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Karyotype Analysis of *Paronychia anatolica* subsp. *anatolica* (Caryophyllaceae, Paronychioideae)

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ABSTRACT: In the study, the chromosomal data of *Paronychia anatolica* subsp. *anatolica* are provided for the first time. The diploid chromosome number and karyotype formula are $2n = 2x = 18 =$ 16m + 2sm with metacentric chromosomes out of submetacentric chromosome 1. No all chromosomes have satellite and secondary constriction. The values of total haploid length and mean chromosome length are very low with 14.47 and 1.61 µm, respectively; because the taxon has low numbers and relatively small of chromosomes, which are range from 1.21 to 1.84 µm. The karyotype is symmetrical type. Within the scope of all the results, the data will provide important contributions to the cytotaxonomy of genus *Paronychia*. Especially, taxon contains very different karyotypic data from other subspecies and it is very important knowledge for cytotaxonomic point of view.

Keywords: Chromosome, karyotype asymmetry, *Paronychia*, Turkey

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INTRODUCTION

The genus *Paronychia* Miller consists of approximately 110 species widespread all over the world. Turkey together with Peru-Bolivia and America are one of the most important distribution centers of *Paronychia* species (Bittrich, 1993). The flora of Turkey currently includes 29 species and 12 subspecies and varieties with 16 endemic taxa in genus *Paronychia* (Chaudhri, 1967, 1968; Eroğlu et al., 2017; Eroğlu et al., 2020).

Paronychia anatolica Czecz. is a perennial herb with flowers white; stems much branched at the base, decumbent to ascending, 5-10 cm length; base diameter 3-6 mm (Figure 1). The species is endemic to Turkey and grows at rocky-stone areas and forest openings in 980-1500 m. Vernacular names are "etyaran" or "ana etyaran" (Eroğlu et al., 2017).

