 28, 52*	[18,25,31, 35]	26	13	2*x*	1.06	27.49			Equal number
G. robertianum	32, 52, 54, 56, 64,128*	[21,24,35]	64	8	8*x*					Equal number
G. rotundifolium	26, 46	[21,23-24,27,35]	28	14	2*x*	1.78	24.88	25.28	14.43	New count
G. sanguineum	52,56, 82,84*	[20-21,29]	84	12	7*x*	1.70	71.56	25.32	19.31	Equal number
G. sibiricum	28	[35]	28	14	2*x*					Equal number
G. sintenisii	26	[26]	26	13	2*x*					Equal number
G. subcaulescens	28, 56	[26,31,33]	28	14	2*x*	0.96	26.91			Equal number
G. sylvaticum	24, 28, 56*	[30,34]	28	14	2*x*	1.58	22.10	20.49	15.20	Equal number
G. tuberosum	28	[28]	28	14	2*x*					Equal number

Table 4. The karyological features of the studied *Geranium* taxa.

Taxa	KF	SC (μm)	LC (μm)	RL (%) SC–LC	CI (min–max)
G. asphodeloides	24m + 2sm	1.21	3.77	3.72–11.60	35.74–47.32
G. collinum	22m + 6sm	1.40	2.19	5.77–9.02	27.32–47.95
G. columbinum	14m + 4sm	1.33	2.97	7.00–15.64	35.43–46.20
G. dissectum	22m	1.13	2.53	5.99–13.40	37.43–47.16
G. lucidum	18m + 2sm	1.36	2.31	7.38–12.53	32.03–49.17
G. molle subsp. *brutium*	20m + 6sm	1.04	2.17	4.68–9.77	33.55–47.12
G. molle subsp. *molle*	26m + 2sm	0.95	2.36	3.97–9.87	35.91–48.59
G. petri-davisii	26m + 2sm	1.51	3.45	4.66–10.64	36.00–46.42
G. platypetalum	22m + 6sm	1.01	2.76	3.79–10.35	26.73–48.67
G. pusillum	22m + 4sm	1.31	3.12	5.07–12.08	29.11–47.60
G. rotundifolium	2M + 22m + 4sm	0.87	2.47	3.50–9.93	26.32–50.00
G. sanguineum	66m + 16sm + 2st	0.90	2.73	1.26–3.81	24.84–48.21
G. sylvaticum	2M + 22m + 4sm	1.09	2.25	4.93–10.18	34.22–50.00

Abbreviations: karyotype formula (KF), shortest chromosome (SC), longest chromosome (LC), relative length (RL), total haploid length (THL), mean chromosome length (MCL), centromeric index (CI), coefficient of variation of chromosome length (CV_{CL}), mean centromeric asymmetry (M_{CA}), median point (M), median (m), submedian (sm).

DISCUSSION

Variations of chromosome number

Various chromosome numbers such as 2*n* = 18, 20, 22, 26, 28, 30, 32, 46, 48, 64, and 84 are detected with dominant numbers of 2*n* = 26 and 28. The chromosome numbers of nine taxa are reported here for the first time: *G. eginense*, *G. gracile*, *G. lasiopus*, *G. ponticum*, and *G. psilostemon* (2*n* = 26), *G. ibericum* subsp. *jubatum*, *G. libani*, *G. libanoticum*, and *G. petri-davisii* (2*n* = 28). These are the most common diploid chromosome numbers of genus.

The chromosome numbers of five taxa represent new cytotypes: *G. asphodeloides* and *G. pratense* (2*n* = 26), *G. ibericum* subsp. *ibericum*, *G. molle* subsp. *molle*, and *G. rotundifolium* (2*n* = 28). The chromosome numbers are 2*n* = 24, 28 in *G. asphodeloides*, 2*n* = 56 in *G. ibericum* subsp. *ibericum*, 2*n* = 26 in *G. molle* subsp. *molle*, and 2*n* = 26, 46 in *G. rotundifolium* [18, 20-21, 23-25, 27, 31, 35, 37]. The chromosome numbers of 24 taxa are the same as in previous reports. Our study did not confirm far-reaching discrepancies of chromosome numbers in *Geranium* taxa that were reported by previous studies (Table 3: *G. lucidum*, *G. robertianum* etc). Winterfeld and coauthors [17] reported that such findings might result from the preparation and identification of the studied samples or use of obsolete species description and would allow observation of chromosome number variations in the future.

