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## Karyotype Analysis of Two Varieties of *Paronychia amani* (Caryophyllaceae, Paronychioideae)

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**ABSTRACT:** The chromosome number and karyotype analysis of *Paronychia amani* var. *amani* and *P. amani* var. *minutiflora* are described for the first time. The diploid chromosome numbers and karyotype formulae are  $2n = 4x = 36 = 32m + 4sm$  in taxa. The karyotypes are symmetrical type including metacentric and submetacentric chromosomes. The small chromosomes varied from 0.96  $\mu\text{m}$  (var. *amani*) to 2.74  $\mu\text{m}$  (var. *minutiflora*). *P. amani* var. *amani* and *P. amani* var. *minutiflora* are very little different varieties morphologically. There are some similarities between varieties in the karyological data. (i) equal number of chromosomes, (ii) same karyotype formula, (iii) very close karyotype asymmetry values, (iv) polyploidy by ploidy level of  $4x$ . These seem to support morphological closeness.

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

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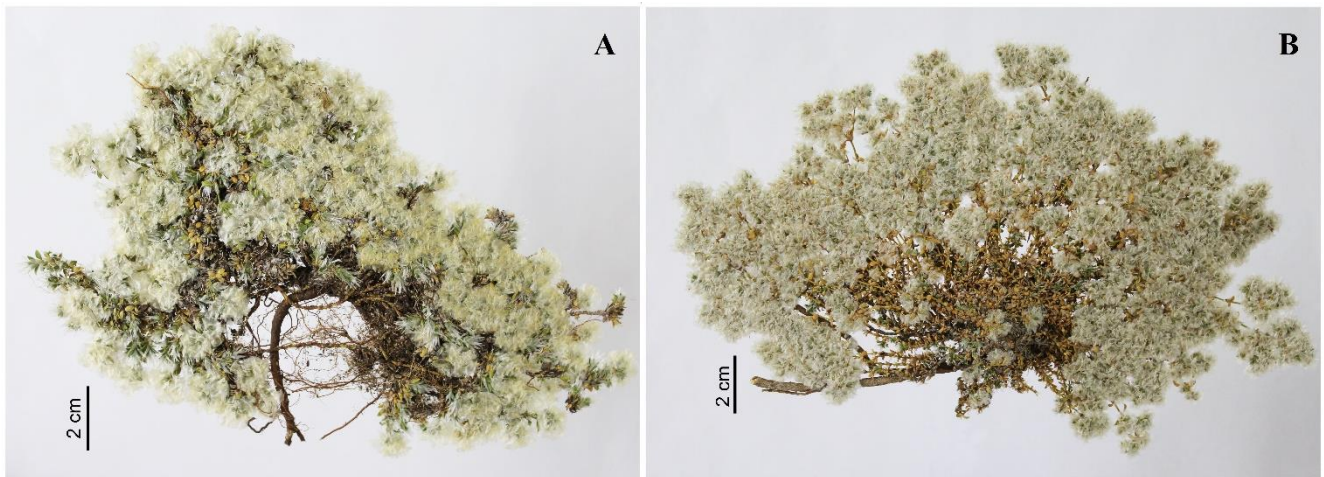
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## INTRODUCTION

The genus *Paronychia* Miller is placed in the subfamily Paronychioideae Meisn. of the family Caryophyllaceae. It contains approximately 110 species of annual or perennial found all over the world except Southern Africa, Southeast Asia (Chaudhri, 1968). The distribution centers of the species are Turkey, America and Peru-Bolivia (Bittrich, 1993; Eroğlu et al., 2017, 2020).

*Paronychia amani* Chaudhri is a perennial herb with flowers white; stems much branched at the base, decumbent to ascending, 3-18 cm length; base diameter 1-3 mm. It contains perennial three varieties. *P. amani* var. *amani* and *P. amani* var. *minutiflora* Chaudhri are narrow endemic taxa in Turkey (Figure 1). *P. amani* var. *amani* shows the distribution in 800-2345 m on rocky-stony place and forest open areas. *P. amani* var. *minutiflora* shows the distribution in 1350-1370 m on rocky-stony place. The variety *amani* is distinguished from other variety with calyx length (Chaudhri, 1967, 1968; Bittrich, 1993).



