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Karyotype evolution and new chromosomal data in *Erodium*: chromosome alteration, polyploidy, dysploidy, and symmetrical karyotypes

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Abstract: Chromosomal data are valuable and very useful for revealing evolution and speciation processes. Due to its wide distribution throughout the world, morphological differences, and chromosomal alterations, *Erodium* L'Hér. is an important genus for investigating the relationship between chromosomal alterations and karyotype evolution. In the present study, the chromosome records of 15 taxa are provided; three are reported here for the first time (*E. birandianum*, *E. gaillardotii*, and *E. hendrikii*), seven present new chromosome numbers, and five are in agreement with previous reports. Karyotype evolution is summarized in the light of this data, and four different genomes are presented in the genus. Millions of years ago the ancestral karyotype was $x = 9$ in Asia (Genome I). Then, karyotypes $x = 8$ (Genome II) and $x = 10$ (Genome III) were shaped through dysploidy in Anatolia and Asia. They were distributed in the Mediterranean Basin through the Anatolian land bridge and in North and South America via the Bering land bridge and the North Atlantic land bridge. Finally, a high proportion of polyploidization was observed in secondary centers, especially the Mediterranean Basin and Australia (Genome IV).

Key words: Anatolia, ancestral, *Erodium*, Geraniaceae, Mediterranean Basin

1. Introduction

Chromosomal data support the characteristics determining karyotype evolution and karyotypic relationships. The primary characteristics of karyotype are basic chromosome number (x), diploid chromosome number ($2n$), and chromosome length. These characteristics could be modified numerically through polyploidy and aneuploidy, as well as through structural rearrangements including translocations (which could modify the chromosome number through dysploidy), deletions, and inversions. All of these events generate intraspecific and interspecific variability of karyotypes, alter chromosome morphology, mainly by changing the centromere position, and affect all karyotype asymmetry indexes as mean centromeric asymmetry (M_{CA}) and coefficient of variation of chromosome length (CV_{CL}) (Schubert, 2007; Guerra, 2008; Schubert and Lysak, 2011; Guerra, 2012; Şirin et al., 2019). Mechanisms such as hybridization or polyploidization contribute significantly to changing chromosomal behavior (Winterfeld et al., 2018). Karyotypic variations and chromosomal differences produce sudden postzygotic-crossover barriers for speciation and diversification (Baltisberger and Hörandl, 2016). Some genera of the family Geraniaceae are excellent systems to use for determining speciation and diversification.

Geraniaceae is a cosmopolitan family with five genera: *California* Aldasoro & C.Navarro & P.Vargas & L.Sáez & Aedo, *Erodium* L'Hér., *Geranium* L., *Monsonia* L., and *Pelargonium* L'Hér. The genus *Erodium* is distinguished from the others by its five staminodes. Although *Erodium* has fewer species than *Geranium* and *Pelargonium*, it has undergone important shifts in speciation (Fiz-Palacios et al., 2010). Furthermore, members of the genus have high diversity, as they can be annual or perennial; autogamous, mixed-mating, or (usually) allogamous; and hermaphrodite or dioecious. *Erodium* is an important model for examining karyotype evolution due to the global distribution of species, different basic chromosome numbers, and various ploidy levels (Fiz et al., 2008). Anatolia is an important region for determining the karyotype evolution and geographic distribution of *Erodium* species.

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Erodium contains 74 annual and perennial species distributed on all continents except Antarctica. The Mediterranean Basin region is a major center of diversity of the genus and hosts 62 species, unlike other continents which have fewer native species (Figure 1): five in Australia, four in Asia, and one each in North and South America (Alarcón et al., 2003; Fiz et al., 2006; Fiz-Palacios et al., 2010; Coşkunçelebi et al., 2012). In terms of species diversity in *Erodium*, the Mediterranean Basin is divided into two subregions: the western Mediterranean, with section *Barbata* (Boiss.) Guitt., and the Eastern Mediterranean, with subsection *Absinthioidea* (Brumh.) Guitt. (Fiz-Palacios et al., 2010). Southwest Asia is one of the main centers of *Erodium* diversity, and Turkey has the greatest diversity, hosting 30 taxa including 16 endemics (Oskay, 2017).

The chromosomal data are reported from 68 species. Forty-six species are only diploid; however, they reveal three different basic numbers: $x = 8$ ($2n = 16$), $x = 9$ ($2n = 18$), and $x = 10$ ($2n = 20$) (Fiz et al., 2006). Seven species are polyploid and reveal three different polyploidy levels: tetraploidy ($2n = 4x = 36$ and 40), hexaploidy ($2n = 6x = 60$), and octoploidy ($2n = 8x = 80$) (Carolin, 1958; Guittonneau, 1965a, 1966, 1967; Kentzinger, 1974; Diaz et al., 1992; Fiz et al., 2006). *Erodium macrocalyx* (G.López) López Udias & Fabregat & Mateo shows a high ploidy level ($2n$ = ca. 160) (Fiz et al., 2006). Fifteen species are diploid and polyploid (Fiz et al., 2006). In addition, several

