Karyotypic phylogeny and polyploidy variations of *Paronychia* (Caryophyllaceae) taxa in Turkey

Halil Erhan EROĞLU[1,*] , **Derya ALTAY**[2] , **Ümit BUDAK**[1] , **Esra MARTİN**[3]

[1]Department of Biology, Faculty of Science and Art, Yozgat Bozok University, Yozgat, Turkey
[2]Department of Complementary and Integrative Medicine, Public Health Institute, Yıldırım Beyazıt University, Ankara, Turkey
[3]Department of Biotechnology, Faculty of Science, Necmettin Erbakan University, Konya, Turkey

Abstract: Chromosomal data can provide very valuable information about karyotypic phylogeny and speciation. This is the first study on karyotype phylogeny and polyploidy variations of the genus *Paronychia*. In this context, the results are these: (1) in 14 taxa, the first report on chromosomes numbers; (2) in 2 taxa, equal chromosome numbers as in the previous report; (3) in all taxa, the first report of detailed karyotype analyses; (4) karyotype asymmetry data and generally symmetrical karyotypes; (5) karyotypic variations by mechanisms of dysploidy and polyploidy; and (6) phylogenetic relationships in *Paronychia*. The data indicate that Anatolia is an important area for the distribution of *Paronychia*. In light of all data, karyotype evolution is briefly summarized. The ancestral karyotype was $x = 9$ (millions of years ago). The karyotypes ($x = 8$ and $x = 7$) were then shaped by dysploidy. The rate of polyploidization then significantly increased in the genus. However, data should be supported by molecular analysis. In addition, the chromosome numbers of 8 species of Turkish *Paronychia* is still unknown. The determination of the karyological data of all species is very important to understand karyotype evolution and chromosomal phylogeny in *Paronychia*.

Key words: Karyology, *Paronychia*, phylogeny, polyploidy, Anatolia

1. Introduction

Karyotype evolution can be constructed with chromosomal features based on size, shape, and number. Karyotypic differences are one of the most important mechanisms supporting speciation as they may create transitional barriers between species (Baltisberger and Hörandl, 2016). Therefore, chromosomal characters are used to elucidate phylogenetic relationships in plant cytotaxonomy. (Eroğlu et al., 2013; Eroğlu, 2015). These characters are basic number (x), diploid number ($2n$), chromosome length, relative length (RL), total haploid length (THL), centromeric index (CI), karyotype formula (KF), chromosome structure changes including deletions, inversions and translocations, chromosome number variations including dysploidy and polyploidy, karyotype asymmetry including symmetry/asymmetry index (S/A_I), the coefficient of variation of chromosome length (CV_{CL}), and mean centromeric asymmetry (M_{CA}) (Peruzzi and Eroğlu, 2013; Eroğlu, 2015; Harpke et al., 2015; Baltisberger and Hörandl, 2016; Peruzzi et al., 2017; Şirin et al., 2019).

Karyotypic variations containing hybridization or polyploidization mechanisms are considered postcrossover

barriers to speciation, diversification, and evolution of higher plants (Baltisberger and Hörandl, 2016). Almost all of the higher plants have undergone genomic duplication at least once in the history of evolution (Zhang et al., 2014). Glaciation, climate change, high altitudes, and high latitudes are important factors increasing polyploidy rates (Metzgar et al., 2016; Demirci Kayıran and Özhatay, 2017).

Paronychia Miller is a polyphyletic genus of the family Caryophyllaceae. The genus contains approximately 110 annual and perennial species spread out all over the world except in South Asia and South Africa (Chaudhri, 1968). Turkey, Peru, Bolivia, and America are distribution centers of the *Paronychia* species (Bittrich, 1993). In particular, Turkey is a major center, with 29 species, 5 subspecies, 7 varieties, and a total of 41 taxa, including 16 endemics (Chaudhri, 1967; Eroğlu et al., 2017). *Paronychia* is an important model in the determination of karyotype evolution and interspecific phylogenetic relationships due to its global distribution, different chromosome numbers, and especially because of its various ploidy levels. Anatolia, which includes 3 different phytogeographical areas such as the Mediterranean, Euro-Siberian, and Irano-Turanian

* Correspondence: herhan.eroglu@bozok.edu.tr

regions, is an important place for the study of interspecific phylogenetic relations (Eroğlu et al., 2013).

Chromosome numbers are only given for 7 Turkish *Paronychia*. Four species are diploid; however, they exhibit 3 different basic numbers: $x = 5$ ($2n = 10$), $x = 7$ ($2n = 14$), and $x = 9$ ($2n = 18$). Six species are polyploidy and reveal 2 different polyploidy levels of tetraploidy ($2n = 4x = 28$ and 36) and octoploidy ($2n = 8x = 56$). *P. argentea* Lam. and *P. polygonifolia* (Vill.) DC. indicate high polyploidy ($8x$). Three species are both diploid and polyploid (Lorenzo Andreu and García Sanz, 1950; Blackburn and Morton, 1957; Fedorov, 1974; Löve, 1975; Diosdado and Pastor, 1994; Runemark, 1996; Eroğlu et al., 2017). In *Paronychia*, the chromosomal reports are generally based on basic/diploid chromosome number without detailed chromosomal data. In the only existing report based on evolution and phylogeny, Alvarez (2010) examined 34 *Paronychia* species and 5 outgroups with chloroplast rps16 sequences and nuclear ITS sequences and reported that *Paronychia* is a polyphyletic genus divided into 2 different groups. In addition, North and South American species are sisters of the outgroup *Gymnocarpos* (Forssk.).

In *Paronychia*, the lack of chromosomal data and the absence of karyotypic phylogeny studies had an impact on the purpose and importance of this study. In this work, the aim is to contribute to the chromosomal data and karyotypic phylogeny of *Paronychia* taxa using the following marks: (1) chromosome number and karyotype formula, (2) detailed chromosome measurements, (3) karyotype symmetry/asymmetry, (4) polyploidy variations, and (5) karyotypic phylogeny.

2. Materials and methods

2.1. Plant material

Figure 1 shows a distribution map of *Paroncyhia* taxa. The collection information is listed below. All taxa except *P. carica* and *P. chionaea* subsp. *chionaea* are endemic to Turkey.

P. aksoyii Budak. Turkey. Erzurum: Tortum, near Tortumkale village, 1580–1620 m, 05-VII-2014, Budak 3060 & Hamzaoğlu (Bozok Hb.).

P. anatolica Czecz. subsp. *balansae* Chaudhri. Turkey. İzmir: Ödemiş, the east of Bozdağ sky center, 1610 m, 26-VI-2012, Budak 2642 & Hamzaoğlu (Bozok Hb.).