**Figure 1.** *Paronychia amani* var. *amani* (A) and *Paronychia amani* var. *minutiflora* (B).

The chromosomal data are increasingly used to elucidate the origin, speciation and evolutionary and phylogenetic relationships of plants in plant taxonomy. Important cytotaxonomic characters are numbers (basic and diploid), lengths (chromosome, relative and total haploid), chromosome structure and number variations (deletion, inversion, translocation, dysploidy and polyploidy), centromeric data (centromeric index and karyotype formula), and karyotype asymmetry (intrachromosomal and interchromosomal) (Peruzzi and Eroğlu, 2013; Eroğlu, 2015; Baltisberger and Hörandl, 2016; Eroğlu et al., 2020; Martin et al, 2020). In *Paronychia*, the basic number is shaped as  $x = 5, 7, 8, 13$  and more common  $x = 9$  (Eroğlu et al., 2020; Eroğlu and Budak, 2020). The karyological data are recorded in 24 Turkish taxa. Four taxa are only diploid and they reveal only one basic number:  $x = 9$  ( $2n = 18$ ). Polyploidy is represented by 17 taxa with three polyploidy levels, which are tetraploidy ( $2n = 4x = 28, 36$  and  $52$ ), hexaploidy ( $2n = 6x = 54$ ), and octoploidy ( $2n = 8x = 56, 72$  and  $104$ ). Other taxa 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, 2020; Eroğlu and Budak, 2020).

The main aims of this study are to contribute to the cytotaxonomy of *Paronychia* with the following marks: chromosome number, karyotype formula, detailed chromosome measurements, and karyotype asymmetry. In the light of karyological data, two varieties of *Paronychia amani* will be compared and contribution to morphological data will be determined.

## MATERIALS AND METHODS

### Plant Material

*P. amani* var. *amani* and *P. amani* var. *minutiflora* were collected from their natural habitats in Turkey. Collection information regarding two varieties is listed below.

*Paronychia amani* var. *amani* – Bursa: Uludağ, Uludağ top, South slopes, 2214 m, 17.07.2010, Budak 2461 & Hamzaoglu (Bozok Hb.). *Paronychia amani* var. *minutiflora* – Sivas: Yıldızeli, between Yıldızeli and Sivas, 1355 m, 11.06.2012, Budak 2587 & Hamzaoglu (Bozok Hb.).

### Cytogenetic Procedure

The stages of cytogenetic procedure are listed below, respectively. (i) between moist whatman papers in petri dishes at room temperature for germination, (ii) 16 hours  $\alpha$ -monobromonaphthalene for pretreatment, (iii) Carnoy's fixative (3 alcohol: 1 acetic acid) for fixation, (iv) 12 min HCl (1 N) at 60°C for hydrolyze, (v) 2 h aceto orcein (2%) for staining, (vi) acetic acid (45%) for squashing, (vi) DPX for permanent preparation (Elçi and Sancak, 2013; Eroğlu et al., 2020; Martin et al, 2020).

### Karyotype Analysis

At least ten metaphase cells were investigated to determine chromosome numbers. The chromosomal measurements were made using the KaryoType software. The following parameters were used to characterize the chromosomes numerically (Table 1).

**Table 1.** The karyological parameters used for chromosome characterization.

Parameters	Formula
Total chromosome length (TL)	TL = LA (long arm) + SA (short arm)
Arm ratio (AR)	AR = LA / SA
Centromeric index (CI)	CI = [(SA) / (LA + SA)] × 100
Total haploid length (THL)	THL = TL <sub>1</sub> + TL <sub>2</sub> + TL <sub>3</sub> + ..... TL <sub>n</sub>
Mean haploid length (MHL)	MHL = THL / n
Relative length (RL)	RL = [(LA + SA) / THL] × 100
Mean centromeric asymmetry (M <sub>CA</sub> )	M <sub>CA</sub> = mean [( $\Sigma$ LA - $\Sigma$ SA) / ( $\Sigma$ LA + $\Sigma$ SA)] × 100
Coefficient of variation of chromosome length (CV <sub>CL</sub> )	CV <sub>CL</sub> = [SD (standard deviation) / MHL] × 100