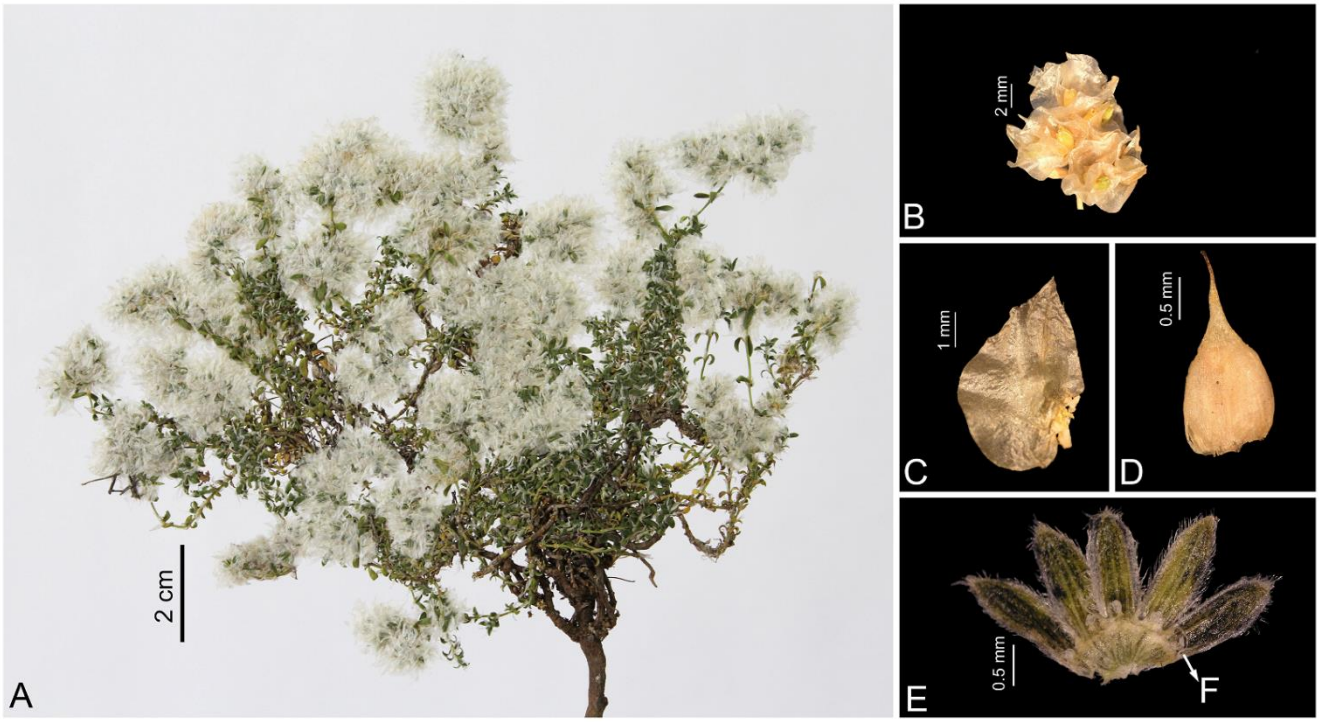

Figure 1. *Paronychia anatolica*: A) habit, B) glomerule, C) bract, D) fruit, E) sepals, F) petals

Within *Paronychia*, the basic chromosome numbers are x = 5, 7, 8, 13 and more common x = 9 (Eroğlu et al., 2020). The chromosomal data are reported from 23 Turkish taxa. Three species are only diploid and they reveal only one basic number: x = 9 ($2n$ = 18). Seventeen species are polyploid and reveal three different polyploidy levels: tetraploidy ($2n = 4x$ = 28, 36 and 52), hexaploidy ($2n = 6x$ = 54), and octoploidy ($2n = 8x$ = 56, 72 and 104). Three species are diploid and polyploid (Lorenzo Andreu and García Sanz, 1950; Blackburn and Morton, 1957; Löve, 1975; Küpfer, 1980; Diosdado and Pastor, 1994; Runemark, 1996; Eroğlu et al., 2017; Eroğlu et al., 2020). In the study, it is aimed to investigate to the parameters of chromosome number, karyotype formula, detailed chromosome measurements, and karyotype asymmetry in *P. anatolica* subsp. *anatolica*.

MATERIALS AND METHODS

Plant Material

P. anatolica subsp. *anatolica* was collected from its natural habitats in Turkey. The collection information: Tokat; Çamlıbel mountain, near Çatalkaya village, 40˚01ˈ43"N 36˚23ˈ28"D, 1140 m,

07.07.2014, Budak 3126 & Hamzaoğlu. Exsicates were deposited at the herbarium of the Department of Biology, at the Yozgat Bozok University in Yozgat.

Cytogenetic Procedure

The cytogenetic procedure consists of six different stages and these are listed below. The first is the germination of seeds in petri dishes at room temperature. The second is pretreatment in α-monobromonaphthalene solution at 4°C for 16 h. The third is fixation in fixative solution (alcohol: acetic acid, 3:1, v:v). The fourth is hydrolysis in 1 N HCl at 60°C for 12 min. The fifth is staining in 2% aceto-orcein for 2 h. The sixth is the preparation with the squash method (Elçi and Sancak, 2013; Martin et al, 2020).

Karyotype Analysis

Photographs of chromosome spreads were taken using a Olympus BX53 microscope fitted with a DP72 digital camera. Ten mitotic cells were analyzed by Software KaryoType. The following formulae were used for karyotype analysis.

$$\text{TL (total chromosome length)} = \text{LA (long arm)} + \text{SA (short arm)} \quad (1)$$

$$\text{AR (arm ratio)} = \text{LA} / \text{SA} \quad (2)$$

$$\text{CI (centromeric index)} = [(\text{SA}) / (\text{LA} + \text{SA}) \times 100] \quad (3)$$

$$\text{THL (total haploid length)} = TL_1 + TL_2 + TL_3 + TL_4 + TL_5 + TL_6 + TL_7 + TL_8 + TL_9 \quad (4)$$

$$\text{MCL (mean chromosome length)} = \text{THL} / n \quad (5)$$

$$\text{RL (relative length)} = [(\text{LA} + \text{SA}) \times 100 / \text{THL}] \quad (6)$$