P. angorensis Chaudhri. Turkey. Ankara: over Beynam village, near Beynam forest entry, 1415 m, 13-VII-2013, Budak 2746 & Hamzaoğlu (Bozok Hb.).

P. argyroloba Stapf. Turkey. Antalya: between Korkuteli and Elmalı, near Ovacık village, 1475 m, 04-VII-2012, Budak 2650 & Hamzaoğlu (Bozok Hb.).

P. beauverdi Czecz. Turkey. Çankırı: between Eldivan and TRT transmitter, 1585 m, 11-VII-2013, Budak 2734 Hamzaoğlu (Bozok Hb.).

P. carica Chaudhri. Turkey. Denizli: Tavas, near Karahisar mine, 1420 m, 13-VII-2013, Budak 2741 & Hamzaoğlu (Bozok Hb.).

P. cataonica Chaudhri. Turkey. Malatya: Darende, near Çukurkaya village, 1340 m, 26-VI-2013, Budak 2718 & Hamzaoğlu (Bozok Hb.).

P. chionaea Boiss subsp. *chionaea*. Turkey. Bursa: below Soğukpınar, 950 m, 31-VII-2013, Budak 2752 & Hamzaoğlu (Bozok Hb.).

P. chionaea Boiss subsp. *kemaliya* Chaudhri. Turkey. Erzincan: Kemaliye, near Salihli village, 1450 m, 10-VII-2013, Budak 2732 & Hamzaoğlu (Bozok Hb.).

P. condensata Chaudhri. Turkey. Kayseri: Bakırdağı, near Yaylacık village, 1570 m, 15-VII-2012, Budak 2703 & Hamzaoğlu (Bozok Hb.).

P. davrazensis Budak. Turkey. Isparta: above Davraz sky center, 1920–1960 m, 10-VII-2014, Budak 3193 & Hamzaoğlu (Bozok Hb.).

P. galatica Chaudhri. Turkey. Kastamonu: near asphalt worksite of highway, 1037 m, 11-VII-2013, Budak 2736 & Hamzaoğlu (Bozok Hb.).

P. kurdica Boiss. subsp. *hausknechtii* Chaudhri. Turkey. Gaziantep: Sof mountain, hillsides, 965 m, 24-VI-2013, Budak 2711 & Hamzaoğlu (Bozok Hb.).

P. kurdica Boiss. subsp. *montis-munzur* Chaudhri. Turkey. Erzincan: between Erzincan and Pülümür, 1560 m, 12-VII-2012, Budak 2684 & Hamzaoğlu (Bozok Hb.).

P. saxatilis Chaudhri. Turkey. Van: Başkale, İspiriz Mountain, 3230-3260 m, 03-VII-2014, Budak 3010 & Hamzaoğlu (Bozok Hb.).

P. turcica Chaudhri. Turkey. Bitlis: between Tatvan and Gevaş, above Koruklu village, 1990 m, 02-VIII-2014, Budak 3298 & Hamzaoğlu (Bozok Hb.).

2.2. Cytogenetic procedure

The seeds were germinated between moist filter papers in petri dishes at room temperature. The root tips were then pretreated in α-monobromonaphthalene solution at 4 °C for 16 h, fixed in Carnoy's fixative at 4 °C for 24 h, hydrolyzed in 1 N HCl at 60 °C for 12 min, and stained in 2% aceto-orcein for 2 h. Preparations were then made with the squash method (Altay et al., 2017; Martin et al., 2019).

2.3. Karyotype analysis

At least 10 well-spread metaphase plates were used to determine chromosome numbers. The chromosomal measurements were obtained by KaryoType software (Altınordu et al., 2016). The following parameters were used to characterize the chromosomes: short-arm length (SA), long-arm length (LA), total chromosome length = (SA + LA), total haploid length (THL), mean chromosome length (MHL), relative length (RL) = [(LA + SA)/THL] × 100, and centromeric index (CI) = [(SA)/(LA + SA)] × 100. Karyotype formulae were determined as described by Levan et al. (1964). The ideograms were drawn based on chromosome arm length.
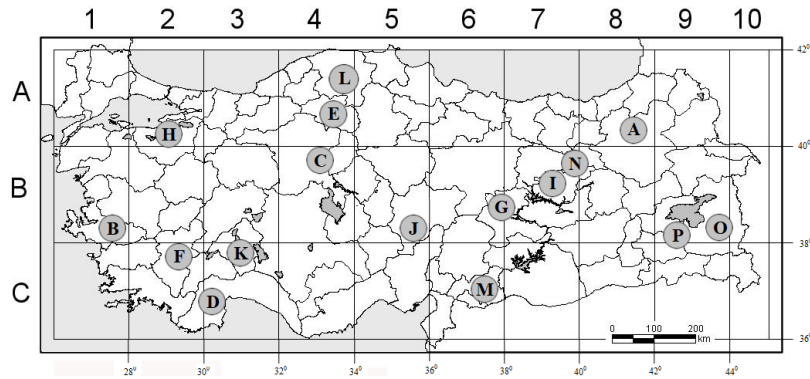
Figure 1. Distribution map of genus *Paronychia*. A: *P. aksoyii*; B: *P. anatolica* subsp. *balansae*; C: *P. angorensis*; D: *P. argyroloba*; E: *P. beauverdi*; F: *P. carica*; G: *P. cataonica*; H: *P. chionaea* subsp. *chionaea*; I: *P. chionaea* subsp. *kemaliya*; J: *P. condensata*; K: *P. davrazensis*; L: *P. galatica*; M: *P. kurdica* subsp. *hausknechtii*; N: *P. kurdica* subsp. *montis-munzur*; O: *P. saxatilis*; P: *P. turcica*.

2.4. Karyological relationships and karyotypic phylogeny

Karyological relationships were determined by following 8 parameters: [1] basic chromosome number (*x*), [2] diploid chromosome number (2*n*), [3] ploidy level (PL), [4] karyotype formula (KF), [5] total haploid length (THL), [6] mean centromeric asymmetry (M_{CA}), [7] coefficient of variation of chromosome length (CV_{CL}), and [8] symmetry/asymmetry index (S/A_I). The following 3 parameters calculated karyotype asymmetry, and these are M_{CA} (intrachromosomal asymmetry), CV_{CL} (interchromosomal asymmetry), and S/A_I. The formulae are given below.

M_{CA} = [mean (L_T–S_T) / (L_T + S_T)] × 100; L_T: total length of long arms and S_T: total length of short arms (Peruzzi and Eroğlu, 2013).

CV_{CL} = (S_{CL}/X_{CL}) × 100; S_{CL}: standard deviation and X_{CL}: mean chromosome length (Paszko, 2006). A scatter diagram was then drawn between the intrachromosomal asymmetry (M_{CA}) and interchromosomal asymmetry (CV_{CL}).

