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Karyological investigation of two Balkan endemic *Centaurea* species

Abstract

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Chromosome numbers, karyotype feature and its numerical data are presented for two Balkan endemic *Centaurea* species, *C. kosaninii* and *C. melanocephala*. The somatic chromosomes number $2n = 22$ was found for both *Centaurea* species. However, the karyotypes showed the significant differences in size and morphology of chromosomes and in number of satellite chromosome pairs, two in *C. melanocephala* and three in *C. kosaninii*.

Keywords: *Centaurea kosaninii*, *Centaurea melanocephala*, chromosome number, karyotype feature, numerical data on karyotype.

Introduction

Genus *Centaurea* L. is one of the largest in the *Asteraceae* [subtribe *Centaureinae* (Cass.) Dumort., tribe *Cardueae* Cass.], and comprises about 250 annual, biannual and perennial species according to the most recent studies (Sussana & Garcia-Jacas 2007). It is widely distributed, mostly in the Eastern Hemisphere, with the diversification center in the Middle East. The genus is well represented in all the Mediterranean region and many species are known from the Balkan Peninsula.

Centaurea is the most complex genus with basic chromosome number ranging from $x = 7$ to $x = 16$ (Siljak-Yakovlev 1986; Garcia-Jacas & al. 1996) and at least four different pollen types (Wagenitz 1955). As in many plant groups, descending dysploidy and polyploidy were found to be the most important evolutionary mechanisms occurring in this genus (Siljak-Yakovlev 1986).

Two investigated species belong to *Acrocentron* (Cass.) DC. section for which only two basic chromosome numbers, $x = 10$ (more often occurring) and $x = 11$, are recorded.

The aim of this study was to verify and eventually confirm the chromosome number, and to characterize, for the first time, the karyotype of two *Centaurea* endemic species from the Balkans.

2010. *Centaurea kosaninii* Hayek — $2n = 2x = 22$; 2C DNA = 3.13 pg (Figs 1, 1a, 1b; Table 1).

Syn. = *Colymbada kosaninii* (Hayek) Holub in Folia Geobot. Phytotax. 7: 315 (1972)

Ko: Kosovo, Brezovica, ultramafite substrate, 22.08.1980, leg. Dr Čedomil Šilić.

The native range of *Centaurea kosaninii* is Western Balkan Peninsula (NE Albania and Kosovo). It grows mostly on serpentine, but also rarely, on limestone substrate and is considered as relict species (Stevanovic & al. 2003). The chromosome number of $2n = 22$ reported already by Siljak-Yakovlev (1981 & 1986) is now confirmed.

The karyotype of *C. kosaninii* is composed of seven metacentric (pairs 1, 2, 3, 4, 5, 8 & 11), three submetacentric (pairs 6, 7 & 10; two of which, pairs 6 & 10, bearing satellites on their short arms) and one subtolocentric chromosome pairs (9) also with satellites (Fig. 1, 1a & 1b). The karyotype formula was as follows: $2n = 14m + 6sm + 2st = 22$.

Numerical data concerning this karyotype are presented in the Table 1. Chromosome types were determined according to the usual recommendation and nomenclature of Levan & al. (1964). Karyotype features were evaluated using the following morphometric parameters: length of the long (l) and short (s) arm, arm ratio (r), total chromosome length (TL), the relative length of each chromosome pair (RL) following Levan & al. (1964), the ratio (R) between the longest and the shortest chromosome pair (Siljak-Yakovlev 1986) and the index of asymmetry, AsI %, following Arano and Saito (1980). Idiogram was constructed from the mean values obtained from at least five metaphase plates corresponding to five different individuals.

Table 1. Numerical data concerning the chromosome set of *Centaurea kosaninii*.

Chrom. pair	Long arm (LA) in μm	Short arm (SA) in μm	Total length (TL) in μm	Relative length	Rapport LA/SA	Centromeric index	Chromosome type
1	1.62 (± 0.02)*	1.48 (± 0.03)*	3.10	120.08	1.10	47.69	m
2	1.62 (± 0.04)	1.43 (± 0.02)	3.05	118.05	1.14	46.79	m
3	1.45 (± 0.05)	1.25 (± 0.03)	2.70	104.43	1.16	46.38	m
4	1.49 (± 0.03)	1.13 (± 0.02)	2.63	101.70	1.32	43.15	m
5	1.25 (± 0.06)	1.25 (± 0.06)	2.50	96.86	1.00	50.00	m
6	1.35 (± 0.03)	0.78 (± 0.03)	2.13	81.73	1.75	37.04	sm-SAT
7	1.33 (± 0.03)	0.75 (± 0.02)	2.08	80.52	1.77	36.09	sm
8	1.09 (± 0.03)	0.94 (± 0.08)	2.03	78.70	1.17	46.15	m
9	1.58 (± 0.02)	0.41 (± 0.12)	1.99	77.19	3.88	20.59	st-SAT
10	1.23 (± 0.02)	0.70 (± 0.09)	1.93	74.92	1.75	36.36	sm-SAT
11	0.94 (± 0.03)	0.76 (± 0.02)	1.70	65.84	1.23	44.83	m
* standard error $\Sigma\text{TL}=51.68 \mu\text{m}$ R=1.82 IAs%=57.86							