The chromosome classifications and karyotype formula were determined according to the Levan et al. (1964). All known methods, mainly M_{CA} (mean centromeric asymmetry) and CV_{CL} (coefficient of variation of chromosome length), were used to calculate karyotype asymmetries (Stebbins, 1971; Paszko, 2006; Peruzzi and Eroğlu, 2013). The following formulae were used for intrachromosomal and interchromosomal asymmetry.

$$M_{CA} = \text{mean}\,[(TOTAL_{LA} - TOTAL_{SA}) / (TOTAL_{LA} + TOTAL_{SA})] \times 100 \quad (7)$$

$$CV_{CL} = [\text{SD (standard deviation)} / \text{MCL}] \times 100 \quad (8)$$

RESULTS AND DISCUSSION

Figure 2 and Figure 3 present the somatic metaphase chromosomes and haploid ideogram in *P. anatolica* subsp. *anatolica*. The karyotype formula is $2n = 2x = 18 = 16m + 2sm$ with metacentric chromosomes out of submetacentric chromosome 1 (Table 1). No all chromosomes have satellite and secondary constriction. THL and MCL values are very low with 14.47 and 1.61 µm, respectively, because the taxon has relatively small and low numbers of chromosomes compared with other taxa given detailed measurements. The detailed chromosomal measurements of *P. anatolica* subsp. *anatolica* are given in Table 2. The values of TL, RL, and CI range from 1.21-1.84 µm, 8.36-12.72%, and 35.33-49.11%, respectively. The high CI is characterized with median region. The chromosome 2 is the most

symmetric chromosome with the highest centromeric index and the lowest arm ratio. Unlike, the low CI is characterized with centromere shift. The chromosome 1 is the most asymmetric chromosome with the lowest centromeric index and the highest arm ratio. Table 3 presents the karyotype asymmetry values with symmetry/asymmetry limits. Stebbins classification is a qualitative parameter and others are quantitative parameters (Stebbins, 1971; Paszko, 2006; Peruzzi and Eroğlu, 2013).