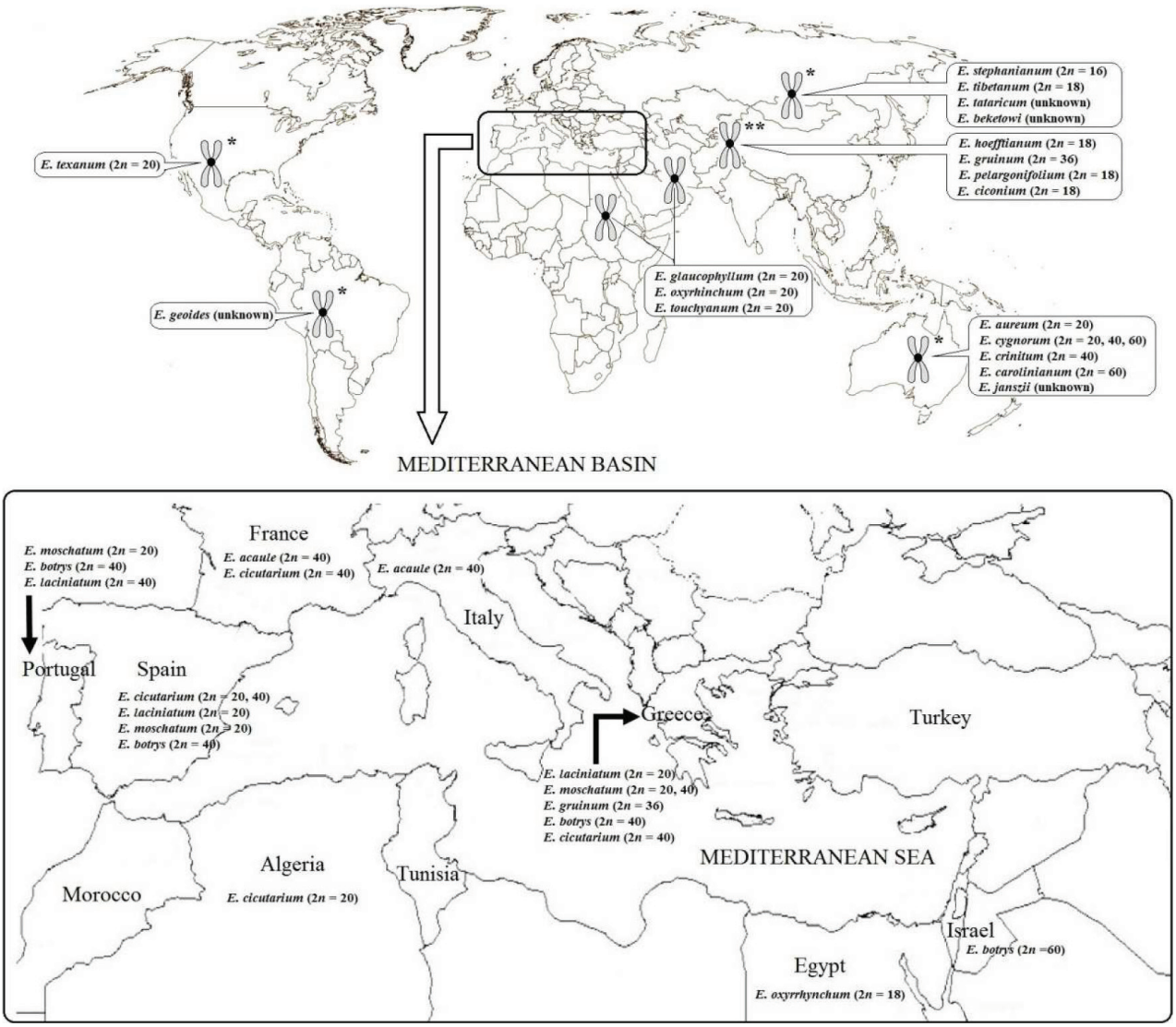

Figure 1. The general distribution map of the genus *Erodium* (single asterisk represents native species, and double asterisk represents species of ancestral region) with the Mediterranean Basin as a major center (including chromosome numbers reported from the Mediterranean Basin species in this study). For detailed references see Fiz et al. (2006).

species show dysploidy, which is an alteration in basic number, generally by fusion, without the significant loss or gain of genetic material (Rottgardt, 1956; Pajaron, 1982; Fiz et al., 2006). In genus *Erodium*, the chromosomal data are generally based on reports of chromosome number, karyotype analysis, and karyotype formula. Based on phylogenetic relationships and evolution, Fiz et al. (2006) examined 68 *Erodium* (66 species and two subspecies) and four outgroups in molecular investigations and chose 74 *Erodium* species and three genera from Geraniaceae for morphological analyses. They reported that the genera *Erodium* and *California* formed a monophyletic group, and the 68 *Erodium* taxa were sister with *California*. Moreover, the trnL analysis showed three clades in a polytomy, whereas the combined morphological and trnL-F analysis displayed *California* as sister to the 68 *Erodium* taxa. The results also indicated the three basic chromosome numbers with two main lineages (clade I: $x = 8, 9, 10$ and clade II: $x = 10$), polyploidy, and rare dysploidy (Fiz et al., 2006). Fiz-Palacios et al. (2010) examined 58 *Erodium* taxa and two outgroups [*California macrophylla* (Hook. & Arn.) Aldasoro & C.Navarro & P.Vargas & L.Sáez & Aedo and *Geranium biuncinatum* Kokwaro] by nrITS sequence combined with existing plastid data for biogeography and phylogenetic relationships. They pointed out that the phylogenetic relationships were similar to previous plastid reconstructions. Additionally, their biogeographical reconstructions pointed to Asia as the ancestral region of *Erodium*, where it appeared approximately 18 million years ago. The basic chromosome number is $x = 9$ in species of the ancestral region (Figure 1). Distribution to secondary centers and chromosomal changes occurred through polyploidy and dysploidy, which causes species of $x = 8$ with fusion. Polyploidy is a particularly important mechanism in the karyotype evolution of *Erodium*.

There are comprehensive chromosomal reports in *Erodium* in the chromosome databases. However, the lack of chromosomal reports from Turkey, which is located in the Mediterranean Basin, may lead to some uncertainties in the cytotaxonomy, karyotype evolution, and phylogeny of *Erodium*. Therefore, we intended to contribute to the karyotype evolution of 15 *Erodium* taxa. The current paper reports: (i) the chromosome numbers of some taxa for the first time; (ii) the karyotypic variations and new chromosome numbers that differ from previous reports; (iii) the detailed chromosome measurements and karyotype analyses of all taxa for the first time; (iv) the karyotype asymmetries through the latest parameters for the first time; and (v) investigates polyploidy, which plays an important role in the karyotype evolution of *Erodium*; (vi) compares the Mediterranean Basin and other regions in terms of chromosomal features; (vii) establishes a phylogenetic tree with combined data analysis to determine the interspecific relationships; and (viii) compares and discusses the hypothetical ancestral karyotype and new data.

2. Materials and methods

2.1. Plant material

Fifteen *Erodium* taxa were collected from their natural habitats across Turkey (Table 1). Exsicates were deposited at the herbarium of the Department of Biological Sciences at Middle East Technical University (METU) in Ankara, and live plants were kept in the Plant Systematics and Phylogenetics Research Laboratory at Uşak University.

2.2. Chromosome preparation

Chromosome spreads were prepared from roots pretreated with α-mono-bromonaphthalene at 4 °C for 16 h, fixed in fixative solution containing absolute alcohol:glacial acetic acid (3:1, v:v) for 24 h, and stored in ethanol (70%) at 4 °C until use. Then, the roots were hydrolyzed in 1 N HCl at 60 °C for 12 min and stained in 2% aceto-orcein. Finally, preparations were made by the squash method (Altay et al., 2017; Martin et al., 2019).

2.3. Karyotype analysis

Ten metaphase plates were analyzed with Software Image Analyses (Bs200ProP) loaded on a personal computer. The following parameters were calculated: long arm (LA), short arm (SA), total length (LA + SA), arm ratio (LA / SA), centromeric index [(SA) / (LA + SA) × 100], total haploid length (THL), relative length [(LA + SA) × 100 / THL], and mean haploid length. The karyotype formulas were determined based on centromere position (Levan et al., 1964). The monoploid ideograms were drawn by chromosome lengths. Some data obtained from Havva Bozkurt's master's thesis were used in the article (Bozkurt, 2018).

Karyotype asymmetry was calculated with two parameters: intrachromosomal asymmetry (M_{CA}) and interchromosomal asymmetry (CV_{CL}) (Paszko, 2006; Eroğlu et al., 2013; Peruzzi and Eroğlu, 2013). The asymmetry formulas are given below. M_{CA} = [mean (L − S) / (L + S)] × 100; L, total length of long arms; and S, total length of short arms. $CV_{CL} = (S_{CL} / X_{CL}) \times 100$; S_{CL}, standard deviation; and X_{CL}, mean chromosome length in a chromosome set.

2.4. Karyological relationships

The following seven parameters were used to determine the karyological relationships: basic chromosome number (x), diploid chromosome number ($2n$), ploidy level (PL), karyotype formula, total haploid length (THL), mean centromeric asymmetry (M_{CA}), and coefficient of variation of chromosome length (CV_{CL}). The phylogenetic trees showing karyological relationships were drawn using *Erodium* taxa and one *California* species as a sister outgroup by bootstrap values with UPGMA software.

Table 1. Localities and collector numbers of studied *Erodium*.