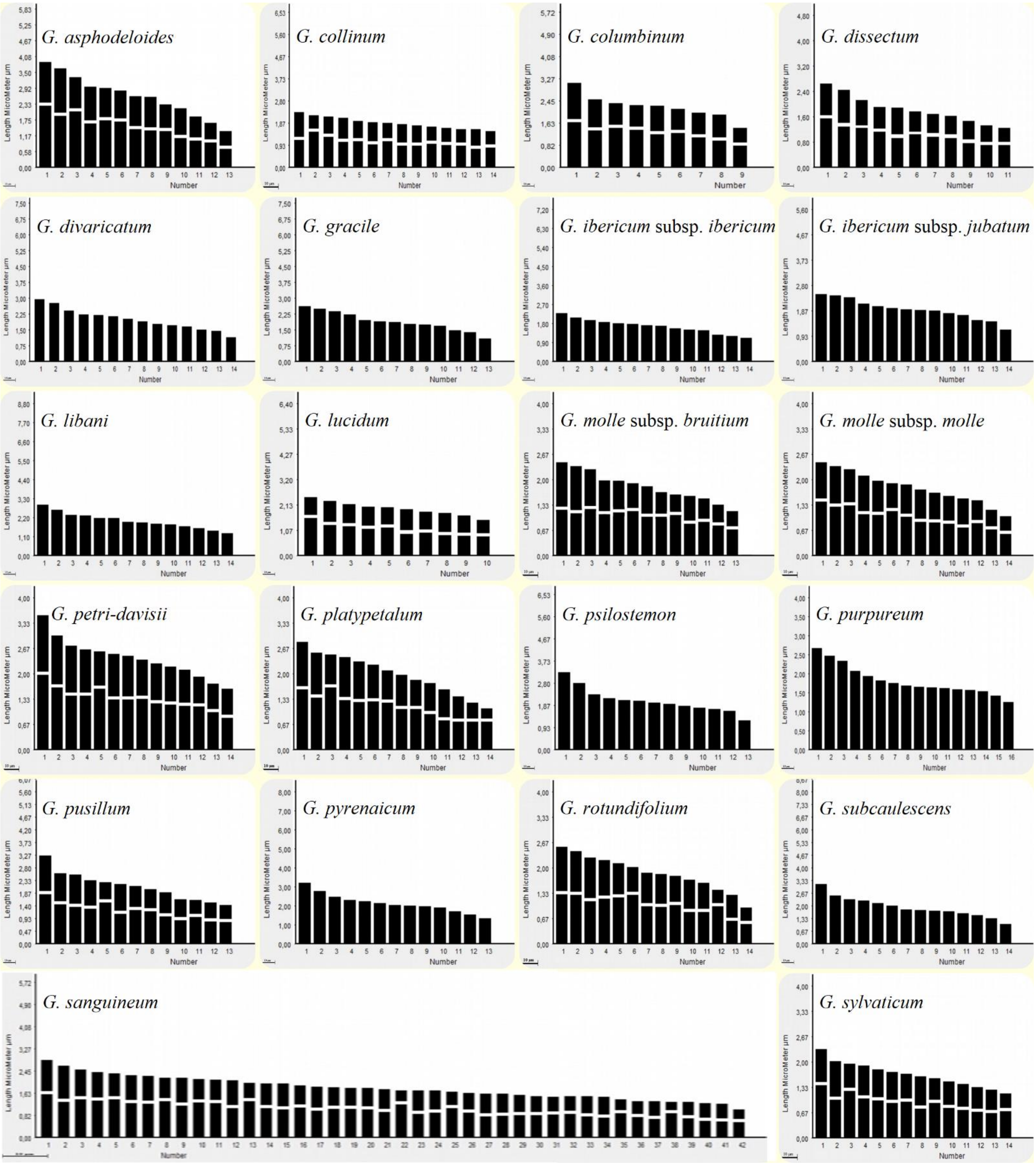

Figure 3. Monoploid ideograms of *Geranium* species.