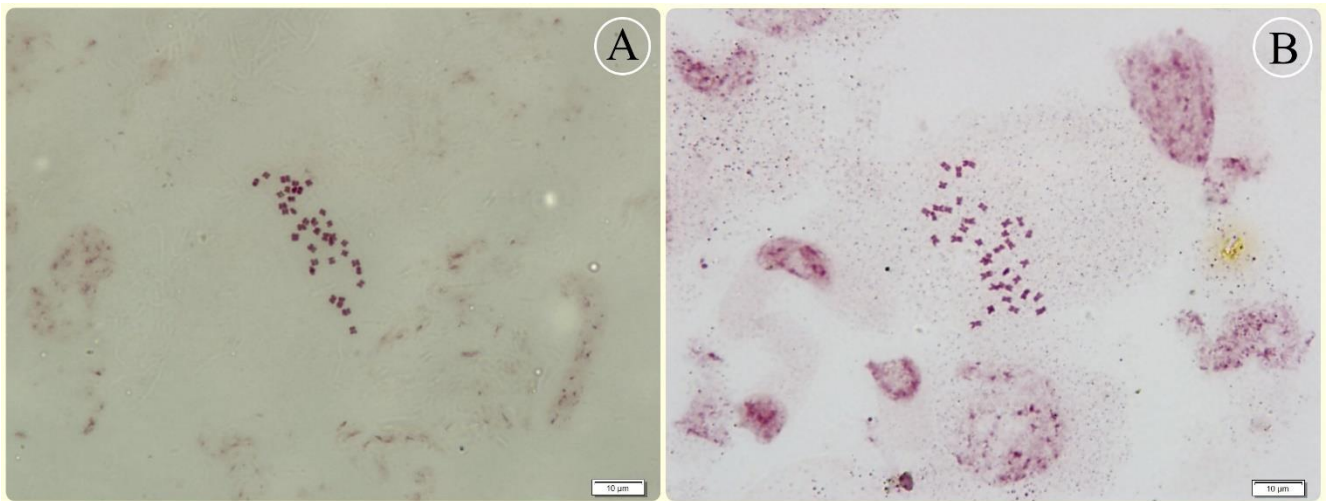
Karyotype formulae were by chromosome morphology based on centromere position according to Levan et al. (1964). The ideograms were drawn based on chromosome arm length (arranged large to small).

Karyotype asymmetries were estimated by many different parameters as the mainly M<sub>CA</sub> (intrachromosomal asymmetry) and CV<sub>CL</sub> (Interchromosomal Asymmetry) (Table 1). These parameters are S Cl. (Stebbins' Classification), AsK (Karyotype Asymmetry Index), TF (total form percent), Syi (Index of Karyotype Symmetry), Rec (Chromosomal Size Resemblance), A1 (Intrachromosomal Asymmetry), A2 (interchromosomal asymmetry), DI (dispersion index), A (degree of karyotype asymmetry), AI (asymmetry index), and CV<sub>CI</sub> (Variation Coefficient of Centromeric Index) (Stebbins, 1971; Paszko, 2006; Peruzzi and Eroğlu, 2013; Eroğlu, 2015).