Taxa	Localities and voucher
E. acaule Bech. & Thell.	Hatay, Yayladağ, Yeditepe, 796 m, 31.5.2015, A. Kahraman 2078A
E. birandianum İlarslan & Yurdak.	Kastamonu, Devrekani, 1850 m, 12.7.2014, A. Kahraman 1859
E. botrys (Cav.) Bertol.	Çanakkale, Lapseki-Biga, 113 m, 14.4.2015, A. Kahraman 2012
E. cedrorum Schott subsp. *cedrorum*	Adana, Aladağ, 1750 m, 29.6.2014, A. Kahraman 1855
E. cicutarium (L.) L'Her. ex Aiton	Uşak, Banaz- Afyon, 1368 m, 20.5.2014, A. Kahraman 1804
E. gaillardotii Boiss.	Malatya, Darende-Gürün, 1465 m, 3.6.2015, A. Kahraman 2085
E. gruinum (L.) L'Hér. -	Antalya, Akseki- Manavgat, 695 m, 13.5.2014, A. Kahraman 1786
E. hendrikii Alpınar	Gümüşhane, Yağmurdere, 1691 m, 5.8.2015, A. Kahraman 2177
E. hoefftianum C.A.Mey.	Burdur, Çavdır-Acıpayam, 1030 m, 4.5.2015, A. Kahraman 2059
E. laciniatum (Cav.) Willd.	Adana, Tuzla, sea level, 26.4.2016, A. Kahraman 2326
E. leucanthum Boiss.	Denizli, Babadağ, 1437 m, 24.5.2014, A. Kahraman 1815
E. moschatum L'Hér. ex Aiton	Manisa, Sard- Ödemiş, 230 m, 6.4.2014, A. Kahraman 1702
E. oxyrrhynchum Bieb.	Erzurum, Uzundere-Yusufeli, 1006 m, 25.6.2015, A. Kahraman 2114
E. pelargoniflorum Boiss. & Heldr.	Karaman, Ermenek, 1680 m, 26.6.2014, A. Kahraman 1852
E. somanum Peşmen	Manisa, Soma, 879 m, 18.5.2014, A. Kahraman 1798

The first phylogenetic tree contains the studied species, and their chromosomal data, from the Mediterranean Basin. The second phylogenetic tree contains the listed species: (i) all studied species; (ii) native species (three Australian, two Asian, and one North American); (iii) the species of the ancestral region; and (iv) the species of the Mediterranean Basin, Northeast Africa, and Asia in Figure 1. Then, using MS-Excel software, the variables were compared by Pearson correlation (Pcor) using the following values: weak correlation (Pcor ≤ 0.25), average correlation (0.25 < Pcor ≤ 0.50), good correlation (0.50 < Pcor ≤ 0.75), and high correlation (0.75 < Pcor) (P < 0.01).

3. Results

3.1. Chromosomal data

Chromosome records of 15 taxa are provided (Figure 2); three are reported here for the first time (*E. birandianum*, *E. gaillardotii*, and *E. hendrikii*), seven present new chromosome numbers, and five agree with previous reports. Table 2 shows chromosome counts from the current and previous studies. Four different chromosome numbers ($2n$ = 18, 20, 30, and 36) were detected. The smallest chromosome size among the taxa is 0.60 µm, in *E. laciniatum*. The largest chromosome size was detected in *E. gaillardotii* (3.26 µm). The smallest value of total haploid length is 12.90 µm, in *E. oxyrrhynchum*, and the highest value is 36.03 µm, in *E. gruinum* (Table 3).

3.2. Basic numbers and ploidy levels

In the genus *Erodium* there are two basic chromosome numbers: x = 9 in four species and (most commonly) x = 10 in the other species, with ploidy levels of 2x in 13 species, 3x in *E. cicutarium*, and 4x in *E. gruinum* (Table 2). The monoploid ideograms are generated by x = 9 and 10 (Figure 3).

3.3. Karyotype formula and karyotype asymmetry

Fourteen taxa have median (m) and submedian (sm) chromosomes and only one taxon has subtelocentric (st) chromosomes; there were no telocentric (t) chromosomes. Five different karyotype samples were observed: M-m, M-m-sm, m, m-sm, and m-sm-st. In intrachromosomal asymmetry, M_{CA} varies from 16.21 (*E. birandianum*) to 22.80 (*E. gruinum*) in x = 9 and from 11.09 (*E. leucanthum*) to 22.48 (*E. gaillardotii*) in x = 10, which refer to symmetrical karyotypes. In interchromosomal asymmetry, CV_{CL} varies from 17.43 (*E. birandianum*) to 31.41 (*E. laciniatum*) in x = 9 and from 15.39 (*E. cicutarium*) to 24.65 (*E. leucanthum*) in x = 10 (Table 3).

3.4. Interspecific relationships

Figure 4 presents a phylogenetic tree containing the studied species from the Mediterranean Basin and their chromosomal data. Seven taxa have variable ploidy levels (2x, 2x/4x, 4x, and 4x/6x) and shape clade I. The diploid taxa are quite dominant in clade II. In general, clade I represents the species of the Mediterranean Basin, except Turkey, and clade II represents the Anatolian species from the Eastern Mediterranean Basin or Eurasia.

Figure 5 demonstrates a phylogenetic tree that includes species from all continents. Firstly, it is devoted to *California macrophylla* (outgroup) and *E. stephanianum* Willd. with x = 8. Secondly, the phylogenetic tree is divided into two