Basic number alterations, dysploidy and ploidy levels, polyploidy

Basic chromosome numbers of x = 13 and 14 dominate in *Geranium* taxa, but basic numbers of x = 8, 9, 10, 11, 12, and 23 characterize several taxa. Many taxa contain basic number variations possibly caused by the dysploidy mechanism. The dysploidy is likely to have happened depending the fusion of metacentric chromosomes or reciprocal translocations in ancestral karyotypes including dominant basic numbers. Basic number alterations are x = 12, 14 in *G. asphodeloides*, *G. lanuginosum*, and *G. sylvaticum*; x = 13, 14 in *G.*

divaricatum and *G. pyrenaicum*; *x* = 10, 11, 14 in *G. lucidum*; *x* = 8, 13, 14 in *G. robertianum*; and *x* = 12 ,13, 14 in *G. pratense* and G*. sanguineum* [18,20-21,24-26,29-31,34-37].

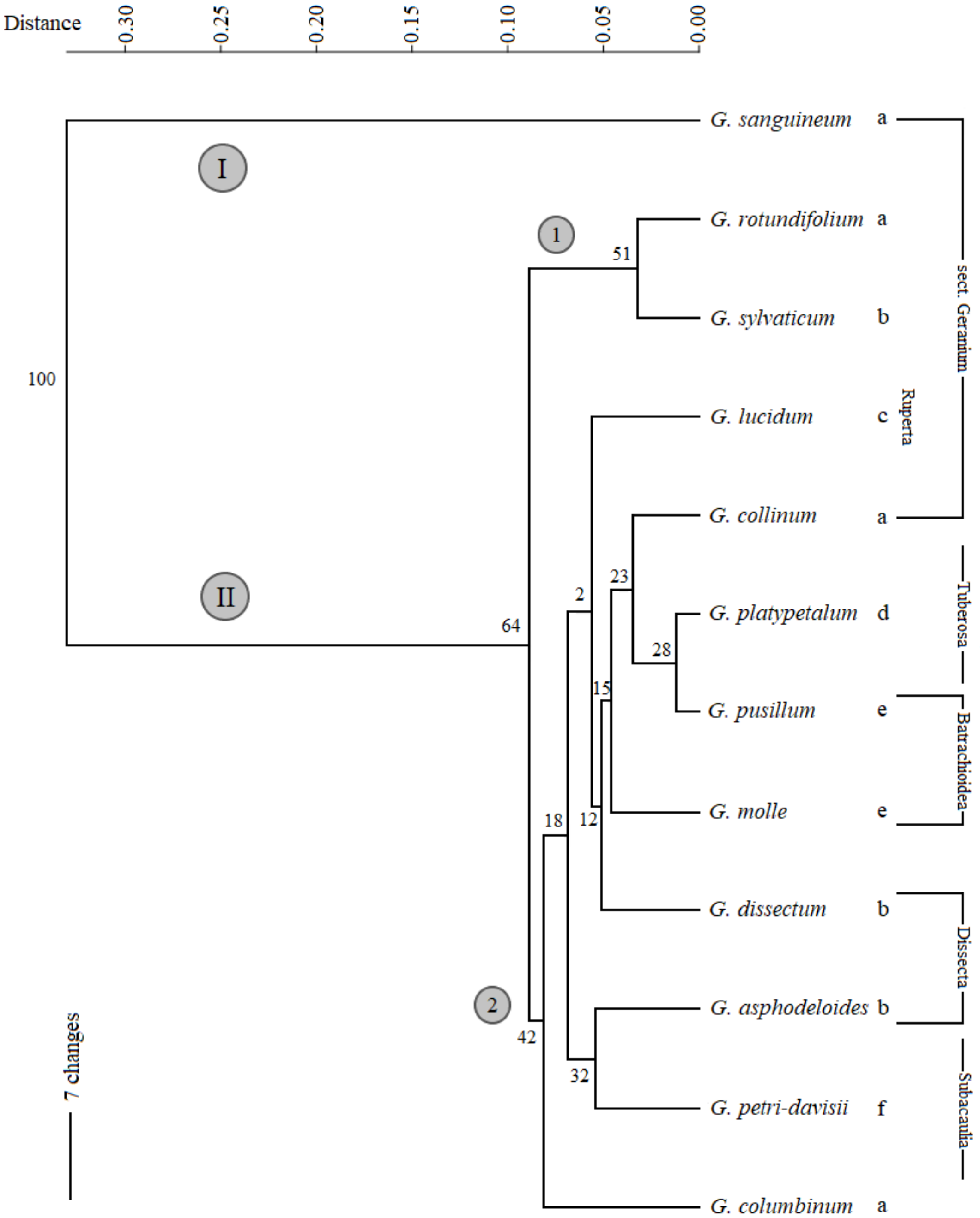

Figure 4. The dendrogram showing karyological relationships of 12 *Geranium* species. Numbers at branches indicate bootstrap values. Main clades and subclades are shown in circle. Seven changes are *x*, 2*n*, ploidy level, karyotype formula, THL [10 < THL ≤ 20 (1), 20 < THL ≤ 30 (2), 30 < THL ≤ 40 (3), 40 < THL (4)]; M_{CA} [10 < M_{CA} ≤ 15 (1), 15 < M_{CA} ≤ 20 (2), 20 < M_{CA} ≤ 25 (3)], and CV_{CL} [10 < CV_{CL} ≤ 20 (1), 20 < CV_{CL} ≤ 30 (2), 30 < CV_{CL} ≤ 40 (3)]. a, sect *Geranium*; b, sect *Dissecta*; c, sect *Ruperta*; d, sect *Tuberosa*; e, sect *Batrachioidea*; f, sect *Subacaulia*.

According to the previous reports, there are many diploid and polyploid reports that include various basic numbers (*x* = 8, 9, 10, 11, 12, 13, 14, and 23) [18,20-37]. In the present study, *G. glaberrimum*, *G. purpureum*, *G. lanuginosum*, *G. sanguineum*, and *G. robertianum* are polyploid species by 2*n* = 3*x* = 30, 2*n* = 4*x* = 32, 2*n* = 4*x* = 48, 2*n* = 7*x* = 84, and 2*n* = 8*x* = 64, respectively. Polyploidy, is an important mechanism regarding speciation and evolution of plants, occurs in two ways, which are autopolyploidy with genome duplication in only one species and allopolyploidy with genome duplication between species. It is reported that polyploidy rates are increased by glaciation, altitudes, and high latitudes although not always [47-48]. For example, polyploid taxa have a wide distribution ranging from 70 to 1470 m.

