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# A karyological study on taxa of *Coronopus* D.C. section of *Plantago* L. genus (Plantaginaceae) in Turkey

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In this study the chromosome number and morphology of five taxa of the *Coronopus* D.C. section – *Plantago coronopus* subsp. *coronopus* subsp. *coronopus* subsp. *coronopus* (QUSS.) PILGER, *P. crassifolia* FORSSKAL, *P. maritima* L., and *P. holosteum* SCOP. (Plantaginaceae) – in Turkey were investigated using karyological techniques. The seeds of taxa were collected from natural habitats. The chromosome numbers are determined for four taxa (*P. coronopus* subsp. *coronopus* subsp. *coronopus* and *P. maritima*) as 2n = 4x = 20 and one taxon (*P. holosteum*) as 2n = 2x = 12. The chromosome numbers and karyotype analysis of the species are reported for the first time for Turkey.

Keywords: chromosome number; karyology; Plantago

#### Introduction

The genus *Plantago* L. includes 483 species distributed around the world (Tutel et al. 2005). There are two subgenera, *Euplantago* and *Psyllium*. The *Psyllium* subgenus contains only the *Psyllium* section, but the *Euplantago* subgenus has 18 sections (Pilger 1937). There are nine sections, 21 species and 23 taxa of *Plantago* in Turkey according to the *Flora of Turkey* (Tutel 1971, 1982, 1993; Davis 1982, 1988; Güner et al. 2000). The genus *Plantago* has a tribasic chromosome number, x = 4, 5 and 6, where x = 6 is the primary base number and the others are considered to be secondary differentiations of it (Dhar and Sharma 1999). The basic number of x = 5 is found in sect. *Leucopsyllium* and sect. *Coronopus*.

Polyploidy is common in *Plantago*: roughly twothirds of *Plantago* species for which chromosome have been counted are polyploid. According to some studies polyploidy does not play a large role in differentiation (Briggs 1973; Rahn 1996), except for sect. *Oliganthos* from subgenus *Plantago* (Rahn 1984). In contrast, Ishikawa et al. (2009) said that the all *Plantago* subgenera have a history of allopolyploidy.

Plantaginaceae has eight sections in Turkey. The *Coronopus* D.C. section contains five taxa (*Plantago coronopus* subsp. *coronopus* L., *Plantago coronopus* subsp. *commutata* (GUSS.) PILGER, *Plantago crassifolia* FORSSKAL, *Plantago maritima* L. and *Plantago holosteum* SCOP.) in Turkey. In this study, we aim to characterize the karyology of these taxa. To date the only studies of these taxa available in the literature are reports of their chromosome numbers (Gregor 1939; Moore 1976; Dalgaard 1989; Mohsenzadeh et al. 2008).

Materials and methods

Plant materials were collected from the localities detailed in Table 1. Voucher specimens are deposited in the herbarium of the Department of Biology at Celal Bayar University. The taxonomical nomenclatures adopted here follow Davis (1982). We used root tips to count chromosome numbers.

For the study of somatic chromosomes, root tips were obtained from germinated seeds in sterilized Petri dishes. They were pre-treated in  $\alpha$ -monobromonaphtalene (16 h) and then fixed in a mixture of ethanol and acetic acid (24 h). Root tips were hydrolyzed with 1 N HCl at 60°C for 15 min, stained in Feulgen reagent for 1 h in darkness, and finally squashed in 45% acetic acid (Elçi 1994). Chromosome measurements were based on five metaphase plates. Slides were examined under a Leica DM 3000 LB photomicroscope (Leica Microsystems, Wetzlar, Germany) and photographs were taken with the same microscope. The karyograms were drawn from 10 mitotic metaphases. Chromosome nomenclature was carried out according to the method described by Levan et al. (1964).

#### Results

#### P. coronopus subsp. coronopus

The chromosome number of this taxon is determined as 2n = 4x = 20 and basic number as x = 5. This taxon consists of one pair of median (M) (4th), two pairs of submedian (sm) (7th and 9th) and seven pairs of median region (m) (1st, 2nd, 3rd, 5th, 6th, 8th and 10th) chromosomes according to centromere position. Satellites were not observed this taxon. Chromosome sizes were 1.83 and 4.25 µm. The longest and shortest arm sizes

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Table 1. Localities of investigated Plantago species used for karyological studies.