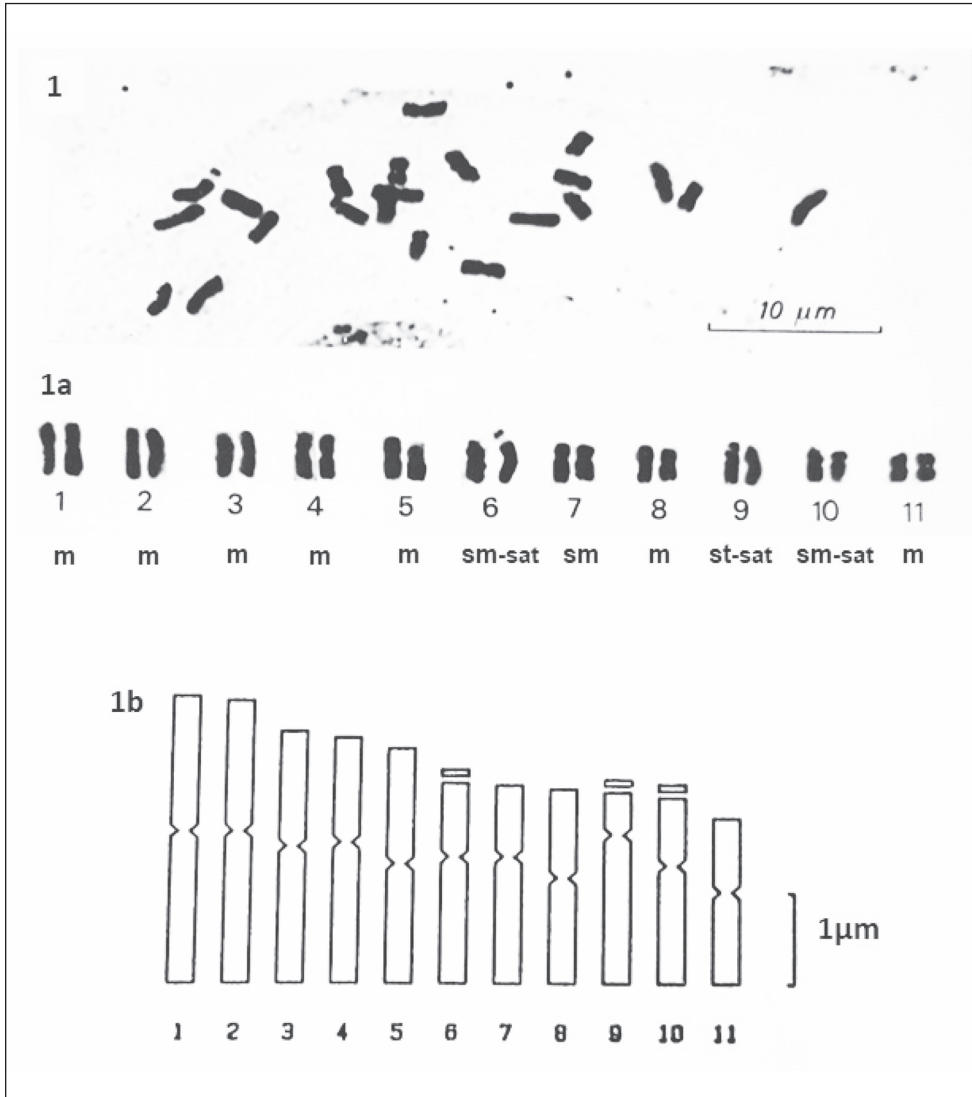


Fig. 1. Mitotic metaphase plate of *Centaurea kosaninii* (1), corresponding karyogram (1a) and idiogram (1b).

The chromosomes are small and total karyotype length (ΣTL) was 51.68 µm. The index of asymmetry was $IAS\% = 57.86$ and ratio (R) between the longest and the shortest chromosome pair was $R = 1.82$.

In addition, the genome size or nuclear DNA amount has been estimated for this species from the same population and $2C$ value was 3.13 pg or 3061 Mbp (Siljak-Yakovlev & al. 2010).

2011. *Centaurea melanocephala* Pančić — $2n = 2x = 22$ (Figs. 2, 2a, 3, 3a & 4; Table 2).