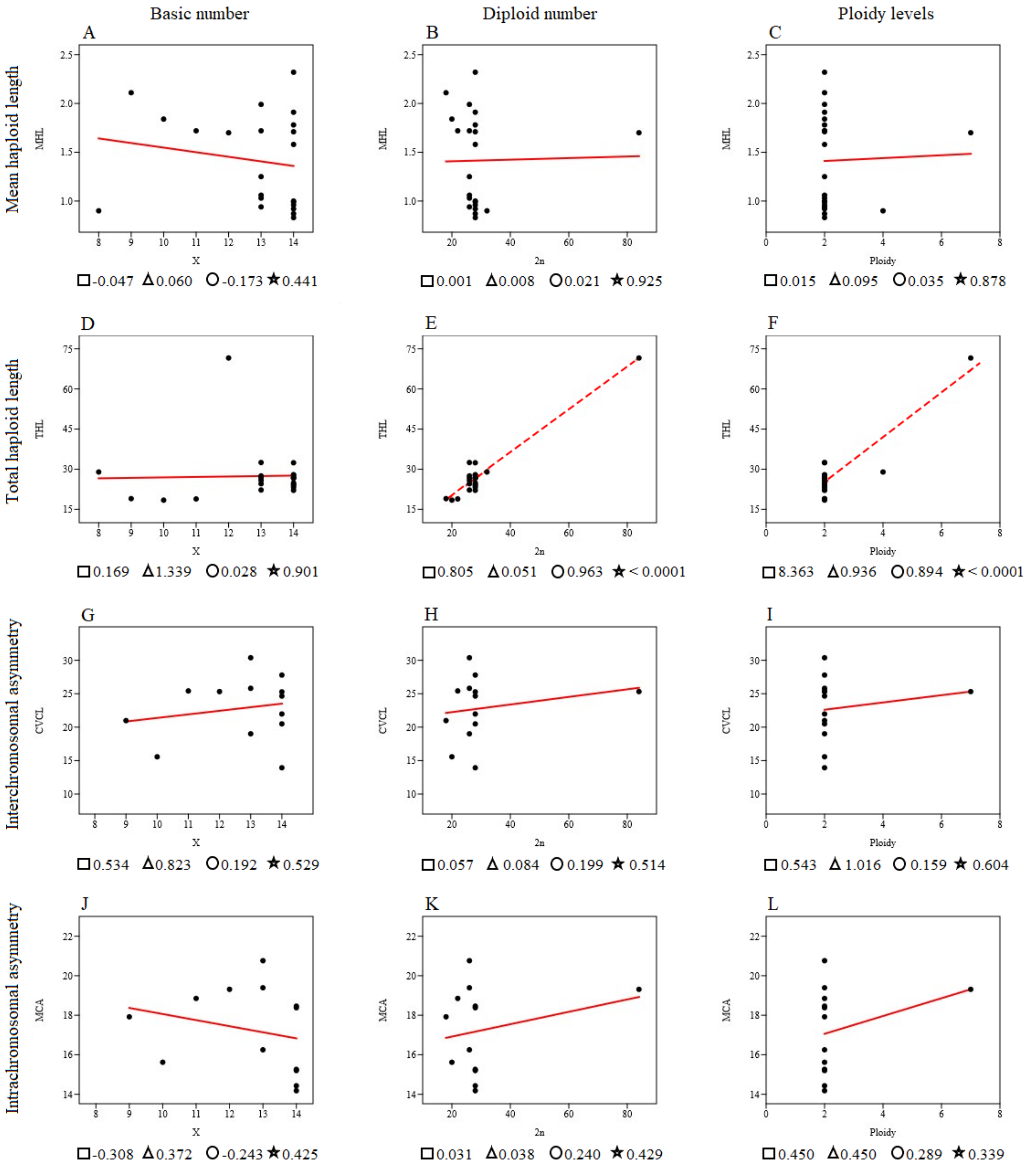

Figure 5. Regression analysis of chromosomal data (basic number, diploid number, ploidy levels) versus karyomorphological data (mean haploid length, total haploid length, interchromosomal asymmetry, and intrachromosomal asymmetry) in *Geranium* taxa. Parameters: square regression slope, triangle standard error, circle r value, star test statistic. Dotted lines (significant linear regression) and regular lines (not significant linear regression).

The comprehensive variations of basic numbers and the relatively low rate of polyploid species showed in the present study promote the evolutionary significance of karyotype alterations by dysploidy mechanism. Despite widespread opposite models, dysploidy may cause relatively long-term persistence in the evolutionary process compared to polyploid changes. The results are inconsistent with previous reports of dysploidy and polyploidy, which are highlighting the evolutionary role of the polyploidy. There are limited studies reporting that the common mechanism in species diversification is dysploidy [16-17,49-50].