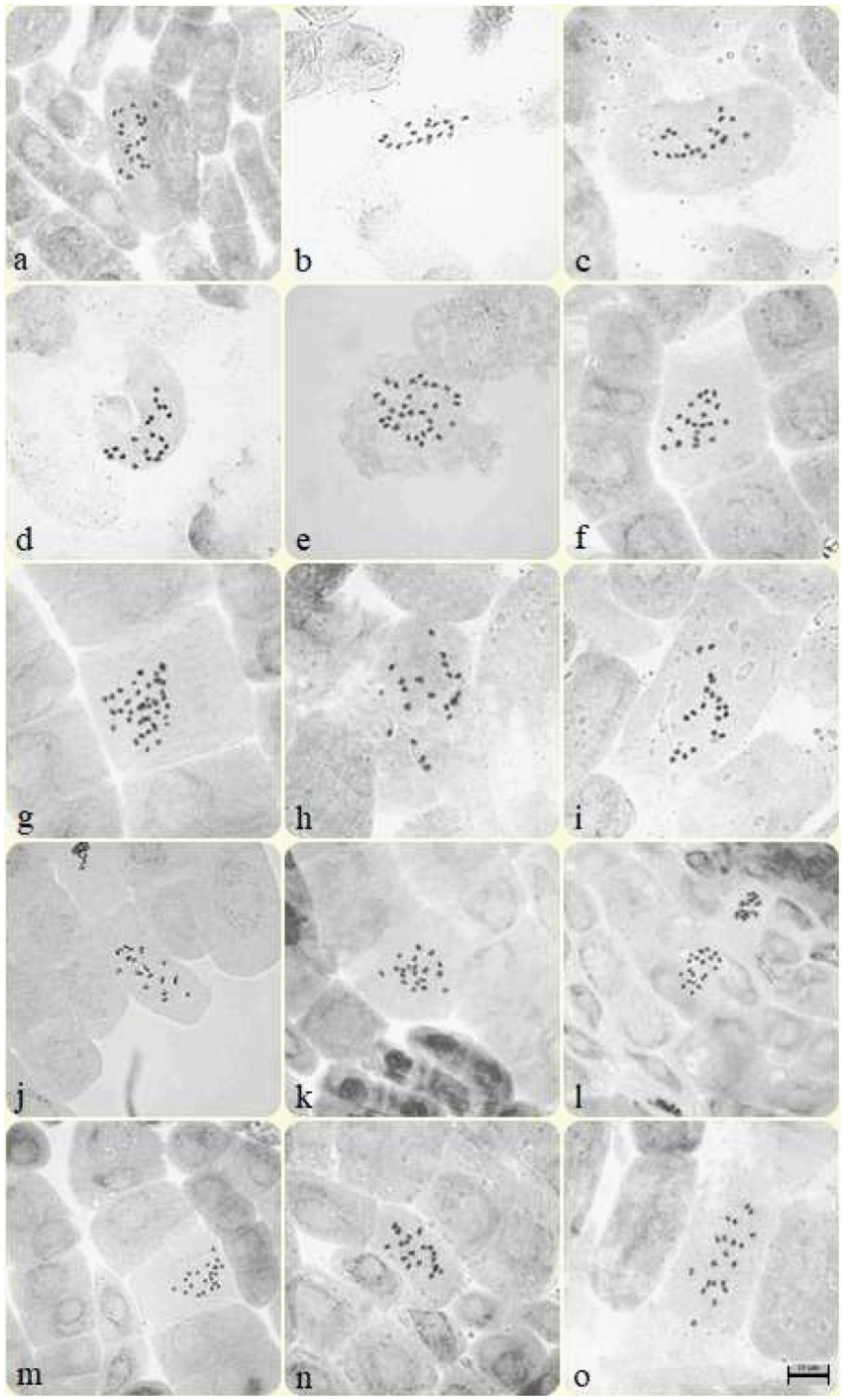

Figure 2. Mitotic metaphase chromosomes of genus *Erodium*: **a** *E. acaule*, **b** *E. birandianum*, **c** *E. botrys*, **d** *E. cedrorum* subsp. *cedrorum*, **e** *E. cicutarium*, **f** *E. gaillardotii*, **g** *E. gruinum*, **h** *E. hendrikii*, **i** *E. hoefftianum*, **j** *E. laciniatum*, **k** *E. leucanthum*, **l** *E. moschatum*, **m** *E. oxyrrhynchum*, **n** *E. pelargoniflorum*, **o** *E. somanum* (scale bar: 10 μm).

main clades: clade I includes the species of other regions, and clade II includes Eurasian species. Finally, subclades are shaped as follows: subclade 1 includes the species of the Central/Western Mediterranean Basin and Australia; subclade 2 includes the species of Central/Eastern Asia, the Middle East, North America, and Australia (one species); subclade 3 includes the species of Eurasia ($2n$ = 20, 30, and 36); and subclade 4 includes the species of Eurasia ($2n$ = 18). While polyploidy is common in subclade 1, diploidy is common in other subclades.

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

Taxa (alphabetically)	Previous results (2*n*) *x*: basic number ploidy in brackets	Present Results (2*n*) *x*: basic number ploidy in brackets	Explanation
E. acaule	*x* = 10 40 (tetraploid) [a]	*x* = 10 20 (diploid)	New count
E. birandianum		*x* = 9 18 (diploid)	First report
E. botrys	*x* = 10 40 (tetraploid) [a] 60 (hexaploid) [b]	*x* = 10 20 (diploid)	New count
E. cedrorum subsp. *cedrorum*	*x* = 10 20 (diploid) [c]	*x* = 10 20 (diploid)	Detailed measurement
E. cicutarium	*x* = 7, 8, 9, 10 20 (diploid) [d] 36, 40 (tetraploid) [d,e,f] 38,42 (probably dysploidy) [g] 48, 54, 60 (hexaploid) [d,h] 56, 80 (octoploid) [d,i]	*x* = 10 30 (triploid)	New count
E. gaillardotii		*x* = 10 20 (diploid)	First report
E. gruinum	*x* = 9, 10 36, 40 (tetraploid) [h,j,k]	*x* = 9 36 (tetraploid)	Detailed measurement
E. hendrikii		*x* = 10 20 (diploid)	First report
E. hoefftianum	*x* = 9 18 (diploid) [k]	*x* = 10 20 (diploid)	New count
E. laciniatum	*x* = 10 20 (diploid) [a,j] 40 (tetraploid) [l]	*x* = 9 18 (diploid)	New count
E. leucanthum	*x* = 9 36 (tetraploid) [c]	*x* = 10 20 (diploid)	New count
E. moschatum	*x* = 10 20 (diploid) [e,f,j] 40 (tetraploid) [j]	*x* = 10 20 (diploid)	Detailed measurement
E. oxyrrhynchum	*x* = 9, 10 18 (diploid) [m] 20 (diploid) [f]	*x* = 10 20 (diploid)	Detailed measurement
E. pelargoniflorum	*x* = 9, 10 18 (diploid) [k] 20 (diploid) [c]	*x* = 9 18 (diploid)	Detailed measurement
E. somanum	x = 9 18 (diploid) [n]	*x* = 10 20 (diploid)	New count

[a] Guittonneau, 1966; [b] Diaz et al., 1992; [c] Kentzinger, 1974; [d] Rottgardt, 1956; [e] Guittonneau, 1965a; [f] Keshavarzi et al., 2015; [g] Pajaron, 1982; [h] Larsen, 1958; [i] Guittonneau, 1965b; [j] Dahlgren, 1980; [k] Fiz et al., 2006; [l] Alves and Leitao, 1976; [m] Badr and Hammund, 1985; [n] Oskay et al., 2011.

Table 3. The karyological features of the studied *Erodium* taxa.

Taxa	KF	SC (μm)	LC (μm)	THL (μm)	CI (min–max)	CV_{CL}	M_{CA}
E. acaule	16m + 4sm	1.26	2.66	20.45	34.31–47.66	20.61	19.32
E. birandianum	2M + 10m + 6sm	1.28	2.30	15.75	33.91–50.00	17.43	16.21
E. botrys	16m + 4sm	1.32	3.02	20.85	35.79–47.58	22.70	20.01
E. cedrorum subsp. *cedrorum*	16m + 4sm	1.25	2.20	16.71	34.40–49.42	17.83	17.02
E. cicutarium	30m	0.99	1.72	19.94	38.64–48.67	15.39	12.95
E. gaillardotii	10m + 8sm + 2st	1.50	3.26	21.74	24.71–47.37	24.57	22.48
E. gruinum	20m + 16sm	1.22	2.78	36.03	28.16–48.02	22.30	22.80
E. hendrikii	16m + 4sm	1.25	2.39	17.61	34.40–45.19	18.17	18.78
E. hoefftianum	14m + 6sm	1.35	2.55	18.66	34.45–48.00	21.30	18.02
E. laciniatum	16m + 2sm	0.60	1.99	12.22	35.00–45.53	31.41	19.13
E. leucanthum	2M + 18m	1.23	2.95	19.36	39.15–50.00	24.65	11.09
E. moschatum	18m + 2sm	0.93	1.75	13.31	36.50–44.80	18.46	17.98
E. oxyrrhynchum	18m + 2sm	0.92	1.62	12.90	34.57–47.24	16.14	16.25
E. pelargoniflorum	2M + 12m + 4sm	1.04	2.21	14.25	34.08–50.00	22.04	20.60
E. somanum	2M + 18m	1.06	2.22	15.60	37.30–50.00	23.29	13.19

Abbreviations: karyotype formula (KF), shortest chromosome length (SC), longest chromosome length (LC), relative length (RL), total haploid chromosome 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 region (m), submedian region (sm), subtelocentric region (st).