S/A_I = [(1 × M) + (2 × SM) + (3 × ST) + (4 × T)]/2*n*; M: median chromosome number, SM: submedian chromosome number: ST: subtelocentric chromosome number; and T: telocentric chromosome number. Unlike other methods, the S/A_I parameter is calculated using chromosome types and is classified into 5 karyotype types; these are [1] full symmetric (S/A_I = 1.0), [2] symmetric (1.0 < S/A_I ≤ 2.0), [3] between symmetric and asymmetric (2.0 < S/A_I ≤ 3.0), [4] asymmetric (3.0 < S/A_I < 4.0), and [5] full asymmetric (S/A_I = 4.0) (Eroğlu, 2015).

The phylogenetic tree showing karyological relationships was drawn by bootstrap values with UPGMA, chord coefficient. The phylogenetic tree contains the comparative phylogeny of the listed species. (1) 16 taxa from the present study, (2) 4 species from previous reports, namely *P. adalia* (Chaudhri), *P. argentea*, *P. echinulata* (Chater), and *P. polygonifolia*, with (3) *Stellaria nemorum* as the sister outgroup. The variables were then compared by Pearson correlations (PC) in Microsoft Excel software. The correlations were classified in the following order: [1] weak correlation (PC ≤ 0.25), [2] average correlation (0.25 < PC ≤ 0.50), [3] good correlation (0.50 < PC ≤ 0.75), and [4] high correlation (0.75 < PC) (P < 0.05).

3. Results

3.1. Chromosomal data

The chromosome records of 16 taxa are herein provided (Figure 2), 14 of which are reported for the first time. In addition, two of them (*P. kurdica* subsp. *hausknechtii* and *P. kurdica* subsp. *montis-munzur*) have similar numbers with previous data reported by Küpfer (1980). The chromosome numbers of Turkish *Paronychia* are given in Table 1, which also includes the results of present and previous studies. Six different chromosome numbers (2*n* = 18, 36, 52, 54, 72, and 104) are detected, 3 of which (2*n* = 52, 72, and 104) are new chromosome numbers for the genus. The smallest chromosome size among the taxa is 0.68 μm in *P. kurdica* subsp. *hausknechtii*. The largest chromosome size was detected in *P. condensata*, with 5.14 μm. The smallest total haploid length is 12.47 μm in *P. kurdica* subsp. *hausknechtii*, and the highest value is 87.48 μm in *P. chionaea* subsp. *chionaea* (Table 2).

3.2. Basic numbers, ploidy levels, and polyploidy

In genus *Paronychia*, there are generally 2 common basic numbers, which are *x* = 7 and most commonly *x* = 9. In this study, the basic chromosome numbers are *x* = 13 in *P. chionaea* subsp. *kemaliya* with ploidy levels of 4*x*/8*x* and *x* = 9 in the other taxa with ploidy levels of 2*x*, 4*x*, 4*x*/8*x*, and 6*x* (Table 1). The monoploid ideograms were generated by *x* = 9, and 13 are given in Figure 3.
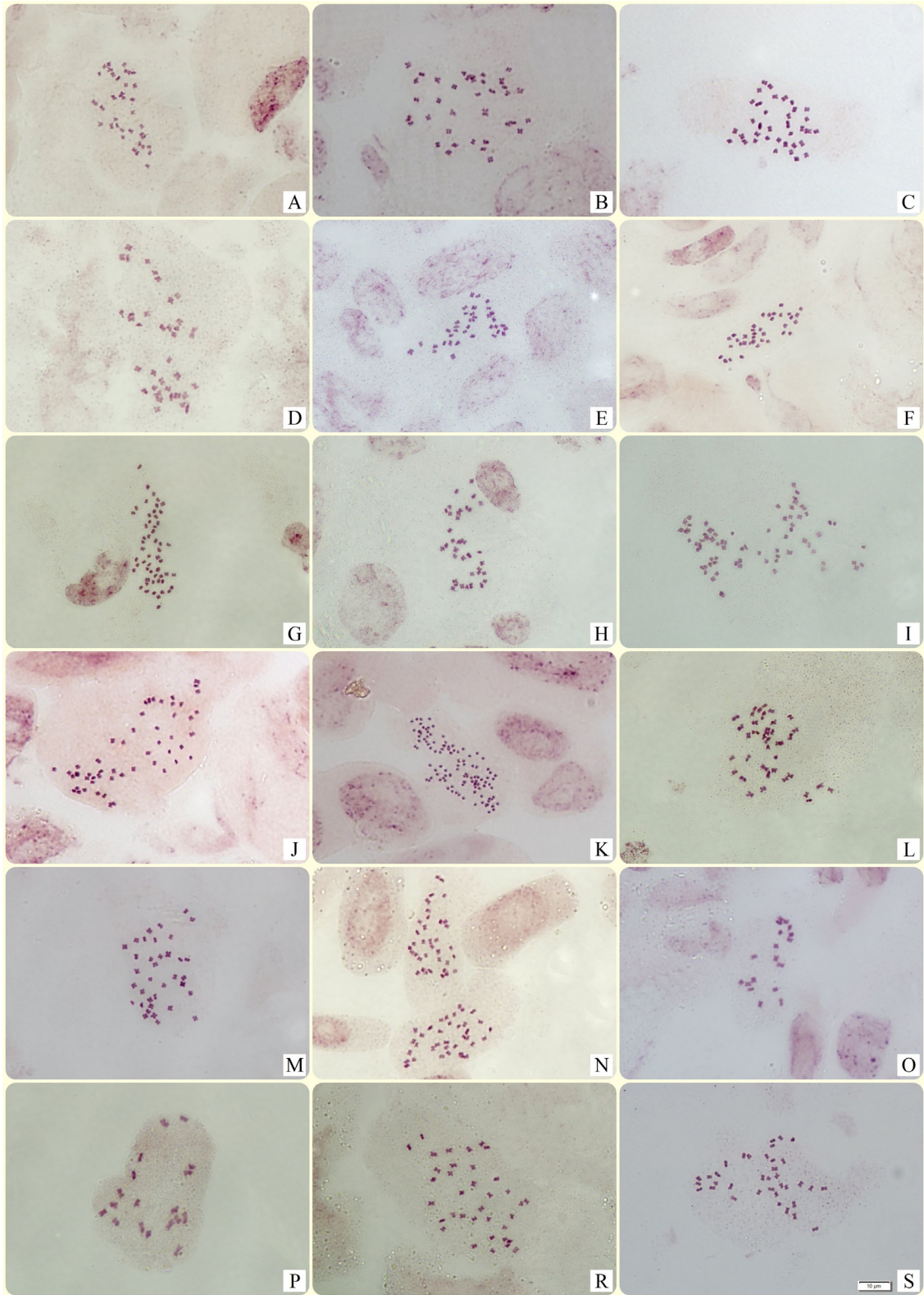
Figure 2. Somatic metaphase chromosomes of A: *P. aksoyii*; B: *P. anatolica* subsp. *balansae*; C: *P. angorensis*; D: *P. argyroloba*; E: *P. beauverdi*; F: *P. carica*; G: *P. cataonica*; H: tetraploid *P. chionaea* subsp. *chionaea*; I: octoploid *P. chionaea* subsp. *chionaea*; J: tetraploid *P. chionaea* subsp. *kemaliya*; K: octoploid *P. chionaea* subsp. *kemaliya*; L: *P. condensata*; M: *P. davrazensis*; N: *P. galatica*; O: *P. kurdica* subsp. *hausknechtii*; P: *P. kurdica* subsp. *montis-munzur*; R: *P. saxatilis*, S: *P. turcica*.