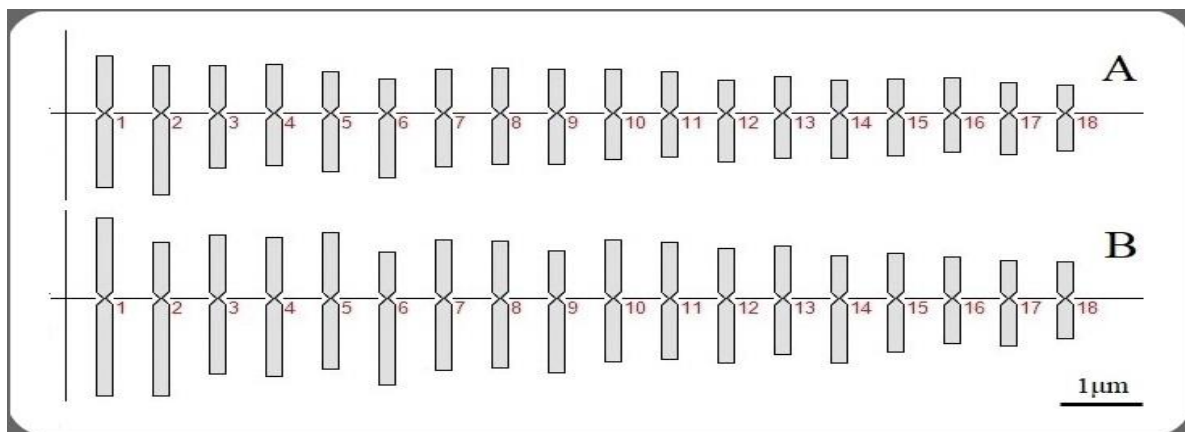
## RESULTS AND DISCUSSION

Figure 2A and Figure 3A represent the metaphase chromosomes and haploid ideogram in *P. amani* var. *amani*. No all chromosomes have satellite and secondary constriction. The karyotype formula is  $2n = 4x = 36 = 32m + 4sm$  by metacentric chromosomes out of submetacentric chromosomes 2 and 6. THL and MHL values are 24.78 and 1.38  $\mu$ m, respectively. Detailed chromosomal measurements of *P. amani* var. *amani* are given in Table 2. The rates of RL and CI range from 3.87-8.07% and 34.23-49.63%,

respectively. The low and high CI are characterized by centromere shift and median region, respectively. The most symmetric chromosome is chromosome 10 by the highest centromeric index (49.63%) and the lowest arm ratio (1.01).



**Figure 2.** Metaphase chromosomes: *P. amani* var. *amani* (A) and *P. amani* var. *minutiflora* (B).



**Figure 3.** The haploid ideogram of *P. amani* var. *amani* (A) and *P. amani* var. *minutiflora* (B).

Figure 2B and Figure 3B represent the metaphase chromosomes and haploid ideogram in *P. amani* var. *minutiflora*. Not all chromosomes have satellite and secondary constriction. The karyotype formula is  $2n = 4x = 36 = 32m + 4sm$  by metacentric chromosomes out of submetacentric chromosomes 11 and 15. THL and MHL values are 33.01 and 1.83  $\mu\text{m}$ , respectively and the chromosomes are slightly larger than the chromosomes of other variety. Detailed chromosomal measurements of *P. amani* var. *minutiflora* are given in Table 3. The rates of RL and CI range from 3.39-8.30% and 34.65-48.57%, respectively. The low and high CI are characterized by centromere shift and median region, respectively. The most symmetric chromosome is chromosome 5 by the highest centromeric index (48.57%) and the lowest arm ratio (1.06).

The diploid number is recorded as  $2n = 36$  for the first time in *P. amani* var. *amani* and *P. amani* var. *minutiflora*. It was reported that diploid chromosome number is  $2n = 36$  in Turkish taxa, which are *P. adalia* Chaudhri, *P. aksoyii* Budak, *P. anatolica* Czeck. subsp. *balansae* Chaudhri, *P. angorensis* Chaudhri, *P. argentea* Lam., *P. argyroloba* Stapf., *P. beauverdii* Czeck., *P. carica* Chaudhri, *P. cephalotes* (M. Bieb.) Besser, *P. chionaea* Boiss subsp. *chionaea*, *P. condensata* Chaudhri, *P. davrazensis* Budak, *P. galatica* Chaudhri, *P. kapela* (Hacq.) A. Kern., *P. saxatilis* Chaudhri, and *P. turcica* Chaudhri, alphabetically (Löve, 1975; Lorenzo Andreu and García Sanz, 1950; Eroğlu et al., 2017, 2020).