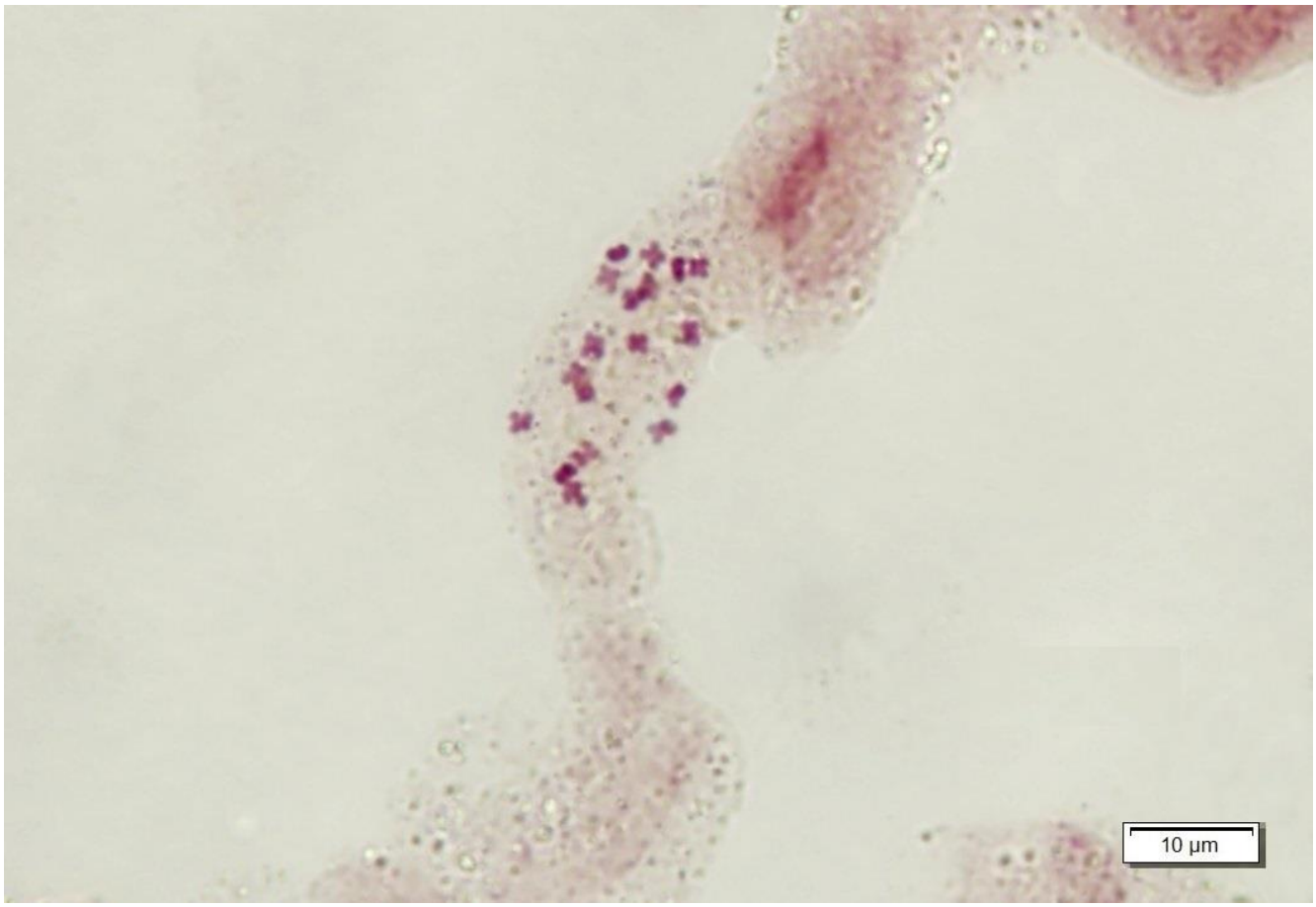

Figure 2. Somatic metaphase chromosomes of *Paronychia anatolica* subsp. *anatolica*

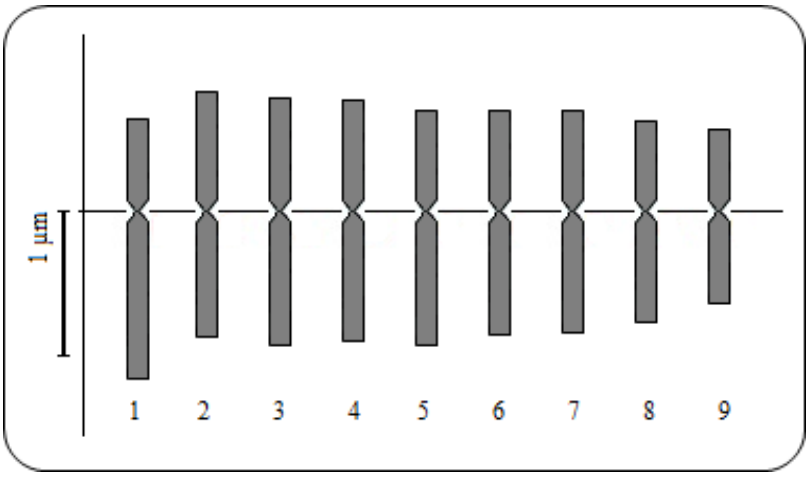

Figure 3. The haploid ideogram of *Paronychia anatolica* subsp. *anatolica*

Table 1. The karyotype parameters of *Paronychia anatolica* subsp. *anatolica*

Karyotype parameters	
x (basic number)	9
2*n* (diploid number)	18
Karyotype formula	16m + 2sm
THL (total haploid length, µm)	14.47
MCL (mean chromosome length, µm)	1.61