Table 1. Chromosome numbers of Turkish *Paronychia* taxa (in alphabetical order).

Paronychia	*x*	2*n*
adalia	9	36[a]
amani	-	Unknown
anatolica subsp. *balansae*	9	36
angorensis	9	36
argentea	7, 9	28[b], 36[c], 56[b]
argyroloba	9	36
aksoyii	9	36
beauverdii	9	36
boissieri	-	Unknown
carica	9	36
cataonica	9	54
cephalotes	9	36[d]
chionaea subsp. *chionaea*	9	36, 72
chionaea subsp. *kemaliya*	13	52, 104
condensata	9	36
davrazensis	9	36
dudleyi	-	Unknown
echinulata	5, 7	10[e], 14[f], 28[b]
euphratica	-	Unknown
galatica	9	36
kapela	9	18, 36[g]
kocii	-	Unknown
kurdica subsp. *hausknechtii*	9	18[h]
kurdica subsp. *montis-munzur*	9	18[h]
macrosepala	9	18[e]
mughlaei	-	Unknown
polygonifolia	7	14, 56[b]
pontica	-	Unknown
saxatilis	9	36
sintenisii	-	Unknown
turcica	9	36

[a]Eroğlu et al. 2017; [b]Blackburn and Morton 1957; [c]Lorenzo Andreu and García Sanz 1950; [d]Fedorov 1974; [e]Runemark 1996; [f]Diosdado and Pastor 1994; [g]Löve 1975; [h]Küpfer 1980 (as only species).

Polyploid nature is demonstrated by the prevalence of cells with $2n = 4x = 36$ (tetraploid) in 12 taxa, $2n = 8x = 72$ (octoploid) in *P. chionaea* subsp. *chionaea*, and $2n = 4x/8x = 52/104$ in *P. chionaea* subsp. *kemaliya* (Table 1). The chromosome arm lengths could not be measured in the octoploid *P. chionaea* subsp. *kemaliya* because the chromosomes are too small and the centromere is indeterminate.

3.3. Karyotype formulae and karyotype asymmetry
All taxa have median (m) and submedian (sm) chromosomes but not subtelocentric (st) and telocentric (t) chromosomes. Three different karyotype formulae are observed, which are M-m, m, and m-sm. In intrachromosomal asymmetry, the M_{CA} value ranges from 2.78 (*P. kurdica* subsp. *montis-munzur*) to 19.82 (*P. argyroloba*), which refers to symmetric karyotypes. In interchromosomal asymmetry, the CV_{CL} value ranges from 13.54 (*P. cataonica*) to 41.99 (*P. kurdica* subsp. *hausknechtii*), which refers to karyotype heterogeneity. In chromosomal type and centromeric position, the S/A_I value ranges from 1.000 to 1.222, which refers to full symmetric and symmetric karyotypes, respectively (Table 2).

3.4. Phylogenetic analyses
Figure 4 shows a phylogenetic tree including the chromosomal data of present and previous studies in Turkish *Paronychia*. Sixteen taxa have variable ploidy levels ($4x$, $6x$, and $4x/8x$) and shape the clade I. The tetraploid taxa are quite dominant in subclade 1. In addition, subclade 2 contains high polyploid ratios and high chromosome numbers. In clade I, *P. saxatilis*, *P. davrazensis*, *P. carica*, *P. argyroloba*, and *P. aksoyii* are karyologically very close to each other. In the same way, *P. turcica*, *P. angorensis*, and *P. anatolica* subsp. *balansae* are also very close to each other karyologically.

Four taxa are diploid ($2x$) and shape the clade II. Subclade 3 contains the most symmetrical karyotypes in terms of intrachromosomal asymmetry. In contrast, *P. echinulata* has the most asymmetric karyotype and is located in subclade 4.

4. Discussion
4.1. Chromosome number
Table 1 demonstrates the chromosome numbers of the taxa investigated in the present study and in previous studies. The chromosome numbers are the first to be reported for 14 taxa. The chromosome numbers of *P. kurdica* subsp. *hausknechtii* and *P. kurdica* subsp. *montis-munzur* are the same as in the previous report, which is $2n = 18$ (Küpfer, 1980). However, this taxon is *P. kurdica*, and it may be different from our subspecies.

Different chromosome numbers such as $2n = 18$, 36, 52, 54, 72, and 104 are determined with a dominant number of $2n = 36$. In the literature, there are different chromosome numbers such as $2n = 10$, 14, 16, 18, 28, 32, 36, 42, 56, and 64 in genus *Paronychia* (for detailed references, see Table 1). Together with the present study, the chromosome numbers of the 8 taxa are still unknown in Turkey, namely *P. amani* Chaudhri, *P. boissieri* Rouy.,

Table 2. Karyological features of the studied *Paronychia* taxa.