4. Discussion

4.1. Chromosome number

The chromosome numbers of three species are reported here for the first time: *E. birandianum* (2*n* = 18), *E. gaillardotii*, and *E. hendrikii* (2*n* = 20). The following chromosome numbers represent new cytotypes for seven species: *E. laciniatum* (2*n* = 18), *E. acaule*, *E. botrys*, *E. hoefftianum*, *E. leucanthum*, *E. somanum* (2*n* = 20), and *E. cicutarium* (2*n* = 30). The chromosome numbers reported in the literature are 2*n* = 20, 40 for *E. laciniatum*; 2*n* = 40 for *E. acaule*; 2*n* = 40, 60 for *E. botrys*; 2*n* = 18 for *E. hoefftianum* and *E. somanum*; 2*n* = 36 for *E. leucanthum*; and 2*n* = 20, 36, 38, 40, 42, 48, 54, 60, 80 for *E. cicutarium*. The chromosome numbers of the other five taxa are the same as in previous reports: *E. pelargoniflorum* (2*n* = 18), *E. cedrorum*, *E. moschatum*, *E. oxyrrhynchum* (2*n* = 20), and *E. gruinum* (2*n* = 36). Although the chromosome number of *E. cedrorum* is same as in a previous report, the studied subspecies are different (for detailed references see Table 2).

4.2. Basic number and ploidy levels

In *Erodium*, according to the chromosome databases, there are many diploid and several polyploid reports that include three basic chromosome numbers (*x* = 8, 9, 10). In the present study, *E. cicutarium* and *E. gruinum* are the polyploid species, with 2*n* = 3*x* = 30 and 2*n* = 4*x* = 36, respectively. In addition, the polyploidy rate is quite high in the Mediterranean Basin and among native Australian species (Figure 1).

4.3. Basic number alterations; dysploidy

Molecular phylogenetic data showed that Asia was the ancestral region of the first *Erodium*, which arose 18 million years ago (Fiz-Palacios et al., 2010). The basic chromosome number is *x* = 9 (Genome I, Figure 6) in species of the ancestral region: *E. hoefftianum*, *E. gruinum*, *E. pelargoniflorum*, and *E. ciconium* (L.) L'Hér. (Figure 1). The distribution to secondary centers (Figure 5) and chromosomal changes occurred by fusion that caused species of *x* = 8. The change from *x* = 9 to *x* = 8 as a result of dysploidy probably occurred in a lineage that left the region early, millions of years ago. The dysploidy is likely to have occurred as a result of reciprocal translocations or the fusion of median chromosomes in ancestral karyotypes (Figure 7). The *x* = 8 karyotype (Genome II, Figure 6) shows very low diversity, with only one species (*E. stephanianum*) worldwide. This species, which has spread in Eastern Asia, is diploid with 2*n* = 2*x* = 16 (Mesicek and Sojak, 1972). Since there is no detailed chromosomal data

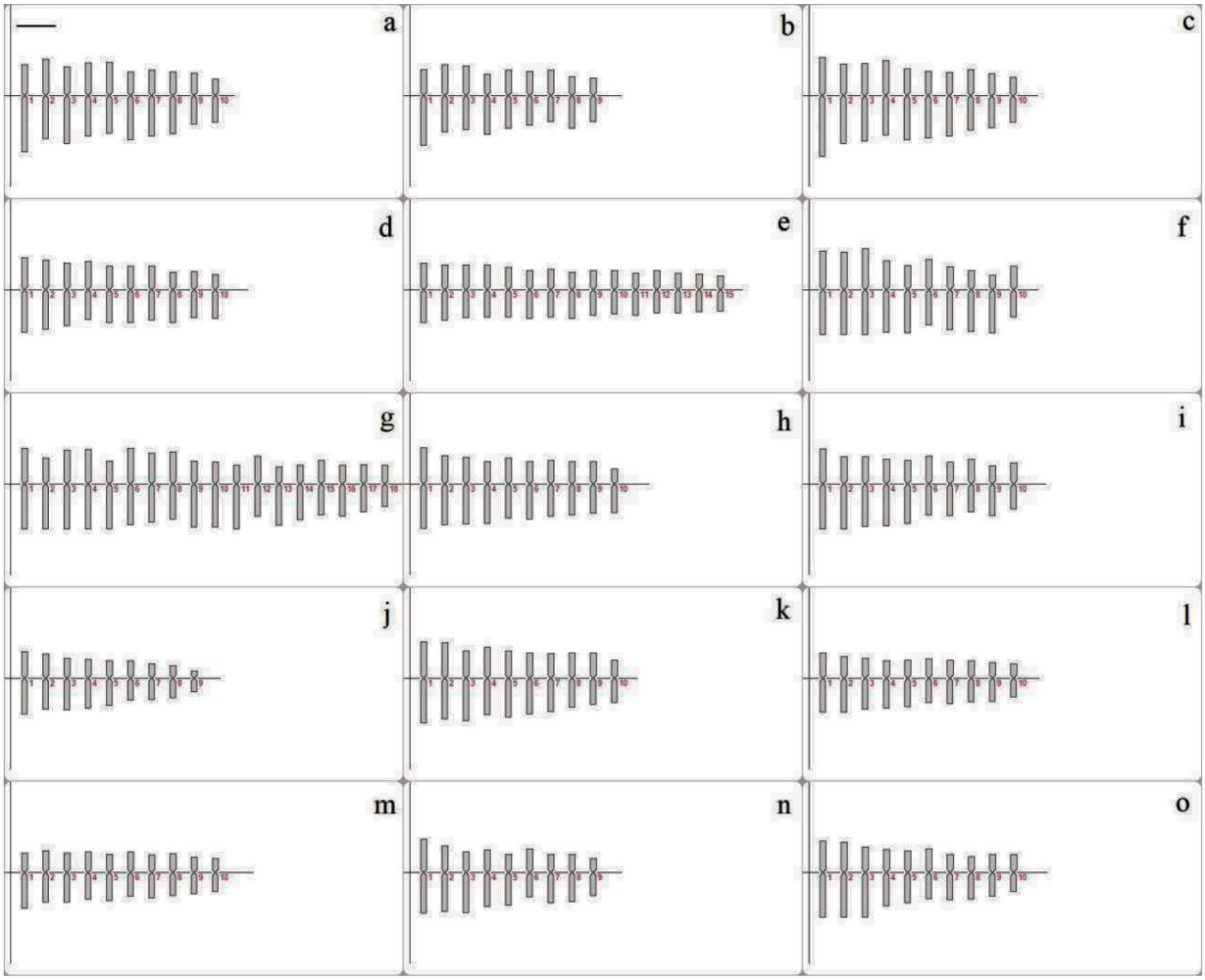

Figure 3. Ideograms of genus *Erodium*: **a** *E. acaule*, **b** *E. birandianum*, **c** *E. botrys*, **d** *E. cedrorum* subsp. *cedrorum*, **e** *E. cicutarium*, **f** *E. gaillardotii*, **g** *E. gruinum*, **h** *E. hendrikii*, **i** *E. hoefftianum*, **j** *E. laciniatum*, **k** *E. leucanthum*, **l** *E. moschatum*, **m** *E. oxyrrhynchum*, **n** *E. pelargoniflorum*, **o** *E. somanum* (scale bar: 1 μm).

on this species, the explanation of karyotype evolution is not clear.

Dysploidy also played an important role in chromosome gain. Figure 5 presents two species, *E. tibetanum* Edgew. & Hook.f. from East Asia and *E. oxyrrhynchum* from West Asia; these have the same karyotypic features as the ancestral karyotypes (*E. hoefftianum* and *E. ciconium*). The first chromosome gain probably occurred in *E. oxyrrhynchum*. In this species, while the basic chromosome number is $x = 9$ in the ancestral region, it is $x = 10$ (Genome III, Figure 6) in Anatolia (Eurasia). The other species of $x = 10$, *E. moschatum*, *E. cedrorum*, *E. hendrikii*, *E. botrys*, and *E. acaule*, mainly formed in Eurasia (Figure 4). Unlike $x = 8$ karyotype, $x = 10$ karyotype shows high diversity. Almost all species in the Eurasian region are characterized by diploid karyotypes (clade II, Figure 5). Only two species in Eurasia have a polyploid karyotype, *E. cicutarium* with $2n = 3x = 30$ and *E. gruinum* with $2n = 4x = 36$ (Figure 4). We think that Eurasia played the role of an Anatolian land bridge that facilitated the distribution of these species into the Mediterranean Basin and then on to other continents. In addition, Anatolia is an important region in terms of plant phytogeography, because it is an intersection of three phytogeographical regions: the Euro-Siberian, Irano-Turanian, and Mediterranean.