Chromosome structure, karyotype asymmetry and regression analysis

In genus *Geranium*, the chromosomal data are generally based on reports of basic and diploid number. Chromosomes are comparatively small (LC < 4 µm and generally MHL < 2 µm). Winterfeld and coauthors [17] reported that the decreasing chromosome length accompanies increasing dysploidy. In addition, total haploid lengths are comparatively small except *G. sanguineum*, which has high chromosome number (2*n* = 84). Centromeric index, intrachromosomal asymmetry and interchromosomal asymmetry in whole complements are variable. In summary, the examined karyotypes show continuous variation.

Regression analyses are established by basic number, diploid number, and ploidy levels versus karyotype data, such as mean haploid length, total haploid length, interchromosomal asymmetry, and intrachromosomal asymmetry. There are significant positive correlations between THL and 2*n*/ploidy levels, meaning that an increase in THL is linked with an increase in 2*n*/ploidy levels. Contrary to the THL, MHL shows very weak positive/negative correlations with all predictor variables. Very weak correlations raise the necessity of comparing chromosome data by a molecular phylogenetic analyses. Asymmetry indices (CV_{CL} and M_{CA}) show weak positive correlations mainly caused by polyploidy with 2*n*/ploidy levels, that the correlation of M_{CA} is twice as high as CV_{CL}. Asymmetry incidences are relatively increasing with genome duplication.

In intrachromosomal asymmetry, the symmetrical karyotypes are dominant. The most asymmetrical karyotypes are *G. molle* subsp. *bruitium*, *G. pusillum*, and *G. sanguineum*, which is a polyploid taxon. In addition, *G. sanguineum* is the only taxon including subtelocentric chromosomes which may be due to the reciprocal translocations of the metacentric/submetacentric chromosomes. In interchromosomal asymmetry, CV_{CL} values show continuous variation. The most symmetric and asymmetric karyotypes are different between CV_{CL} and M_{CA} by very weak positive correlation (r = 0.014) (Figure 6). The most symmetrical karyotypes are *G. petri-davisii* (M_{CA} = 14.18) and *G. collinum* (CV_{CL} = 13.93), which have basic number of *x* = 14. The most asymmetrical karyotypes are *G. molle* subsp. *bruitium* (M_{CA} = 20.76) and *G. asphodeloides* (CV_{CL} = 30.38), which have basic number of *x* = 13.