Table 2. The detailed chromosome measurements of *Paronychia anatolica* subsp. *anatolica*

Chromosome pair	**Total length (µm)**	**Long arm (µm)**	**Short arm (µm)**	**Arm ratio**	**Relative length (%)**	**Centromeric index (%)**	**Chromosome type**
1	1.84	1.19	0.65	1.83	12.72	35.33	submetacentric
2	1.75	0.89	0.86	1.03	12.09	49.14	metacentric
3	1.74	0.94	0.80	1.17	12.02	45.98	metacentric
4	1.71	0.92	0.79	1.16	11.82	46.20	metacentric
5	1.66	0.94	0.72	1.31	11.47	43.37	metacentric
6	1.58	0.87	0.71	1.23	10.92	44.94	metacentric
7	1.57	0.85	0.72	1.18	10.85	45.86	metacentric
8	1.41	0.78	0.63	1.24	9.74	44.68	metacentric
9	1.21	0.64	0.57	1.12	8.36	47.11	metacentric

Table 3. The karyotype asymmetry values in *Paronychia anatolica* subsp. *anatolica*

Parameters	**Value**	**Symmetry–Asymmetry limits***
S Cl. (Stebbins' classification)	1A	1A – 4C
As K (%) (karyotype asymmetry index)	55.42	50 – 100
TF (%) (total form percent)	44.58	50 – 0
Syi (%) (index of karyotype symmetry)	80.42	100 – 0
Rec (%) (chromosomal size resemblance)	87.38	100 – 100
A_1 (intrachromosomal asymmetry)	0.18	0 – 1
A_2 (interchromosomal asymmetry)	0.12	0 – 0
DI (dispersion index)	5.26	0 – 0
A (degree of karyotype asymmetry)	0.11	0 – 1
AI (asymmetry index)	1.05	0 – NA
CV_{CI} (variation coefficient of centromeric index)	8.67	0 – NA
CV_{CL} (variation coefficient of chromosome length)	12.13	0 – 0
M_{CA} (mean centromeric asymmetry)	10.53	0 – 100

* Stebbins, 1971; Paszko, 2006; Peruzzi and Eroğlu, 2013. NA, not applicable

The chromosome number is reported here for the first time for *P. anatolica* subsp. *anatolica*. The taxon has chromosome number of 2*n* = 18 as reported with also earlier Turkish taxa, which are *P. kapela* (Hacq.) A. Kern., *P. kurdica* Boiss. subsp. *hausknechtii* Chaudhri, *P. kurdica* Boiss. subsp. *montis-munzur* Chaudhri, and *P. macrosepala* Boiss., alphabetically (Löve, 1975; Küpfer, 1980; Runemark, 1996; Eroğlu et al., 2020).

In *P. anatolica* subsp. *anatolica*, the basic chromosome number is *x* = 9 with ploidy level of 2*x*. This is the most common basic number in Turkish species. However, the basic number of *x* = 8 dominates in Macaronesia and Spain (Hartman, 1972, 1974; Diosdado and Pastor, 1994; Suda et al., 2003). In addition, the polyploidy is quite common in Turkish species. The basic numbers are *x* = 7 with ploidy levels of 4*x*, 8*x* in *P. argentea* Lam., *P. echinulata* Chater, and *P. polygonifolia* (Vill.) DC.; *x* = 9 with ploidy levels of 4*x*, 6*x*, 8*x* in many species; *x* = 13 with ploidy levels of 4*x*, 8*x* in *P. chionaea* Boiss subsp. *kemaliya* Chaudhri (Lorenzo Andreu and García Sanz, 1950; Blackburn and Morton, 1957; Löve,

1975; Küpfer, 1980; Diosdado and Pastor, 1994; Runemark, 1996; Eroğlu et al., 2017; Eroğlu et al., 2020).