Taxa	KF	SC (μm)	LC (μm)	RL (%) SC–LC	THL	MCL (μm)	CI (min–max)	CV$_{CL}$	M$_{CA}$	S/A$_I$
P. aksoyii	34m + 2sm	1.23	2.86	3.64–8.46	33.79	1.88	35.00–48.78	22.57	13.87	1.056
P. anatolica subsp. *balansae*	36m	1.92	3.67	4.03–7.70	47.64	2.65	39.01–47.18	16.09	11.62	1.000
P. angorensis	36m	1.28	3.31	3.53–9.12	36.29	2.02	39.08–48.28	25.06	12.19	1.000
P. argyroloba	28m + 8sm	1.31	4.24	2.89–9.37	45.27	2.52	27.36–47.76	27.01	19.82	1.222
P. beauverdii	36m	1.30	2.54	3.67–7.18	35.39	1.97	42.35–49.79	16.91	8.77	1.000
P. carica	34m + 2sm	0.83	1.76	3.52–7.47	23.57	1.31	34.94–47.97	18.18	13.92	1.056
P. cataonica	4M + 50m	1.13	1.95	2.84–4.90	39.80	1.47	38.05–50.00	13.54	10.06	1.000
P. chionaea subsp. *chionaea**	36m	1.77	3.29	4.16–7.73	42.58	2.37	38.68–49.33	16.50	10.87	1.000
P. chionaea subsp. *chionaea***	68m + 4sm	1.44	3.73	1.65–4.26	87.48	2.43	35.29–49.06	24.05	12.02	1.056
P. chionaea subsp. *kemaliya**	52m	1.10	2.62	2.36–5.63	46.56	1.79	38.82–49.62	18.29	11.83	1.000
P. chionaea subsp. *kemaliya***	***	***	***	***	82.08	1.58	***	***	***	***
P. condensata	36m	1.77	5.14	3.28–9.54	53.89	2.99	37.36–49.03	26.54	11.96	1.000
P. davrazensis	34m + 2sm	1.35	2.79	3.84–7.94	35.16	1.95	36.84–48.48	17.26	12.27	1.056
P. galatica	2M + 34m	0.99	2.21	3.46–7.72	28.61	1.59	40.40–50.00	21.27	10.57	1.000
P. kurdica subsp. *hausknechtii*	18m	0.68	2.26	5.45–18.12	12.47	1.39	42.86–49.53	41.99	7.24	1.000
P. kurdica subsp. *montis-munzur*	2M + 16m	1.78	3.33	7.64–14.29	23.31	2.59	47.19–50.00	17.76	2.78	1.000
P. saxatilis	34m + 2sm	1.62	3.57	3.90–8.59	41.58	2.31	33.33–48.22	18.44	14.79	1.056
P. turcica	36m	1.61	3.08	3.74–7.16	42.98	2.39	38.04–48.05	15.72	12.99	1.000

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$_{CA}$); symmetry asymmetry index (S/A$_I$); *tetraploid; **octoploid; ***no detailed chromosome measurements.

P. dudleyi Chaudhri, *P. euphratica* (Chaudhri) Chaudhri, *P. kocii* Budak, *P. mughlaei* Chaudhri, *P. pontica* (Borhidi) Chaudhri, and *P. sintenisii* Chaudhri. In this respect, the results of this study provide important contributions to the cytotaxonomy of *Paronychia*.

4.2. Basic number and karyotype formula
A basic chromosome number of $x = 9$ dominates in Turkish *Paronychia* taxa, but basic numbers of $x = 5$, 7, and 13 characterize several taxa. However, a basic chromosome number of $x = 8$ dominates in some regions such as Granada, Almeria in Spain, and Macaronesia, which is a phytogeographical region comprising the Azores, Madeira, the Canary Islands, and the Cape Verde Islands in the eastern North Atlantic. For example, the basic number is $x = 8$ in *P. andina* A. Gray, *P. canariensis* (L.) Link, *P. depressa* (Torr. & A. Gray) Nutt. *ex* A. Nelson, *P. pulvinata* A. Gray, *P. sessiliflora* Nutt., and *P. suffruticosa* (L.) Lam. (Hartman, 1972, 1974; Fedorov, 1974; Diosdado and Pastor, 1994; Suda et al., 2003).

All taxa have karyotypes containing M-m, m, and m-sm. In addition, the genus has the karyotypes with st

chromosomes, which are 12m + 8sm + 2sm/st + 6st in *P. argentea* and m + 4sm + 6st in *P. echinulata* (Diosdado and Pastor, 1994).

4.3. Karyotype evolution; dysploidy and polyploidy
We believe that the ancestral basic number is probably $x = 9$ (Genome I), which is the dominant number. Then, the formations of the basic number such as $x = 8$ (Genome II) and $x = 7$ (Genome III) occurred with chromosomal changes such as fusion. Dysploidy is probably caused by fusion or reciprocal translocations of median chromosomes in ancestral karyotypes. Unlike the $x = 9$ karyotype, the karyotypes of $x = 7$ and 8 show lower diversity.

The polyploidy mechanism, which is quite common in genus *Paronychia*, appears to be the most important mechanism in the karyotype evolution of the genus. The first polyploidy mechanism probably occurred in ancestral karyotypes millions of years ago (Genome IV). Polyploid nature was demonstrated by the prevalence of cells with $2n = 4x = 36$ in many species, $2n = 4x = 52$ in *P. chionaea* subsp. *kemaliya*, $2n = 6x = 54$ in *P. cataonica*, $2n = 8x = 72$ in *P. chionaea* subsp. *chionaea*, and $2n = 8x = 104$ in
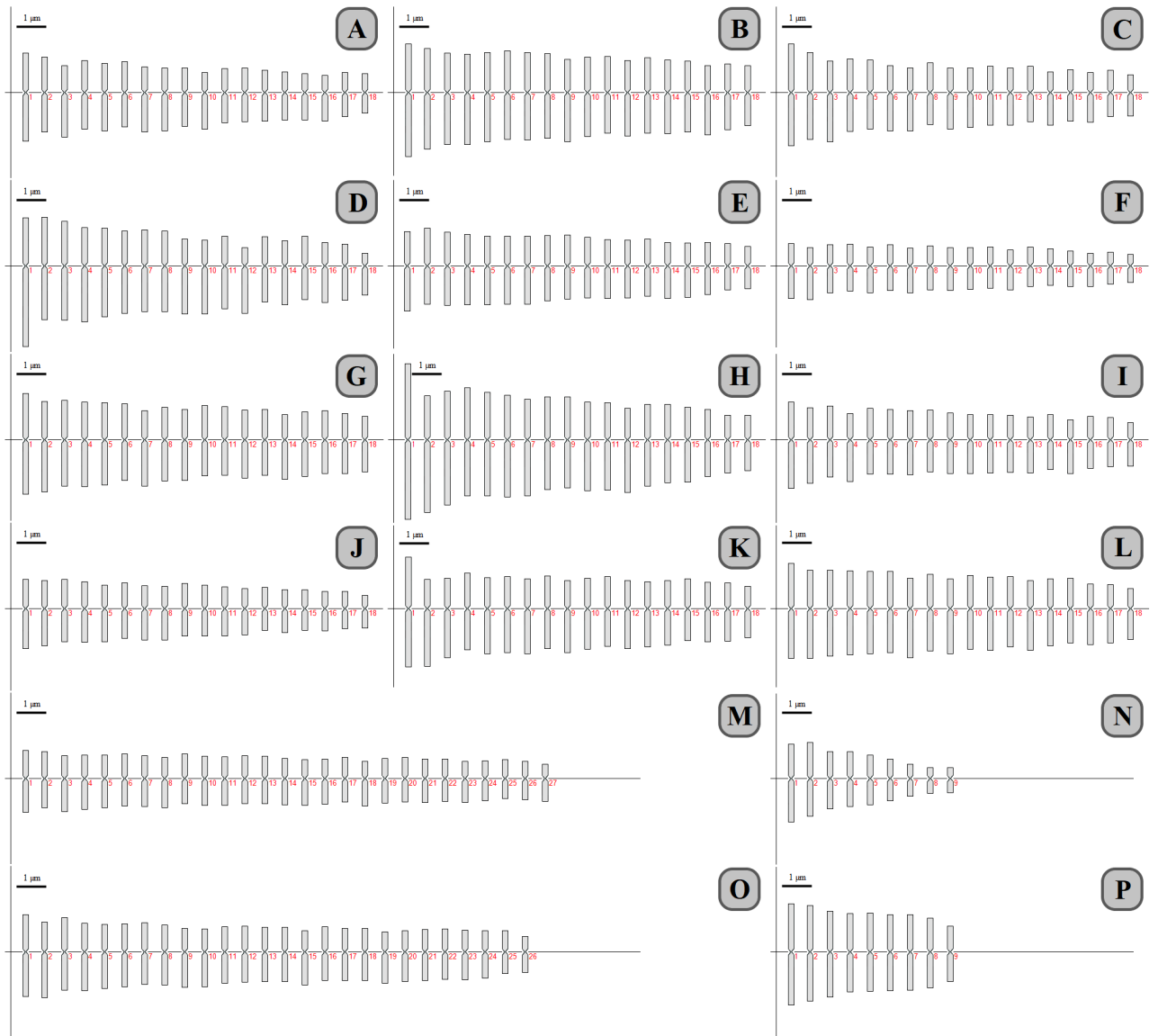
Figure 3. Ideograms of A: *P. aksoyii*; B: *P. anatolica* subsp. *balansae*; C: *P. angorensis*; D: *P. argyroloba*; E: *P. beauverdi*; F: *P. carica*; G: *P. chionaea* subsp. *chionaea*; H: *P. condensata*; I: *P. davrazensis*; J: *p. galatica*; K: *P. saxatilis*; L: *P. turcica*; M: *P. cataonica*; N: *P. kurdica* subsp. *hausknechtii*; O: *P. chionaea* subsp. *kemaliya*; P: *P. kurdica* subsp. *montis-munzur*.