**Table 2.** Detailed chromosomal data of *Paronychia amani* var. *amani*.

Chromosome pair	Length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio	Relative length (%)	Centromeric index (%)	Chromosome type
1	2.00	1.12	0.88	1.27	8.07	44.00	metacentric
2	1.98	1.25	0.73	1.71	7.99	36.87	submetacentric
3	1.53	0.81	0.72	1.12	6.17	47.06	metacentric
4	1.51	0.77	0.74	1.04	6.09	49.01	metacentric
5	1.50	0.88	0.62	1.42	6.05	41.33	metacentric
6	1.49	0.98	0.51	1.92	6.01	34.23	submetacentric
7	1.47	0.80	0.67	1.19	5.93	45.58	metacentric
8	1.44	0.76	0.68	1.12	5.81	47.22	metacentric
9	1.42	0.76	0.66	1.15	5.73	46.48	metacentric
10	1.35	0.68	0.67	1.01	5.45	49.63	metacentric
11	1.28	0.65	0.63	1.03	5.17	49.22	metacentric
12	1.22	0.73	0.49	1.49	4.92	40.16	metacentric
13	1.21	0.66	0.55	1.20	4.88	45.45	metacentric
14	1.16	0.67	0.49	1.37	4.68	42.24	metacentric
15	1.12	0.62	0.50	1.24	4.52	44.64	metacentric
16	1.09	0.56	0.53	1.06	4.40	48.62	metacentric
17	1.05	0.61	0.44	1.39	4.24	41.90	metacentric
18	0.96	0.55	0.41	1.34	3.87	42.71	metacentric

**Table 3.** Detailed chromosomal data of *Paronychia amani* var. *minutiflora*.

Chromosome pair	Length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio	Relative length (%)	Centromeric index (%)	Chromosome type
1	2.74	1.50	1.24	1.21	8.30	45.26	metacentric
2	2.35	1.50	0.85	1.76	7.12	36.17	metacentric
3	2.14	1.16	0.98	1.18	6.48	45.79	metacentric
4	2.13	1.19	0.94	1.27	6.45	44.13	metacentric
5	2.10	1.08	1.02	1.06	6.36	48.57	metacentric
6	2.02	1.32	0.70	1.89	6.12	34.65	metacentric
7	1.98	1.09	0.89	1.22	6.00	44.95	metacentric
8	1.93	1.05	0.88	1.19	5.85	45.60	metacentric
9	1.86	1.14	0.72	1.58	5.63	38.71	metacentric
10	1.86	0.96	0.90	1.07	5.63	48.39	metacentric
11	1.76	0.91	0.85	1.07	5.33	48.30	submetacentric
12	1.74	0.98	0.76	1.29	5.27	43.68	metacentric
13	1.63	0.84	0.79	1.06	4.94	48.47	metacentric
14	1.61	0.97	0.64	1.52	4.88	39.75	metacentric
15	1.49	0.80	0.69	1.16	4.51	46.31	submetacentric
16	1.29	0.67	0.62	1.08	3.91	48.06	metacentric
17	1.26	0.70	0.56	1.25	3.82	44.44	metacentric
18	1.12	0.58	0.54	1.07	3.39	48.21	metacentric

In *P. amani* var. *amani* and *P. amani* var. *minutiflora*, the basic number and ploidy level are recorded as  $x = 9$  and  $4x$ , respectively. The most common basic numbers are  $x = 8$  in Spain and Macaronesia and  $x = 9$  in Turkish taxa (Hartman, 1972, 1974; Diosdado and Pastor, 1994; Suda et al., 2003; Eroğlu et al., 2017, 2020; Eroğlu and Budak, 2020). Polyploid nature is also common. Basic numbers (ploidy levels) are  $x = 7$  ( $4x$ ,  $8x$ ) in *P. argentea*, *P. echinulata* Chater, and *P. polygonifolia* (Vill.) DC.  $x = 9$  ( $4x$ ,  $6x$ ,  $8x$ ) in 17 Turkish taxa, and  $x = 13$  ( $4x$ ,  $8x$ ) *P. chionaea* 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, 2020; Eroğlu and Budak, 2020).

In both taxa, karyotype formula is  $32m + 4sm$ . It is recorded the similar karyotype formulae, which are  $34m + 2sm$  in *P. aksoyii*, *P. carica*, *P. davrazensis*, and *P. saxatilis*;  $28m + 8sm$  in *P. argyroloba*; and  $68m + 4sm$  in *P. chionaea* subsp. *chionaea* (Eroğlu et al., 2020). In addition, Diosdado and Pastor (1994) reported the karyotypes including st chromosomes in *P. argentea* and *P. echinulata*.