Species	Locality	Specimen number
P. coronopus subsp. coronopus	İzmir, Aliağa, back beach area, sea level, 18 May 2012	BBOZDAG 1150
P. coronopus subsp. commutata	İzmir, Gümüldür, near forest camping, sea level, 25 May 2012	BBOZDAG 1170
P. crassifolia	İzmir, Çeşme, sea level, 22 June 2012	BBOZDAG 1250
P. maritima	İzmir, Çeşme, sea level, 22 June 2012	BBOZDAG 1260
P. holosteum	Kütahya, Murat Mounth, Kesiksöğüt area, 30 June 2012	BBOZDAG 1280

were 2.33 and 0.75  $\mu$ m, respectively. Total chromosome size was determined as 25.78  $\mu$ m (Figures 1A, B, 2A, Table 2).

#### P. coronopus subsp. commutata

The chromosome number of this taxon is determined as 2n = 4x = 20 and basic number as x = 5. According to the karyotype analysis, taxon has two pairs of median (M) (1st and 10th), three pairs of submedian (sm) (2nd, 4th and 7th) and five pairs of median region (m) (3rd, 5th, 6th, 8th and 9th) chromosomes. Satellites were not observed. Chromosome sizes were 2.00 and 4.00 µm. The longest and shortest arm sizes were 2.00 and 1.00 µm, respectively. Total chromosome size was determined as 26.07 µm (Figures 1C, D, 2B, Table 3).

# P. crassifolia

The chromosome number of this species is determined as 2n = 4x = 20 and the basic number as x = 5. The karyotype of this species consists of nine pairs of median region (m), and one pair of median (M) chromosomes. The centromeres of the 1st, 2nd, 3rd, 4th, 6th, 7th, 8th, 9th and 10th chromosomes are at the median region (m); the 5th chromosomes are at the median (M). No satellites were observed in the karyotype of this species. Chromosome sizes vary from 2.37 to 4.66 µm. The longest arm is 2.78 µm and the shortest arm is 1.00 µm (Figures 1E, F, 2C, Table 4).

#### P. maritima

The chromosome number of this species is determined as 2n = 4x = 20 and the basic number as x = 5. The karyotype of this species consists of nine pairs of median region (m), and one pair of median (M) chromosomes. The centromeres of the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 9th and 10th chromosomes are at the median region (m); and of the 8th chromosomes are at the median (M). No satellites were observed in the karyotype of this species. Chromosome sizes vary from 2.40 to 5.18 µm. The longest arm is 3.03 µm and the shortest arm is 0.98 µm (Figures 1G, H, 2D, Table 5).

# P. holosteum

The chromosome number of this species is determined as 2n = 2x = 12 and basic number as x = 6. According to the karyotype analysis, this taxon has one pair of median (M) (4th), two pairs of submedian (sm) (5th and 6th) and three pairs of median region (m) (1st, 2nd and 3rd) chromosomes. Satellites were not observed. The chromosome sizes were 2.17 and 4.08  $\mu$ m. The longest and shortest arm sizes were 2.33 and 0.59  $\mu$ m, respectively. The total chromosome size was determined as 18.66  $\mu$ m (Figures 1I, J, 2E, Table 6).

# Discussion

In this study, we determined chromosome numbers and detailed measurements of taxa of Coronopus section of the genus *Plantago* in Turkey. No previous studies have been performed on the chromosomes except for determination of the chromosome numbers of these species. We found that four tetraploid taxa (P. coronopus subsp. coronopus, P. coronopus subsp. commutata, P. crassifolia, and P. maritima) have chromosome numbers of 2n = 4x = 20 and a basic number of x = 5. P. holosteum has 2n = 2x = 12 and x = 6. Taxa have three different chromosome types - median (M), submedian (sm) and median region (m) - according to centromere position. We did not observe satellites in any investigated taxa. Variation was found in total chromosome size, short and long arm size, and chromosome arm ratios among the taxa. The longest chromosome was determined in P. maritima as 5.18 µm and the shortest chromosome was determined in P. coronopus subsp. coronopus as 1.83 μm.

According to one of the few studies, *P. crassifolia* has 2n = 24 and *P. maritima* group the numbers 2n = 12 and 24 chromosome number (Gregor 1939). In another study, the chromosome number of *P. maritima* L. subsp. *borealis* (Lange) Blytt & Dahl was determined as 2n = 12 (Dalgaard 1989). According to these studies, these plants have x = 6 as a basic number, which is different from our results. We determined the chromosome number of *P. crassifolia* and *P. maritima* to be 2n = 20 and the basic number x = 5.