P. chionaea subsp. *kemaliya*. We are of the opinion that Anatolia has a role in the distribution to other regions of *Paronychia* species for 2 reasons. The majority of taxa have a basic number of $x = 9$ and have a high polyploidy ratio.

Polyploidy originates from autopolyploidy by genome duplication in a species and allopolyploidy by genome duplication between species and has played a major role in the speciation and evolution of higher plants (Demirci Kayıran and Özhatay, 2017). Polyploidy may affect the speciation of subspecies. Metzgar et al. (2016) reported that glaciation and associated climate shifts increased polyploidy rates. Demirci Kayıran and Özhatay (2017)

reported that altitudes and high latitudes might have increased the polyploidy rates, although not always. All taxa have a distribution between 950 and 3260 m. High altitudes may have affected polyploidy levels, but this is not the only reason. For example, the adjacent cells of *P. chionaea* subsp**.** *kemaliya* have different patterns in terms of chromosome sets, which is the mixoploidy.

4.4. Karyotype asymmetry
In intrachromosomal asymmetry, all karyotypes are symmetrical except *P. echinulata. P. kurdica* subsp. *montis-munzur, P. kurdica* subsp. *hausknechtii*, and *P. beauverdii*, which have the most symmetrical karyotypes with 0
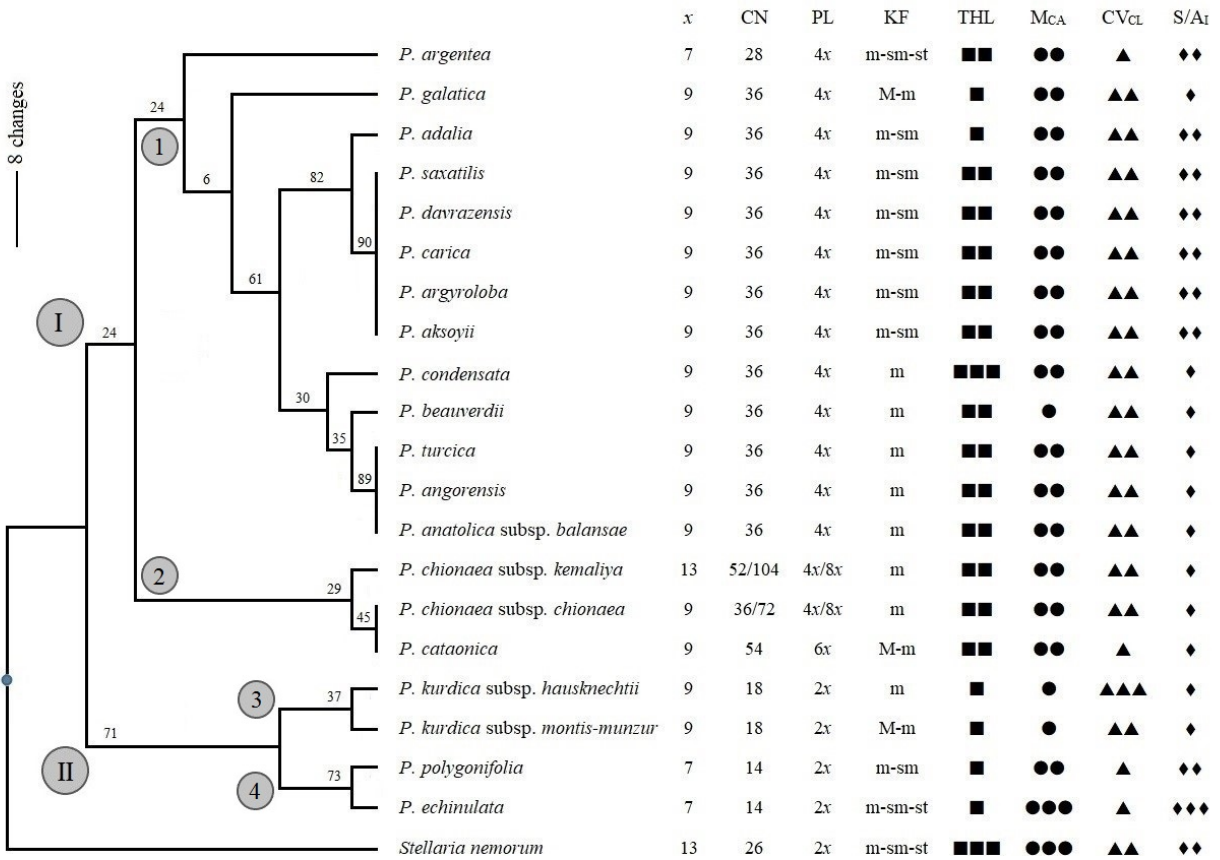
Figure 4. The phylogenetic tree contains the comparative karyotypic phylogeny of Turkish *Paronychia* taxa given the karyotype data with this and previous studies. The numbers above the branches indicate bootstrap values. Main clades and subclades are shown in the circle. $0 < THL \leq 25.00$ (■); $25.00 < THL \leq 50.00$ (■■); $50.00 < THL \leq 75.00$ (■■■); $0 < M_{CA} \leq 10.00$ (●); $10.00 < M_{CA} \leq 20.00$ (●●); $20.00 < M_{CA}$ (●●●); $0 < CV_{CL} \leq 15.00$ (▲); $15.00 < CV_{CL} \leq 30.00$ (▲▲); $30.00 < CV_{CL}$ (▲▲▲); $1.0 = S/A_I$ (♦, full symmetric); $1.0 < S/A_I \leq 2.0$ (♦♦, symmetric); $2.0 < S/A_I \leq 3.0$ (♦♦♦, between symmetric and asymmetric).