Karyotype asymmetry is one of the most important parameters in chromosome studies (Eroğlu, 2015). The  $M_{CA}$  and  $CV_{CL}$  are the most reliable parameters among asymmetry indexes (Peruzzi and Eroğlu, 2013).  $M_{CA}$  values of *P. amani* var. *amani* and *P. amani* var. *minutiflora* are 11.52 and 11.17, respectively.  $CV_{CL}$  values of *P. amani* var. *amani* and *P. amani* var. *minutiflora* are 20.65 and 21.99, respectively (Table 4). A perfect positive correlation was calculated between  $M_{CA}$  and  $CV_{CL}$  ( $r = 1.000$ ). However, according to the  $M_{CA}$  and  $CV_{CL}$  values, symmetrical and asymmetrical karyotypes are different. While *P. amani* var. *minutiflora* is little more symmetrical than *P. amani* var. *amani* in  $M_{CA}$ , As K, TF, Syi, Rec, A1 and A, it is opposite in  $CV_{CL}$  together with only A2 (Table 4). Peruzzi and Eroğlu (2013) reported that the  $CV_{CL}$  gives the reliable results together with  $M_{CA}$ .

**Table 4.** The karyotype asymmetry values in *P. amani* var. *amani* and *P. amani* var. *minutiflora*.

Parameters	<i>P. amani</i> var. <i>amani</i>	<i>P. amani</i> var. <i>minutiflora</i>	Minimum–Maximum values*
S Cl.	1B	1B	1A – 4C
As K (%)	55.93	55.86	50 – 100
TF (%)	44.07	44.14	50 – 0
Syi (%)	78.79	79.01	100 – 0
Rec (%)	68.83	66.93	100 – 100
A <sub>1</sub>	0.20	0.19	0 – 1
A <sub>2</sub>	0.21	0.22	0 – 0
DI	9.32	9.70	0 – 0
A	0.12	0.11	0 – 1
AI	2.00	2.14	0 – NA
$CV_{CI}$	9.69	9.74	0 – NA
$CV_{CL}$	20.65	21.99	0 – 0
$M_{CA}$	11.52	11.17	0 – 100

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

In intrachromosomal asymmetry, both varieties have symmetrical karyotypes ( $M_{CA} = 11.52$  and 11.17). It was reported the similar index values in *P. galatica* (10.57), *P. anatolica* subsp. *balansae* (11.62), *P. chionaea* subsp. *kemaliya* (11.83), and *P. condensata* (11.96) (Eroğlu et al., 2020). According to present and previous studies, Turkish *Paronychia* have symmetric karyotypes, which indicate beginning phase of karyotype evolution.

*Paronychia amani* var. *amani* and *P. amani* var. *minutiflora* are very little different varieties morphologically. A taxonomic key is given below with distinctive character for two taxa.

1. Calyx least 2.75 mm long ..... *P. amani* var. *amani*
1. Calyx up to 2.25 mm long ..... *P. amani* var. *minutiflora*

**Table 5.** Comparison of morphological and karyological data of varieties.

<i>P. amani</i> var. <i>amani</i>	<i>P. amani</i> var. <i>minutiflora</i>	Data type
Calyx least 2.75 mm long	Calyx up to 2.25 mm long	Morphological
$x = 9$	$x = 9$	Karyological
$2n = 36$	$2n = 36$	Karyological
Tetraploid (4x)	Tetraploid (4x)	Karyological
$32m + 4sm$	$32m + 4sm$	Karyological
Symmetrical karyotype	Symmetrical karyotype	Karyological

There are some similarities between varieties in the karyological data. (i) equal number of chromosomes, (ii) same karyotype formula, (iii) very close karyotype asymmetry values. These seem to support morphological closeness. A comparison list containing morphological and karyological data is given in Table 5. In our opinion, there is no problem regarding systematic position of the varieties.

## CONCLUSION

In this study, the karyological data of *Paronychia amani* var. *amani* and *P. amani* var. *minutiflora* were showed for the first time. The present results supply significant contributions to cytotaxonomy: (i) diploid number,  $2n = 36$ ; (ii) basic number,  $x = 9$ ; (iii) symmetrical karyotypes,  $32m + 4sm$ ; and (iv) polyploidy by ploidy level of  $4x$ . Two varieties include quite close karyological data and there is no problem regarding systematic position of the varieties according to these data.

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## Conflict of Interest

The article authors declare that there is no conflict of interest between them.

## Author's Contributions

The authors declare that they have contributed equally to the article.

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