The chromosome numbers of *P. coronopus* subsp. *commutata* and *P. coronopus* subsp. *coronopus* were determined as 2n = 20 and 2n = 10, respectively (Moore 1976). In another study from Iran, the chromosome numbers of *P. coronopus* subsp. *commutata* and *P. coronopus* subsp. *coronopus* subsp. *coronopus* subsp. *coronopus* and 2n = 20, respectively (Mohsenzadeh et al. 2008). The basic

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Figure 1. Microphotographs of somatic metaphases of taxa of *Coronopus* section. (A, B) *P. coronopus* subsp. *coronopus* (2n = 20); (C, D) *P. coronopus* subsp. *commutata* (2n = 20); (E, F) *P. crassifolia* (2n = 20); (G, H) *P. maritima* (2n = 20); (I, J) *P. holosteum* (2n = 12). Scale bars: 10 µm.



Figure 2. Haploid idiograms of taxa. (A) *P. coronopus* subsp. *coronopus* (2n = 20); (B) *P. coronopus* subsp. *commutata* (2n = 20); (C) *P. crassifolia* (2n = 20); (D) *P. maritima* (2n = 20); (E) *P. holosteum* (2n = 12). Scale bars: 1 µm.

number of these plant samples is x = 5. In this study, we determined the chromosome number of these taxa to be 2n = 20 and basic number to be x = 5.

There are many different studies of the chromosome number in *Plantago* species. Roughly 75% of the studied species have x = 6 as a base number, and the remaining 25% have x = 5 (Rahn 1957, 1996). Badr (1999) explained that evolution in *Plantago* has involved a reduction in the nuclear DNA content with a decrease in the

number of chromosomes from x = 6 to x = 5 to x = 4. Turkey (*P. coronopus* subsp. *commutata*, *P. coronopus* subsp. *coronopus*, *P. crassifolia* and *P. maritima*) with the basic number of x = 5 evolved before *P. holosteum*. *P. holosteum* has a basic chromosome number of x = 6. The rate of evolution of this taxon is progressing more slowly than other *Coronopus* taxa in Turkey.

This study presents the chromosome number of *Coronopus* sect. in Turkey. The chromosome numbers

Cn	C (µm)	L (µm)	S (µm)	R	I (μm)	RS	СР
1	4.25	2.33	1.92	1.21	45.17	16.48	Median region (m)
2	3.08	1.83	1.25	1.46	40.58	11.94	Median region (m)
3	2.75	1.66	1.09	1.52	39.63	10.66	Median region (m)
4	2.66	1.33	1.33	1.00	50.00	10.31	Median (M)
5	2.58	1.50	1.08	1.38	41.86	10.01	Median region (m)
6	2.42	1.42	1.00	1.42	41.32	9.38	Median region (m)
7	2.16	1.41	0.75	1.88	34.72	8.38	Submedian (sm)
8	2.13	1.30	0.83	1.56	38.96	8.26	Median region (m)
9	1.92	1.32	0.60	2.20	31.25	7.45	Submedian (sm)
10	1.83	1.08	0.75	1.44	40.98	7.10	Median region (m)
Total ch	romosome size: 2	5.78 μm.					6 ( )

Table 2. Parameters of mitotic metaphase chromosomes of *P. coronopus* subsp. coronopus.

Abbreviations: Cn, chromosome number; C, total chromosome length; L, long arm length; S, short arm length; R, arm ratio = L/S; I, centromeric index =  $(S/C) \times 100$ ; RS, relative size; CP, centromeric position.

Table 3. Parameters of mitotic metaphase chromosomes of P. coronopus subsp. commutate.

Cn	C (µm)	L (µm)	S (µm)	R	I (μm)	RS	СР
1	4.00	2.00	2.00	1.00	50.00	15.34	Median (M)
2	3.25	2.10	1.15	1.82	35.38	12.46	Sub-median (sm)
3	2.83	1.58	1.25	1.26	44.16	10.85	Median region (m)
4	2.50	1.58	0.92	1.72	36.80	9.59	Sub-median (sm)
5	2.50	1.42	1.08	1.31	43.20	9.59	Median region (m)
6	2.42	1.50	0.92	1.63	38.01	9.28	Median region (m)
7	2.25	1.50	0.75	2.00	33.33	8.63	Sub-median (sm)
8	2.16	1.33	0.83	1.60	38.42	8.28	Median region (m)
9	2.16	1.25	0.91	1.37	42.13	8.28	Median region (m)
10	2.00	1.00	1.00	1.00	50.00	7.67	Median (M)
Total chi	romosome size: 2	6.07 μm.					