$< M_{CA} \leq 10.00$, respectively. *P. kurdica* subsp. *montis-munzur* and *P. kurdica* subsp. *hausknechtii* are diploid taxa (Table 2 and Figure 4). Table 3 presents a weak positive correlation between M_{CA} values and ploidy levels (r = 0.233). In addition, the M_{CA} value shows the correlations as weak or average in all parameters except with the karyotype formula and S/A_I value, for which it correlates well. *P. argentea* and *P. echinulata* are rare species with subtelocentric chromosomes (Diosdado and Pastor, 1994), possibly due to the reciprocal translocations of the median/submedian chromosomes.

In interchromosomal asymmetry, all karyotypes are symmetrical except *P. kurdica* subsp. *hausknechtii*. *P. polygonifolia*, *P. echinulata*, *P. argentea*, and *P. cataonica*, which have the most symmetrical karyotypes with $0 < CV_{CL} \leq 15.00$, respectively. These taxa show diploidy in *P. polygonifolia* and *P. echinulata*, polyploidy in *P. argentea*, and high polyploidy in *P. cataonica* (Table 2 and Figure 4). Table 3 presents a negative average correlation between CV_{CL} values and ploidy levels (r = –0.452). In addition,

the CV_{CL} value shows the correlations as weak or average in all parameters.

The most symmetric and asymmetric karyotypes are completely different between M_{CA} and CV_{CL} with a very weak correlation (r = 0.014) (Figure 5). All taxa have symmetrical karyotypes with only median/submedian chromosomes. As karyotype evolution progresses, chromosomal asymmetry continues to increase (Baltisberger and Hörandl, 2016). The fact that Anatolian *Paronychia* taxa have symmetrical karyotypes may indicate that these taxa are in the early stages of karyotype evolution. These data support our opinion that Anatolia plays an important role in the distribution of the *Paronychia* species. The asymmetric karyotypes are probably higher in other regions where the genus *Paronychia* spreads.

4.5. Conclusion

In this study, the chromosomal data, polyploidy variations, and karyotypic phylogeny of 16 Turkish *Paronychia* are shown. The data make up the first report for 14 taxa. The

Table 3. Pearson correlations for variables.

	x	CN	PL	KF	THL	M_{CA}	CV_{CL}	S/A_I
x	1							
CN	0.807	1						
PL	0.579	0.949*	1					
KF	−0.046	−0.048	−0.042	1				
THL	0.244	0.484	0.538	0.122	1			
M_{CA}	0.017	0.176	0.233	0.713	0.499	1		
CV_{CL}	−0.098	−0.365	−0.452	0.124	−0.410	−0.013	1	
S/A_I	−0.130	−0.135	−0.116	0.631	0.124	1.732	0.167	1

*Correlation is significant at the 0.05 level.
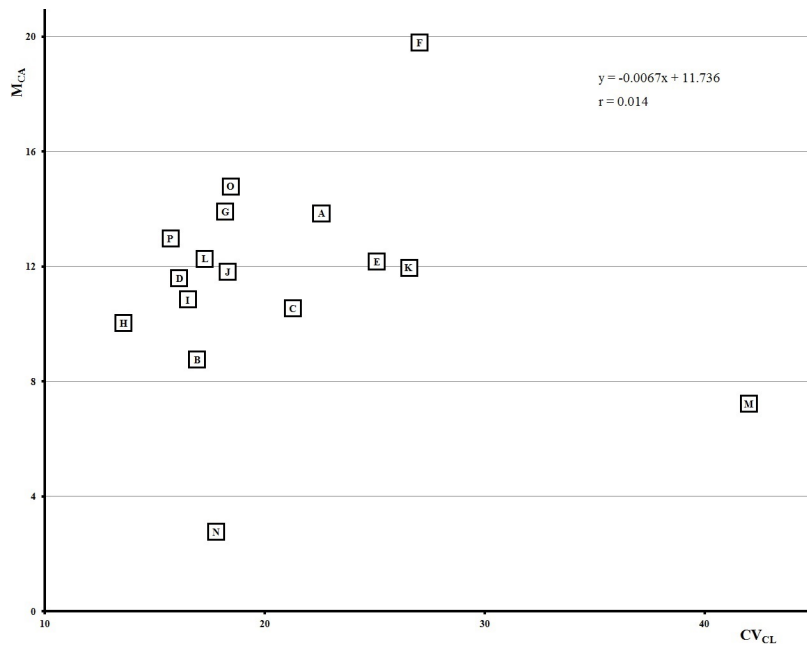
Figure 5. Scatter diagram between M_{CA} and CV_{CL}. A: *P. aksoyii*; B: *P. beauverdi*; C: *P. galatica*; D: *P. anatolica* subsp. *balansae*; E: *P. angorensis*; F: *P. argyroloba*; G: *P. carica*; H: *P. cataonica*; I: *P. chionaea* subsp. *chionaea*; J: *P. chionaea* subsp. *kemaliya*; K: *P. condensata*; L: *P. davrazensis*; M: *P. kurdica* subsp. *Hausknechtii*; N: *P. kurdica* subsp. *montis-munzur*; O: *P. saxatilis*; P: *P. turcica*.

listed data provide important contributions to karyotype phylogeny and cytotaxonomy of *Paronychia*: (1) the majority of taxa have a basic number of $x = 9$, (2) high polyploidy rates, and (3) symmetrical karyotypes. The data support the fact that Anatolia is an important distribution center of *Paroncyhia*. However, it should be supported by molecular analysis. In addition, the chromosome numbers of 8 species from Turkey are still unknown. Determining the karyological data of all species is very important to understand karyotype evolution and chromosomal phylogeny of *Paronychia*.