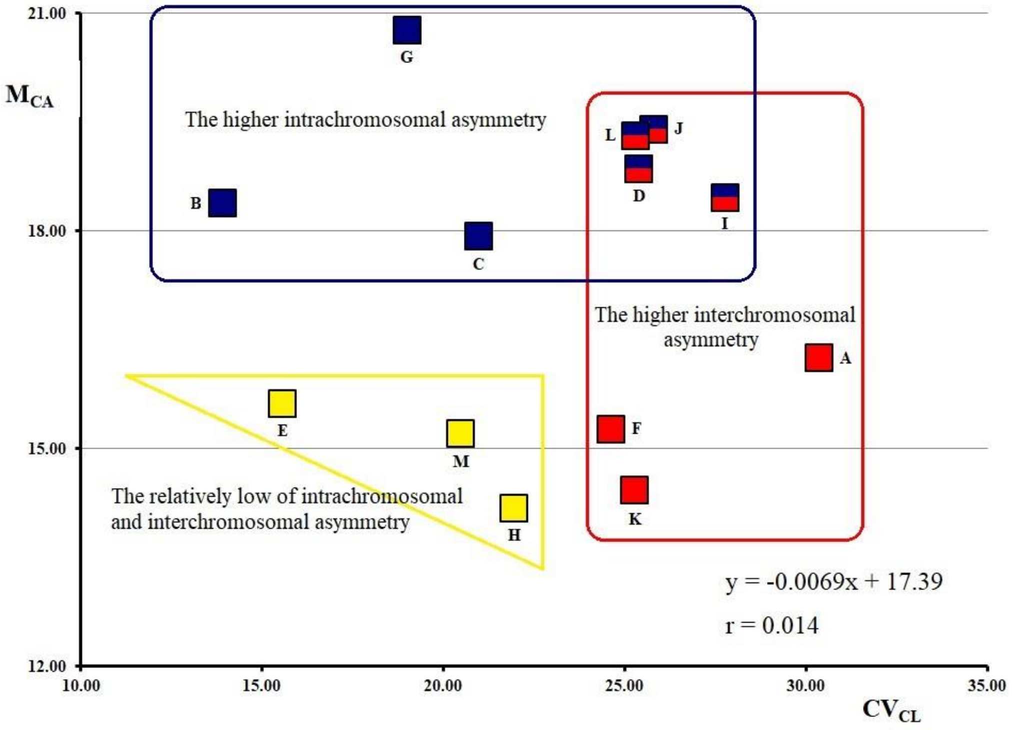

Figure 6. Scatter diagram between M_{CA} and CV_{CL}. (A) *G. asphodeloides*; (B) *G. collinum*; (C) *G. columbinum*; (D) *G. dissectum*; (E) *G. lucidum*; (F) *G. molle* subsp. *molle*; (G) *G. molle* subsp. *bruitium*; (H) *G. petri-davisii*; (I) *G. platypetalum*; (J) *G. pusillum;* (K) *G. rotundifolium*; (L) *G. sanguineum*; (M) *G. sylvaticum*.

Geranium taxa have different patterns in terms of asymmetry degrees: *G. lucidum*, *G. petri-davisii*, and *G. sylvaticum* possess relatively low intrachromosomal and interchromosomal asymmetry; seven taxa have a higher interchromosomal asymmetry; and seven taxa have a higher intrachromosomal asymmetry. *G. dissectum*, *G. platypetalum*, *G. pusillum*, and *G. sanguineum* show distribution both higher interchromosomal and higher intrachromosomal asymmetry. All taxa except *G. sanguineum* have symmetric karyotypes including metacentric or submetacentric chromosomes. It was recorded that the chromosome asymmetry increases in karyotype evolution [51]. The fact that Turkish *Geranium* taxa have symmetrical karyotypes may indicate that these taxa are at the first levels of karyotype evolution and Turkey is an important distribution center for genus *Geranium*.

Interspecific relationships

There is no detailed research recording karyotype evolution and karyological relationships among Turkish *Geranium* taxa. The studies consist of some karyotype analyses including basic and diploid chromosome numbers. *G. sanguineum* forms a monophyletic group by quite different karyotypic features such as different basic and diploid numbers, subtelocentric chromosomes, and high polyploidization (clade I). The other species form a medium monophyletic group (clade II). *G. rotundifolium* and *G. sylvaticum* shape the subclade 1 by some variations, which are different karyotype sample and relatively more intrachromosomal asymmetry. Subclade 2 is branched by low bootstrap values and contains nine taxa by strong karyological variations. First branched species are *G. lucidum* ($x = 10$, $2n = 20$) and *G. collinum* (lowest karyotype heterogeneity). The five species that have been separated so far are species of the sect. *Geranium* and sect. *Ruperta*. Then other sections are separated, which are sect. *Tuberosa*, sect. *Batrachioidea*, sect. *Dissecta*, and sect. *Subcaulia*. The only exception to the sect. *Geranium* is *G. columbinum* ($x = 9$, $2n = 18$).

CONCLUSION

The following is an overview of the data included in the present study: (i) first record of diploid chromosome numbers in nine taxa; (ii) new diploid counts different from previous records in five taxa; (iii) detailed chromosomal data in 13 taxa; (iv) first record of karyotype asymmetry by symmetric karyotypes; and (v) karyological variations as a result of polyploidy and especially dysploidy. Dysploidy and polyploidy variations may be the main factors in karyotype evolution of the genus as our results indicate to some degree.

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