

Morphological, Karyological Features and Pollen Morphology of Endemic *Psephellus Pyrrhoblephara* Boiss. From Turkey

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Abstract

In this study, morphological, morphometrical, karyological and detailed pollen morphology of the endemic *Psephellus pyrrhoblephara* Boiss. was studied for the first time. In the morphologic study of the plant, some morphological and morphometrical features was observed and compared with the Flora of Turkey records. Karyogram and idiograms of the plant were also prepared. The chromosome number of *Psephellus pyrrhoblephara* was found as $2n = 30$ and haploid karyotype formula $8m + 2sm + 5M$. Metaphase chromosome length ranging from 2.36 to 1.34 μm and the total haploid chromosome length was 26, 16 μm . The results of the light microscope investigation of the pollen revealed that they have radially symmetrica, iso-polar tricolporate, spheroidal type and exine ornamentation was also determined scabrate. The results were discussed with genus patterns.

Key words: Turkish endemic, *Psephellus pyrrhoblephara*, morphology, pollen, Karyotype.

1. Introduction

Recently, *Centaurea* L. has been divided into four genera. According to the revised system these genera are *Centaurea*, *Rhaponticoides* Vaill., *Psephellus* Cass. and *Cyanus* Mill. One of these is *Psephellus* that has 75–80 species and a distribution with a centre in East Anatolia, Caucasian and northwest Iran; only few species occur outside this area [1].

In Wagenitz & Hellwig (2000), 12 sections that had been included in the genus *Centaurea* were transferred to the genus *Psephellus*. Some of these species occur only in Turkey. The former sections of the genus *Centaurea* were: sect. *Psephelloideae*, *Psephellus*, *Hyalinella*, *Aetheopappus*, *Amblyopogon*, *Heterolophus*, *Czerniakovskya*, *Odontolophoideae*, *Odontolophus*, *Xanthopsis*, *Uralepis* and *Sosnovskya*. New combinations under the genus *Psephellus* are provided for these sections and 35 species, especially from Turkey and Iran.

In recent taxonomic revisions of the genus *Centaurea* [1-2-3] several of our investigated species were excluded from this genus and

shifted to the genus *Psephellus* (*C. appendicigera*, *C. pecho*) and to the genus *Cyanus*, [*C. pichleri* subsp. *pichleri* Boiss., *C. pichleri* subsp. *extrarosularis* (Hayek and Siehe) [4].

The members of sect. *Psephelloideae* have membraneous or hyaline phyllary appendages of very variable size and form, and are sometimes slightly decurrent, entire, dentate or ciliate, but never with a prominent terminal mucro or spinule. Their achenes are large and their pappus scabrous. Sect. *Hyalinella* is similar to sect. *Psephelloideae* but the leaves are distinctly discolorous, tomentose below, green and more or less arachnoid above and the phyllary appendages nearly circular to broadly ovate, not decurrent or scarcely so, and with denticulate or ciliate margins [5].

Psephellus pyrrhoblephara is perennial with sterile shoots and several stems with 1-2 capitula, leaves floccose-tomentose and flowers rose-purple, marginal strongly radiant which belongs to sect. *Psephelloideae* (Boiss.) Sosn. *Psephellus pyrrhoblephara* is an endemic species for Turkey [6].

A close correlation among karyology, pollen morphology and systematic in the subtribe Centaureinae has been demonstrated where basic chromosome numbers are considered a key character for sectional classification [7-8]. The basic chromosome number of *Centaurea* is known to be $x=7, 8, 9, 10, 11, 12, 13, 14$ and 15 [9]. The chromosome number is an important karyological feature for plant taxonomy and there is close correlation between karyology and systematics in *Centaurea* [8].

In Turkey, the first detailed morphological analyses of pollen grains in *Centaurea* were made by Wagenitz (1975) in an attempt to solve phylogenetic problems in this genus. Wagenitz (1955) typified *Centaurea* pollen grains by morphological features. He used the following features to group acetolyzed pollen: pollen shape, exine ornamentation, internal and external layers of columellae, and length of colpus, pore shape and costae.

The analysis of karyotype, morphological and pollen have much important impacts for the revision and systematics of this species. A literature survey showed no previous reports of karyological and morphological features of the endemic *Psephellus pyrrhoblephara* species in Turkey.

2. Material and Method

2.1. Materials

Psephellus pyrrhoblephara Boiss. specimens were collected during to flowering stage in June, 2010, on Ankuzu Baba Mountain, at an altitude of 1200 m, Harput (Elazig-Turkey). Voucher specimens are kept at the Firat University Herbarium (FUH).

2.2. Morphological Studies

The morphological observations and biometric measurements were made on fresh and also with herbarium specimens. Herbarium specimens of *Psephellus pyrrhoblephara* were showed in Figure 1.