References

Altay D, Eroğlu HE, Hamzaoğlu E, Koç M (2017). Karyotype analysis of some taxa of *Dianthus* section *Verruculosi* (Caryophyllaceae, Sileneae). Turkish Journal of Botany 41: 367-374. doi: 10.3906/bot-1612-30

Altınordu F, Peruzzi L, Yu Y, He XJ (2016). A tool for the analysis of chromosomes: KaryoType. Taxon 65: 586-592. doi: 10.12705/653.9

Alvarez S (2010). Evolution and systematics of locally endemic *Paronychia* (Caryophyllaceae) in South America. MS, Uppsala Universitet, Uppsala, Sweden.

Baltisberger M, Hörandl E (2016). Karyotype evolution supports the molecular phylogeny in the genus *Ranunculus* (Ranunculaceae). Perspectives in Plant Ecology, Evolution and Systematics 18: 1-14. doi: 10.1016/j.ppees.2015.11.001

Bittrich V (1993). Caryophyllaceae. In: Kubitzki K, Rohwer J, Bittrich V (editors). The Families and Genera of Vascular Plants, Magnoliid, Hamamelid, and Caryophyllid Families. Vol. 2. Berlin, Germany: Springer, pp. 206-236.

Blackburn KB, Morton JK (1957). The incidence of polyploidy in the Caryophyllaceae of Britain and of Portugal. New Phytologist 56: 344-351. doi: 10.1111/j.1469-8137.1957.tb06979.x

Chaudhri MN (1967). *Paronychia* Mill. In: Davis PH (editor). Flora of Turkey and the East Aegean Islands, Vol. 2. Edinburgh, UK: Edinburgh University Press, pp. 250-262.

Chaudhri MN (1968). A Revision of the Paronychinae. Utrecht, Netherlands: Tilburg, H. Gianotten.

Demirci Kayıran S, Özhatay FN (2017). A karyomorphological study on the genus *Muscari* Mill. growing in Kahramanmaraş (Turkey). Turkish Journal of Botany 41: 289-298. doi: 10.3906/bot-1605-6

Diosdado JC, Pastor J (1994). Estudio cariologico del gĕnero *Paronychia* Miller (Caryophyllaceae) en Andalucĭa (Espana). Acta Botanica Malacitana 19: 89-95 (in Spanish).

Eroğlu HE, Şimşek N, Koç M, Hamzaoğlu E (2013). Karyotype analysis of some *Minuartia* L. (Caryophyllaceae) taxa. Plant Systematics and Evolution 299: 67-73. doi: 10.1007/s00606-012-0703-8

Eroğlu HE (2015). Which chromosomes are subtelocentric or acrocentric? A new karyotype symmetry/asymmetry index. Caryologia 68: 239-245. doi: 10.1080/00087114.2015.1032614

Eroğlu HE, Doğan H, Altay D, Budak Ü (2017). The medicinal plant of genus *Paronychia* and the karyotype analysis of *Paronychia adalia*. International Journal of Secondary Metabolite 4 (3): 103-107. doi: 10.21448/ijsm.363827

Fedorov AA (1974). Chromosome Numbers of Flowering Plants. Leningrad, USSR: Komarov Botanical Institute.

Harpke D, Carta A, Tomović G, Ranđelović V, Ranđelović N et al. (2015). Phylogeny, karyotype evolution and taxonomy of *Crocus* series *Verni* (Iridaceae). Plant Systematics and Evolution 301: 309-325. doi: 10.1007/s00606-014-1074-0

Hartman RL (1972). *Paronychia pulvinata – P. sessiliflora*: A cytological and chemical study. Journal of the Colorado-Wyoming Academy of Science 7: 102-102.

Hartman RL (1974). Rocky mountain species of *Paronychia* (Caryophyllaceal. A morphological, cytological, and chemical study. Brittonia 26: 256-263.

Küpfer P (1980). Contribution à la cytotaxonomie de quelques orophytes Iraniens. Revue de Biologie et d'Ecologie Mediterranéenne 7: 37-48 (in French).

Levan AK, Fredga K, Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220. doi: 10.1111/j.1601-5223.1964.tb01953.x

Lorenzo Andreu A, García Sanz MP (1950). Cromosomas somáticos de plantas espontáneas en la estepa de Aragón. II. Anales de la Estación Experimental de Aula Dei 2: 12-63 (in Spanish).

Löve Á (1975). IOPB Chromosome Number Reports XLIX. Taxon 24: 501-516.

Martin E, İçyer Doğan G, Karaman Erkul S, Eroğlu HE (2019). Karyotype analyses of 25 Turkish taxa of *Astragalus* from the sections *Macrophyllium*, *Hymenostegis*, *Hymenocoleus*, and *Anthylloidei* (Fabaceae). Turkish Journal of Botany 43: 232-242. doi: 10.3906/bot-1807-1

Metzgar J, Stamey M, Ickert-Bond S (2016). Genetic differentiation and polyploid formation within the *Cryptogramma crispa* complex (Polypodiales: Pteridaceae) Turkish Journal of Botany 40: 231-240. doi: 10.3906/bot-1501-54

Paszko B (2006). A critical review and a new proposal of karyotype asymmetry indices. Plant Systematics and Evolution 258: 39-48. doi: 10.1007/s00606-005-0389-2

Peruzzi L, Eroğlu HE (2013). Karyotype asymmetry: again, how to measure and what to measure? Comparative Cytogenetics 7: 1-9. doi: 10.3897/CompCytogen.v7i1.4431

Peruzzi L, Carta A, Altınordu F (2017). Chromosome diversity and evolution in *Allium* (Allioideae, Amaryllidaceae). Plant Biosystems 151: 212-220. doi: 10.1080/11263504.2016.1149123

Runemark H (1996). Mediterranean chromosome number reports 6 (590-678). Flora Mediterranea 6: 223-243.

Suda J, Kyncl T, Freiova R (2003). Nuclear DNA amounts in Macaronesian angiosperms. Annals of Botany 92: 153-164.

Şirin E, Bozkurt M, Uysal T, Ertuğrul K (2019). Karyomorphological features of Turkish *Centaurea* (subgenus *Cyanus*, Asteraceae) species and its taxonomic importance. Turkish Journal of Botany 43: 538-550. doi: 10.3906/bot-1811-28

Zhang C, Cao D, Kang L, Duan J, Ma X et al. (2014). Ploidy variation and karyotype analysis in *Hemerocallis* spp. (Xanthorrhoeaceae) and implications on daylily breeding. New Zealand Journal of Crop and Horticultural Science 42: 183-193. doi: 10.1080/01140671.2013.877040