Abbreviations: Cn, chromosome number; C, total chromosome length; L, long arm length; S, short arm length; R, arm ratio = L/S; I, centromeric index =  $(S/C) \times 100$ ; RS, relative size; CP, centromeric position.

Table 4. Parameters of mitotic metaphase chromosomes of P. crassifolia.

Cn	C (µm)	L (µm)	S (µm)	R	I (μm)	RS	СР
1	4.66	2.78	1.88	1.48	40.34	13.33	Median region (m)
2	4.44	2.75	1.69	1.63	38.06	12.70	Median region (m)
3	4.13	2.50	1.63	1.53	39.46	11.81	Median region (m)
4	3.70	2.20	1.50	1.47	40.54	10.58	Median region (m)
5	3.34	2.03	1.31	1.55	39.22	9.55	Median (M)
6	3.26	1.63	1.63	1.00	50.00	9.32	Median region (m)
7	3.22	1.84	1.38	1.33	42.86	9.21	Median region (m)
8	3.03	1.78	1.25	1.42	41.25	8.67	Median region (m)
9	2.81	1.62	1.19	1.36	42.35	8.03	Median region (m)
10	2.37	1.37	1.00	1.37	42.20	6.78	Median region (m)
Total ch	romosome size: 34	4.96 μm.					5 ()

Abbreviations: Cn, chromosome number; C, total chromosome length; L, long arm length; S, short arm length; R, arm ratio = L/S; I, centromeric index =  $(S/C) \times 100$ ; RS, relative size; CP, centromeric position.

Table 5. Parameters of mitotic metaphase chromosomes of P. maritime.

Cn	C (µm)	L (µm)	S (µm)	R	I (μm)	RS	СР
1	5.18	3.03	2.15	1.41	41.50	14.96	Median region (m)
2	4.35	2.60	1.75	1.48	40.23	12.56	Median region (m)
3	3.88	2.25	1.63	1.38	42.01	11.21	Median region (m)
4	3.55	2.08	1.47	1.41	41.41	10.25	Median region (m)
5	3.38	2.00	1.38	1.45	40.83	9.76	Median region (m)
6	3.15	1.88	1.27	1.48	40.32	9.10	Median region (m)
7	3.00	1.75	1.25	1.40	41.66	8.66	Median region (m)
8	3.00	1.50	1.50	1.00	50.00	8.66	Median (M)
9	2.73	1.55	1.18	1.31	43.22	7.88	Median region (m)
10	2.40	1.42	0.98	1.45	40.83	6.93	Median region (m)
Total ch	romosome size: 34	4.62 um.					2 ()

Abbreviations: Cn, chromosome number; C, total chromosome length; L, long arm length; S, short arm length; R, arm ratio = L/S; I, centromeric index =  $(S/C) \times 100$ ; RS, relative size; CP, centromeric position.

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Cn	C (µm)	L (µm)	S (µm)	R	I (μm)	RS	СР
1	4.08	2.33	1.75	1.33	42.89	21.86	Median region (m)
2	3.58	2.08	1.50	1.38	41.90	19.18	Median region (m)
3	3.33	1.92	1.41	1.36	42.34	17.84	Median region (m)
4	3.00	1.50	1.50	1.00	50.00	16.07	Median (M)
5	2.50	1.85	0.67	2.76	26.80	13.39	Submedian (sm)
6	2.17	1.58	0.59	2.68	27.19	11.63	Submedian (sm)
Total ch	romosome size: 13	8.66 um					( )

Table 6. Parameters of mitotic metaphase chromosomes of P. holosteum.

Abbreviations: Cn, chromosome number; C, total chromosome length; L, long arm length; S, short arm length; R, arm ratio = L/S; I, centromeric index =  $(S/C) \times 100$ ; RS, relative size; CP, centromeric position.

of four taxa were determined as 2n = 20 and one taxon was determined as 2n = 12; also idiograms and karyograms of these taxa are given here for the first time.

## **Disclosure statement**

No potential conflict of interest was reported by the authors.

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