Figure 1. Image of *P. pyrrhoblephara*

2.3. Palynological Analysis

Pollen material was obtained from dried flower specimens. The pollen morphology of this taxon was investigated through light microscope. [10] Terminology was used for naming the exine layers. For light microscopic investigations the method of preparation described by Wodehouse [11]. Pollen identifications and counts have done by Prior binocular microscope with a 10x ocular, 10, 40 and 100x plain oil immersion objectives. The exine and intine thickness of pollen were measured using 50 replicates. From the measurements, a natural mathematical mean is calculated. For scanning electron microscopy (SEM), dry pollen grains were mounted on stubs and coated with gold.

2.4. Karyological Studies

The localities are presented in the results according to the grid system adopted for the Flora of Turkey by Davis [6], geographical position, altitude and voucher number of the investigated samples. Seeds were germinated at 25 °C on moist filter paper in Petri dishes. Actively-growing root tips, 1 cm in length, were excised from the germinating seeds and pretreated with aqueous colchicine (0.05%) for 3–3.5 h at room temperature. They were fixed with Carnoy (1:3 glacial acetic acid/absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1N HCl at 60°C for 15 min and then rinsed in tap water for a minimum of 3–5 min. Staining was carried out in Feulgen for 1 h and squash preparations were made with 45% acetic acid [12]. Microphotographs of good quality

metaphase plates of each specimen (normally at least three) were taken using an Olympus BX51 microscope and were recorded with an Olympus Camedia C-4000 digital camera. Karyotypes were obtained from well-spread metaphase plates. Long arm, short arm and total length of each chromosome were measured and relative lengths, arm ratios ($r = l/s$) and centromeric indices ($I = 100 \cdot s/c$) were calculated and used to classify and determine homologous chromosomes. The chromosome nomenclature followed Levan et al. [13]. The variation in chromosome length and chromosome arm ratio within the karyotype was estimated by calculating the standard deviation (SD) of these parameters.

3. Discussion

Psephellus pyrrhoblephara is endemic to the Irano-Turanian element of Turkey. The somatic chromosome number of this species was found to be $2n=30$ and this is the first report on the chromosome number and morphology of this species (Figure 1).

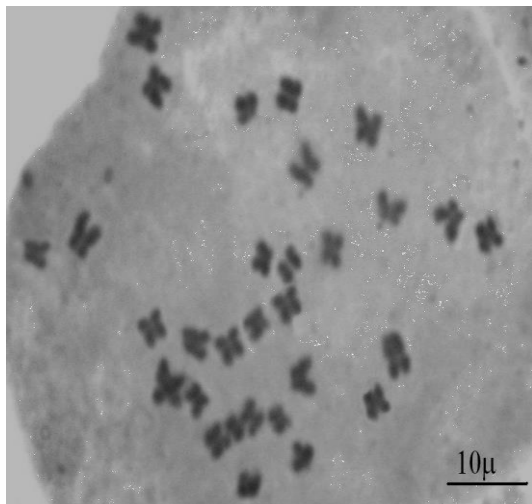


Figure 2. Somatic metaphase chromosomes of *Psephellus pyrrhoblephara* ($2n = 30$). Bar represents 10 μm .

The haploid karyotype Formula is $n = 8m + 2sm + 5M$. Long and short arm with SD, total length of the chromosome, arm ratio ($r = l/s$) and relative length are given in Table 1.

The range of metaphase chromosome lengths was 2.36 to 1.34 μm . The total length of

the haploid set was 26, 16 μm . The idiogram of haploid chromosome set are presented in Figs 2.

Table I. Morphometric data on chromosomes of *P. pyrrhoblephara*

Chromosome no.	Total Chromosome length (μm)	Long arm (μm)	Short arm (μm)	Arm ratio (long/short)	Centromeric index $I = 100 \cdot (S/C)$	Relative length (%)	Chromosome morphology
1	2,36	1,37	0,99	1,39	41,87	9,03	m
2	2,18	1,38	0,81	1,71	36,94	8,35	sm
3	2,03	1,06	0,96	1,10	47,64	7,74	m
4	1,90	0,95	0,95	1,00	50,00	7,25	M
5	1,86	0,98	0,87	1,13	47,03	7,09	m
6	1,80	0,90	0,90	1,00	50,00	6,90	M
7	1,74	0,90	0,84	1,08	48,04	6,65	m
8	1,70	0,93	0,77	1,22	45,08	6,49	m
9	1,69	0,86	0,83	1,04	49,13	6,47	m
10	1,60	0,83	0,77	1,08	48,15	6,12	m
11	1,56	1,00	0,56	1,80	35,77	5,95	sm
12	1,50	0,75	0,75	1,00	50,00	5,73	M
13	1,46	0,74	0,72	1,02	49,45	5,57	m
14	1,45	0,72	0,72	1,00	50,00	5,53	M
15	1,34	0,67	0,67	1,00	50,00	5,13	M