4.4. Karyotype evolution; polyploidy

Polyploidy is one of the most important mechanisms in the karyotype evolution of *Erodium*. The first polyploidy mechanism probably occurred in *E. gruinum* from ancestral karyotypes, and this taxon could have been $2n = 18$ millions of years ago. In Figures 4 and 5, the results point to two types of polyploidy. The first is autopolyploidy with tetraploid *E. gruinum* containing fourfold Genome I and tetraploid species such as *E. botrys* containing fourfold

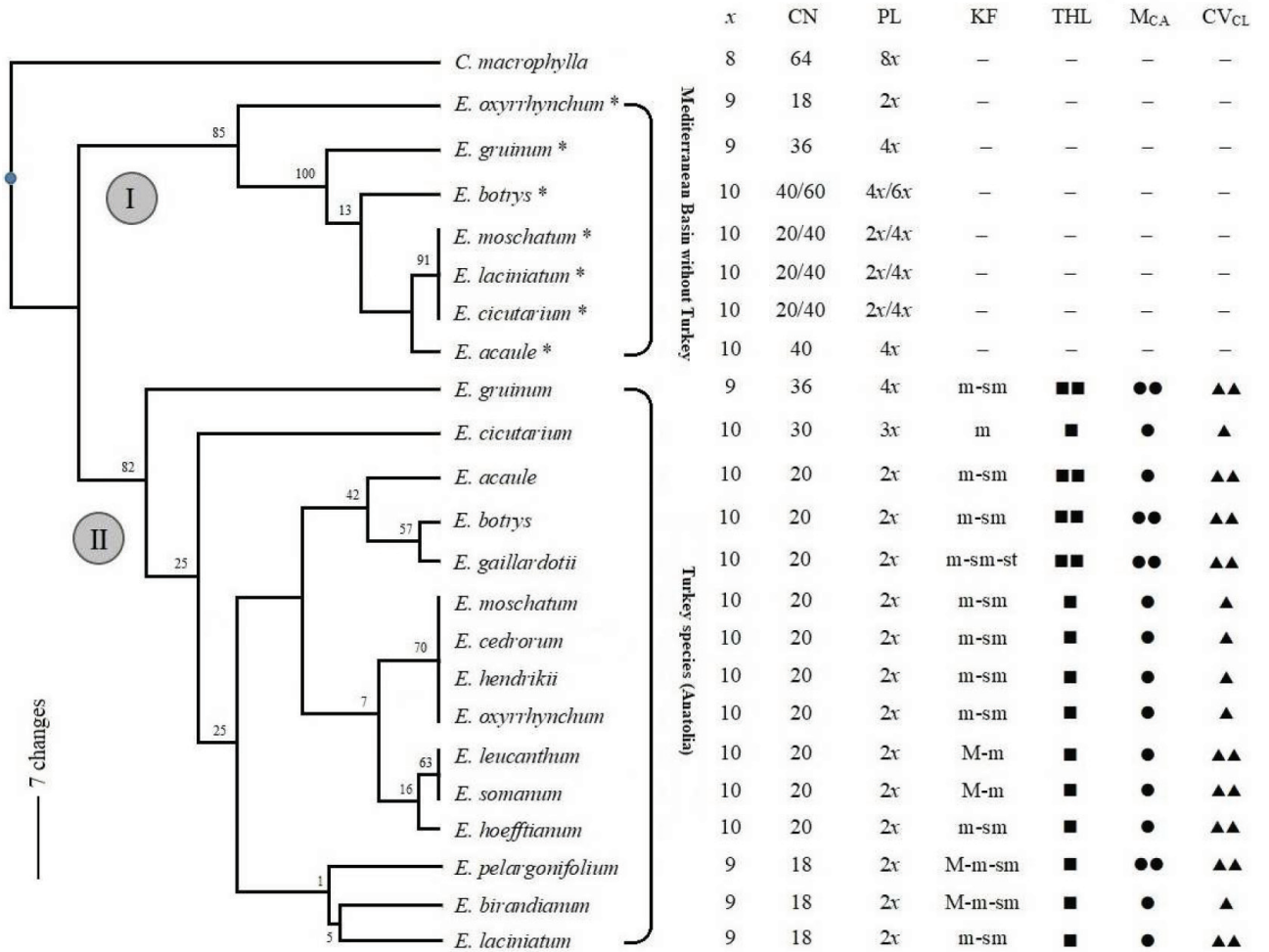

Figure 4. Phylogenetic tree containing the comparative phylogeny of the studied species and their chromosomal data in the Mediterranean Basin (marked with an asterisk). Numbers above branches indicate bootstrap values. Main clads are shown in circle. *x*: basic chromosome number, CN: chromosome number, PL: ploidy level, KF: karyotype formula, THL: total haploid length, M_{CA}: mean centromeric asymmetry, CV_{CL}: coefficient of variation of chromosome length. $0 < THL \leq 20.00$ (▪), $20.00 < THL \leq 40.00$ (▪▪), $0 < M_{CA} \leq 20.00$ (●), $20.00 < M_{CA} \leq 40.00$ (●●), $0 < CV_{CL} \leq 20.00$ (▲), $20.00 < CV_{CL} \leq 40.00$ (▲▲).

Genome III. The tetraploid *E. gruinum* is represented by $x = 9$ in Anatolia and $x = 9, 10$ in the Mediterranean Basin; *E. botrys* is represented by a diploid karyotype in Anatolia and a polyploid karyotype in the Mediterranean Basin. These data may provide evidence for the autopolyploidy view and should be supported by molecular data such as GISH and a phylogeny based on sequences from two genomes (nuclear and plastid). The second type of polyploidy is allopolyploidy (Genome IV, Figure 6) which probably occurred between diploid *E. moschatum* and diploid *E. cicutarium*, *E. laciniatum*, or *E. acaule*. These taxa are represented by a diploid karyotype in Anatolia and diploid or polyploid karyotypes in the Mediterranean Basin. In addition, *E. moschatum* and *E. cicutarium* are morphologically and cytotaxonomically close species, and their geographic distribution is similar (Alarcón et al., 2003). These views, which must be supported by molecular data, are consistent with previous phylogenetic reports (Fiz et al., 2006; Fiz-Palacios et al., 2010).

Four taxa spread to all continents with habitats similar to the Mediterranean phytogeographical region. These taxa, *E. botrys* ($2n = 40, 60$), *E. brachycarpum* Thell. ($2n = 40$), *E. cicutarium* ($2n = 40, 60$), and *E. moschatum* ($2n = 40$) (Table 2) show high polyploidy rates. In addition, polyploidy is quite common in the species of the Mediterranean Basin and Australia, unlike Turkish species which have diploid karyotypes (Figures 4 and 5). This karyotype distribution also supports the idea of an Anatolian land bridge. The species later moved into North and South America via the Bering land bridge and the North Atlantic land bridge (Milne, 2006; Fiz-Palacios et al., 2010).