The taxon has karyotype formula containing metacentric (m) and submetacentric (sm) chromosomes. The similar karyotypes have been showed, namely 18m in *P. kurdica* (Mirzadeh Vaghefi et al., 2014) and 34m + 2sm in *P. adalia* Chaudhri (Eroğlu et al., 2017). Eroğlu et al. (2020) reported that 16 taxa have karyotypes containing only m or m-sm but not subtelocentric (st) and telocentric (t) chromosomes. However, karyotypes containing subtelocentric chromosomes have been reported, namely 12m + 8sm + 2sm/st + 6st in *P. argentea* and m + 4sm + 6st in *P. echinulata* (Diosdado and Pastor, 1994).

According to the all parameters, *P. anatolica* subsp. *anatolica* shows the low intrachromosomal and interchromosomal asymmetry with a quite symmetrical karyotype. The values of AsK, TF, Syi, A1, A, and especially M_{CA} refer the intrachromosomal asymmetry. In addition, the values of Rec, A2, and especially CV_{CL} refer the interchromosomal asymmetry. The values of the Stebbins classification, CV_{CL}, and M_{CA} are 1A, 12.13, and 10.53, respectively. In literature, *P. adalia* has a symmetrical karyotype (Eroğlu et al., 2017); unlike, *P. argentea* and *P. echinulata* have more asymmetric karyotypes by several subtelocentric chromosomes (Diosdado and Pastor, 1994). In the most comprehensive study, 16 karyotypes are symmetrical (Eroğlu et al., 2020). Turkish *Paronychia* have symmetrical karyotypes. The symmetric karyotypes suggest early stages of karyotype evolution. This shows that Anatolia is an important distribution center of the genus.

The taxon contains quite different karyotypic data compared to other subspecies, which is *P. anatolica* subsp. *balansae* Chaudhri. In the literature, *P. anatolica* subsp. *balansae* is tetraploid taxon with $2n = 4x = 36$ chromosomes (Eroğlu et al., 2020). Whereas *P. anatolica* subsp. *anatolica* is diploid taxon with $2n = 2x = 18$ chromomes. In addition, *P. anatolica* subsp. *anatolica* and *P. anatolica* subsp. *balansae* are different taxa morphologically. A key to closely relate to the taxonomic status of subspecies is given below. The key was made from plant samples collected for cytogenetic analysis.

Glomerules 5-7 × 8-11 mm; fruits 2.5-3 mm ..... **subsp. *balansae***
Glomerules 10-18 × 10-18 mm; fruits 2-2.4 mm ..... **subsp. *anatolica***

The cytotaxonomic differences seemed to support morphological differences. A comparison list including karyological and morphological data is given in Table 4. In our opinion, systematic status of two subspecies should be reviewed.

Table 4. Comparison of karyological and morphological data of subspecies

P. anatolica* subsp. *anatolica	***P. anatolica* subsp. *balansae***	**Data type**
Glomerules 10-18 × 10-18 mm	Glomerules 5-7 × 8-11 mm	Morphological
Fruits 2-2.4 mm	Fruits 2.5-3 mm	Morphological
$2n = 18$	$2n = 36$*	Karyological
Diploid ($2x$)	Tetraploid ($4x$)*	Karyological
Symmetrical karyotype	Symmetrical karyotype*	Karyological

* Eroğlu et al., 2020

CONCLUSION

In the study, the chromosomal data of *P. anatolica* subsp. *anatolica* are showed and the data are the first report. The listed data provide important contributions to the cytotaxonomy of genus *Paronychia*: (1) the diploid chromosome number of $2n = 18$, (2) the basic chromosome number of $x = 9$, (3) no polyploidy with ploidy level of $2x$, and (4) symmetrical karyotype. Especially, taxon contains

very different karyotypic data from other subspecies and it is very important knowledge for cytotaxonomic point of view.

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