m= Median, sm = submedian M = median point

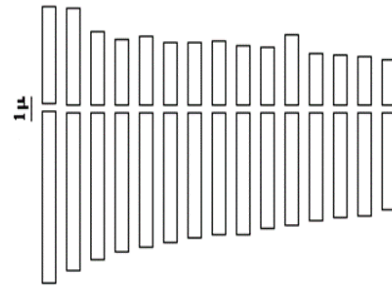


Figure 3. Haploid idiogram of *P. pyrrhoblephara*

The palynological characteristics of the examined specimens are given in Table 2.

Table II. The palynological characteristics of the examined of *P. Pyrrhoblephara*.

P	E	P/E	Clg	Clt	Plg	Plt	Exine	Intine
31,14	29,82	1,04	24,58	9,67	10,89	5,50	4,34	1,55

(range - μm)

P = Polar axis, E = Equatorial axis, Clg = Length of colp, Clt = Width of colp, Plg = Length of porus, Plt = Width of porus, $\pm$ = Standard deviation.

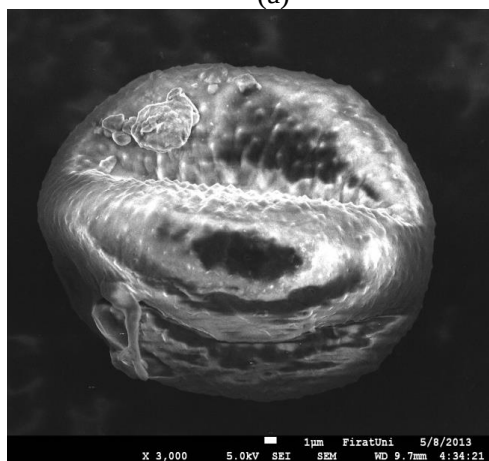
The pollen grains of examined *P. pyrrhoblephara* are radially symmetric, isopolar, tricolporate and spheroidal in polar view. The polar axis (P) is 31,14 μm , equatorial axis (E) 29,82 μm and P/E 1,04 μm .

The colpi are long and with distinct margin; Clg 24,58 μm , Clt 9,67 μm . The pori are circular and with distinct margin; Plg 10,89 μm , Plt 5,50 μm . The exine is 4,34 μm thick. Exine ornamentation is scabrate. The intine is 1,55 μm .

The surface ornamentation is microechinate under SEM. (Figure 3 a-b)



(a)



(b)

Figure 4. Pollen microphotography of *P. pyrrhoblephara*. (a) Polar view of a non acetolysed pollen in SEM, (b) Equatorial view of a non acetolysed pollen in SEM.

The morphological findings and the growing environment for the species are the following:

Biennial with thick fleshy taproot and erect stem, 25 – 40 cm, arachnoid-tomentose, at last glabrescent. Basal leaves long petiolate,

ovatesubcordate or rarely lyrate, up to 9,4 $\times$ 4 cm. median leaves broadly lanceolate or oblong, 80 $\times$ 30 mm; Inflorescence raceme; capitula 30 x 15 mm; involucre 25 x 24 mm subglobose; phyllaries multiseriate, coriaceous-scarious, external phyllaries 10 $\times$ 5 mm. included cilia and cilia number 13. medium phyllaries 15 $\times$ 10 mm, included cilia and cilia number 30. internal phyllaries 20 $\times$ 3 mm. included cilia and cilia number 20. appendages large, ovate or broadly triangular, concealing basal part of phyllaries, white or straw-coloured to brownish purple 70 mm. Flowers purple, 4,5 cm. Achenes 20 $\times$ 10 mm, shiny. Pappus double (inner row shorter), barbellate, straw-coloured to brownish, 70 x 30 mm. Flower between April and July. Although, *P. pyrrhoblephara* is similar to *P. yildizii* different in features such as habitat, leaf shape, leaf segments, involucre size and colour, phyllary appendage indumentum [5]. In addition, *P. yildizii* leaf are pinnatipartite and its phyllary appendages glabrous. These features are very important morphological characters for the distinguish between *P. yildizii* and *P. pyrrhoblephara*.

4. Results

Nearly 300 species of the genus *Centaurea* are problematic and none of the early attempts to subdivide the genus has been widely accepted [7-14-15]. However, no study has been reported on the chromosome number and morphology of *P. pyrrhoblephara*. This study allowed us to define the morphological and karyological features of *P. pyrrhoblephara*.

Finally, the karyological and morphological description of the investigated taxon in this work presents the first data available in the literature.

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