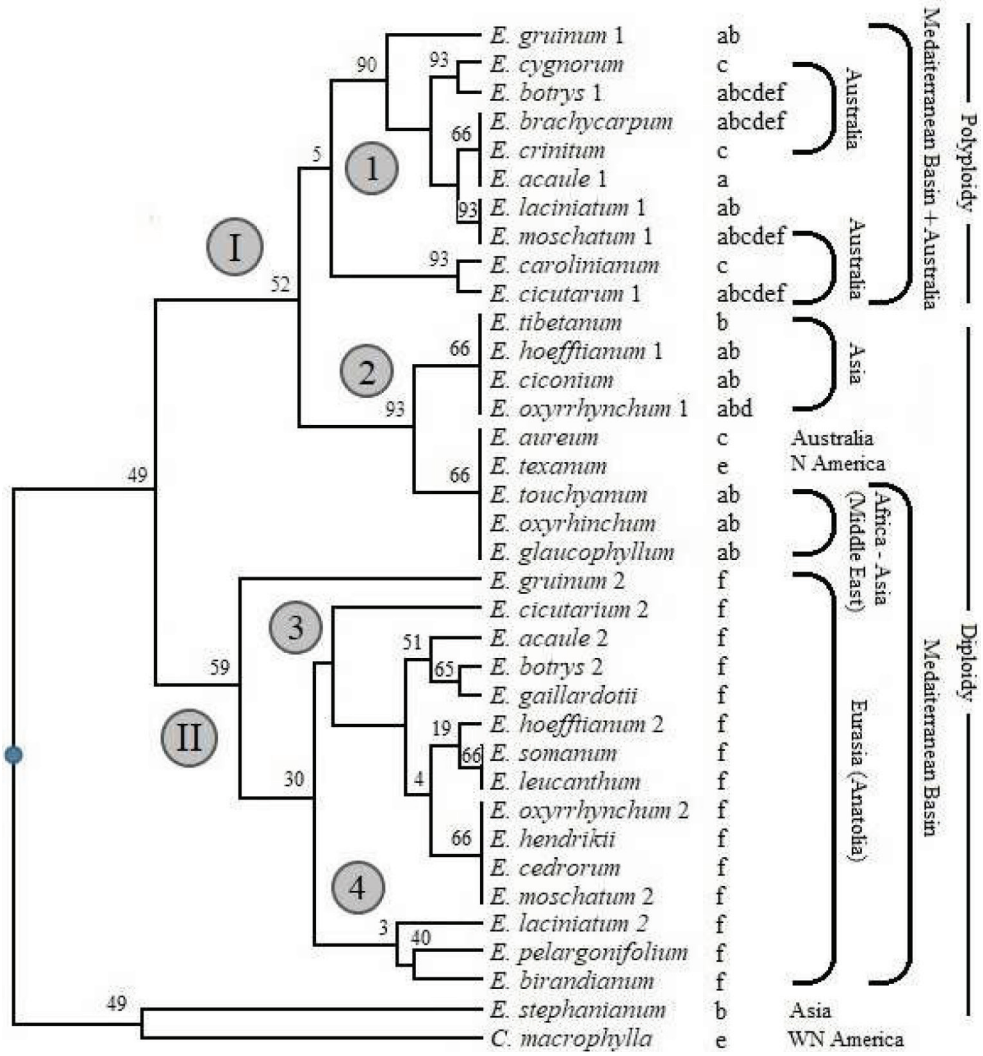

Figure 5. Phylogenetic tree including the comparative phylogeny of all studied species, native species (three Australian species, two Asian species, and one North American species); the species of the ancestral region; and some species of the Mediterranean Basin, Northeast Africa, and Asia. Numbers above branches indicate bootstrap values. Main clads and subclades are shown in circle. a: Africa, b: Asia, c: Australia, d: Mediterranean Basin, e: America, f: Eurasia (Anatolia).

4.5. Karyotype asymmetry

In intrachromosomal asymmetry, the karyotypes are mostly symmetric. The most asymmetrical karyotypes are the karyotypes of *E. gruinum* and *E. gaillardotii*. Although *E. gruinum* is a polyploid taxon with an asymmetric karyotype, the other polyploid taxon (*E. cicutarium*) has a symmetric karyotype. Table 4 presents a weak positive correlation between ploidy levels and M_{CA} (r = 0.080). In addition, the M_{CA} value correlates weakly with all parameters except THL, which has an average correlation with the M_{CA} value. *Erodium gaillardotii* is the only species with subtelocentric chromosomes which may be due to the reciprocal translocations of the median/submedian chromosomes.

In interchromosomal asymmetry, the karyotypes are mostly symmetric. The most asymmetrical karyotypes are the karyotypes of *E. laciniatum*, *E. leucanthum*, and *E. gaillardotii*. In addition, they are diploid with $x = 9$ and $x = 10$. The polyploid taxa have a symmetric karyotype. Table 4 presents a weak negative correlation between ploidy levels and CV_{CL} (r = –0.178). In addition, the CV_{CL} value correlates weakly with all parameters except KF, which has an average correlation with CV_{CL}.

The most symmetrical and asymmetrical karyotypes are different between M_{CA} and CV_{CL} with weak positive correlation (r = 0.246) (Figure 8). All taxa except *E. gaillardotii* have symmetrical karyotypes with median or submedian chromosomes. Baltisberger and Hörandl

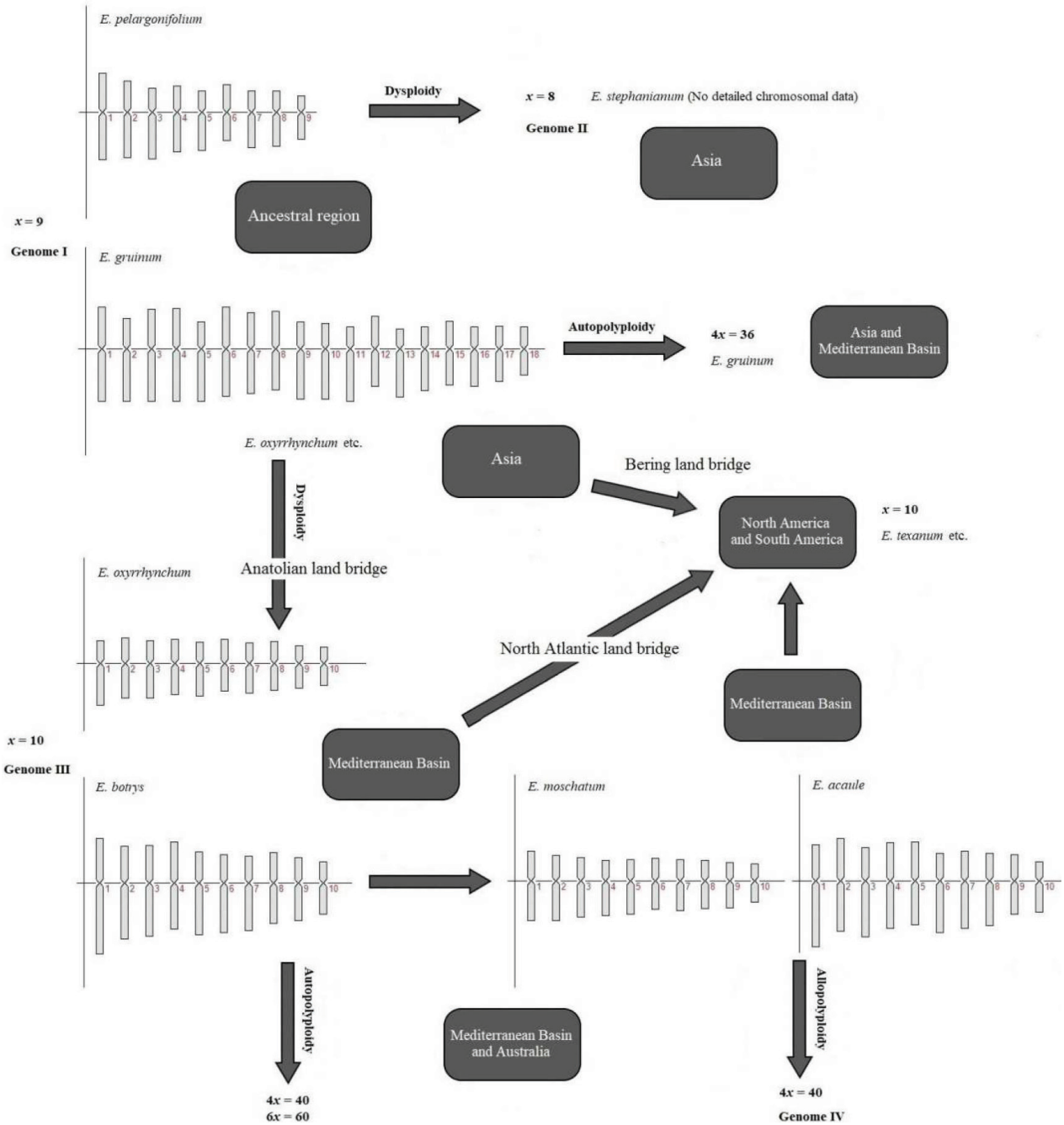

Figure 6. The karyotype evolution and karyotype distributions of *Erodium* from the ancestral region to other centers via the Anatolian land bridge, Bering land bridge, and North Atlantic land bridge and the genome shaping with dysploidy and polyploidy mechanisms.

(2016) reported that karyotype evolution drives increasing asymmetry of chromosomes. The fact that Anatolian *Erodium* species have symmetrical karyotypes may indicate that these species are at the first levels of karyotype evolution. These data support our idea of an Anatolian land bridge. In the Central and Western Mediterranean Basin and Australia the polyploidy ratio, as well as the asymmetric karyotype ratio, is probably higher. However, all of these considerations can be tested for *Erodium* when a phylogenetic hypothesis based on DNA sequences is taken and compared to chromosome data and biogeography.

4.6. Overview

The following is an overview of the data included in this study: (i) in three species, the first report of chromosome

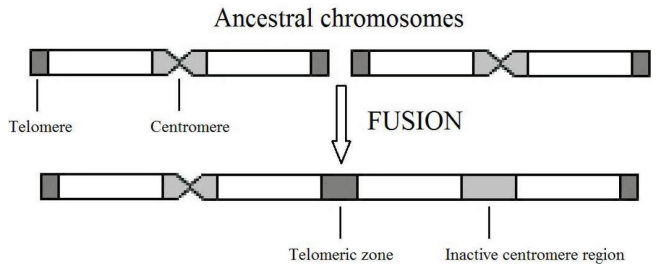

Figure 7. Dysploidy due to fusion of median chromosomes in ancestral karyotypes. First, the fusion reaction occurs in the telomere regions of the ancestral chromosomes. Then a subtelocentric chromosome comprising the telomeric zone and the inactive centromere region arises.

Table 4. The Pearson correlations of variables.

	x	CN	PL	KF	THL	M_{CA}	CV_{CL}
x	1						
CN	–0.147	1					
PL	–0.334	0.981*	1				
KF	–0.492	–0.456	–0.327	1			
THL	0.470	–0.088	–0.177	–0.250	1		
M_{CA}	0.132	0.113	0.080	–0.213	0.381	1	
CV_{CL}	0.167	–0.152	–0.178	0.424	0.045	0.246	1

* Correlation is significant at the 0.01 level. Abbreviations: basic chromosome number (*x*), chromosome number (CN), ploidy level (PL), karyotype formula (KF), total haploid length (THL), mean centromeric asymmetry (M_{CA}), coefficient of variation of chromosome length (CV_{CL}).

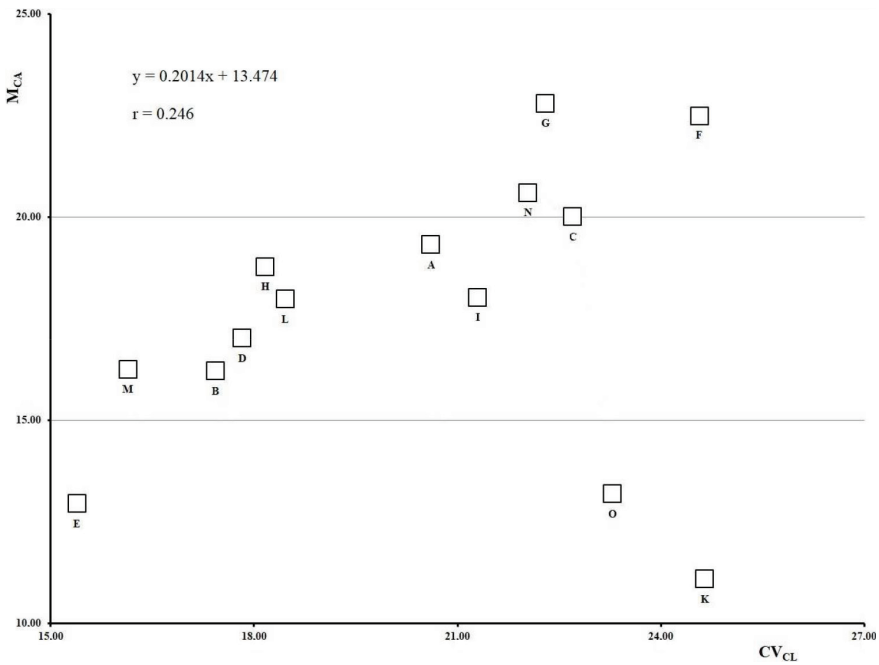

Figure 8. Scatter diagram between M_{CA} and CV_{CL}: **a** *E. acaule*, **b** *E. birandianum*, **c** *E. botrys*, **d** *E. cedrorum* subsp. *cedrorum*, **e** *E. cicutarium*, **f** *E. gaillardotii*, **g** *E. gruinum*, **h** *E. hendrikii*, **i** *E. hoefftianum*, **j** *E. laciniatum*, **k** *E. leucanthum*, **l** *E. moschatum*, **m** *E. oxyrrhynchum*, **n** *E. pelargoniflorum*, **o** *E. somanum*.

numbers; (ii) in seven species, new chromosome counts that differ from previous reports; (iii) in all taxa, the first report of detailed karyotype analyses; (iv) the first report of karyotype asymmetry and generally symmetrical karyotypes; (v) karyotypic variations as a result of dysploidy and polyploidy; and (vi) the karyotype evolution of genus *Erodium*. In Figure 6, the karyotype evolution is summarized. In Asia millions of years ago the basic number of the ancestral karyotype was $x = 9$. Then, karyotypes of $x = 8$ and $x = 10$ were shaped through dysploidy in Anatolia and Asia. Dysploidy likely occurred as a result of reciprocal translocations or fusion of median chromosomes in ancestral karyotypes. Then, they were distributed into the Mediterranean Basin via the Anatolian land bridge and into North and South America via the Bering land bridge and the North Atlantic land bridge. Finally, a high proportion of polyploidization was observed in the secondary centers, especially in the Mediterranean Basin and Australia.

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