AI: Prov. Kukës: Kolsh, 24.07.2002, leg. *Tone Wraber*).

Centaurea melanocephala Pančić, Fl. Serbiae 443 (1874). This species presents two synonyms:

= *Centaurea candelabrum* Hayek & Košanin in Repert. Spec. Nov. Regni Veg. Beih. 30(2): 746 (1931)

= *Colymbada candelabrum* (Hayek & Košanin) Holub in Folia Geobot. Phytotax. 7: 315 (1972)

Centaurea melanocephala is also an endemic species from West Balkan Peninsula (Albania, Serbia). It grows in the mountain rocky grasslands on ultramafite substrate at an altitude from 800 to 1000 m.a.s.l.

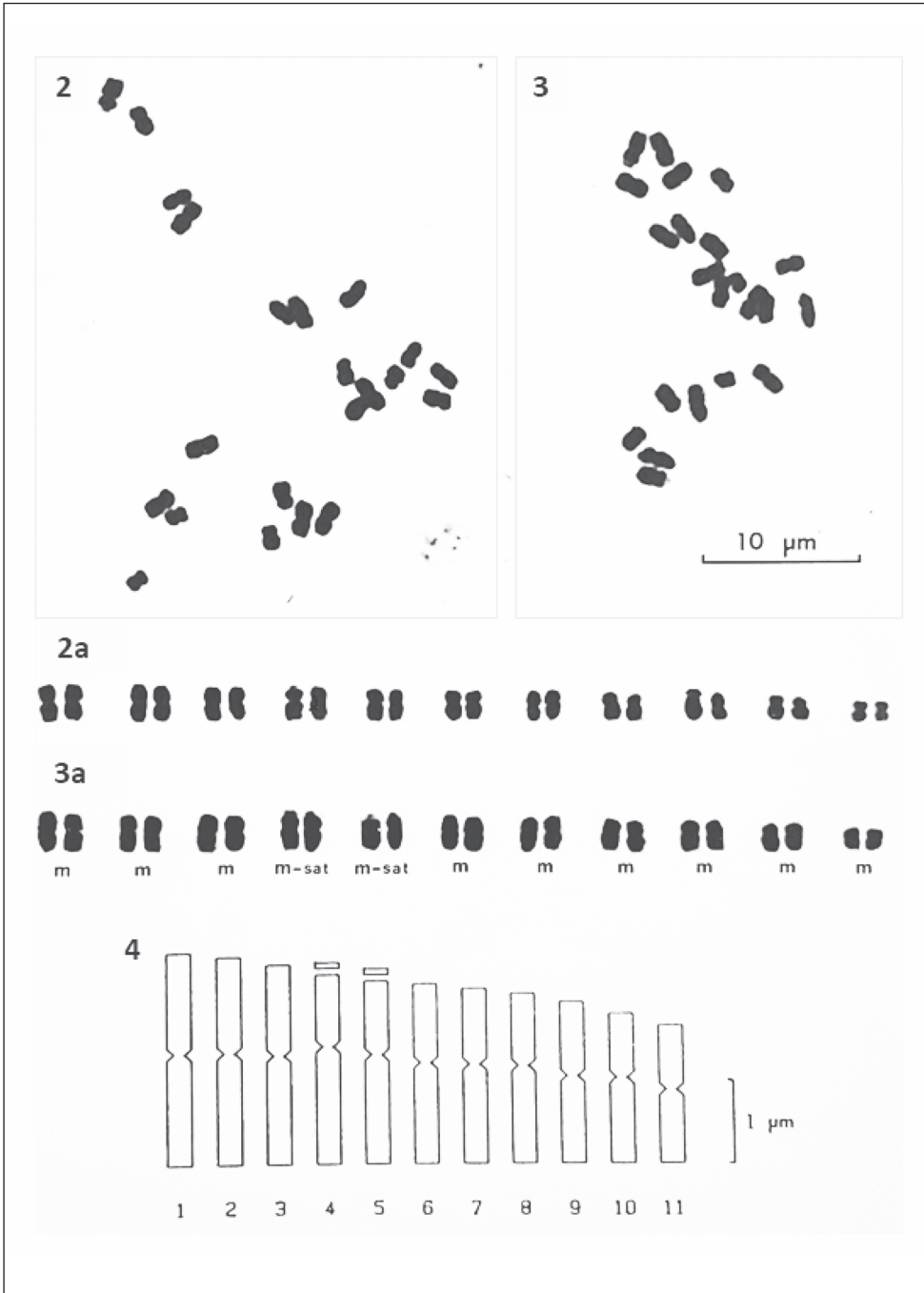
The chromosome number of this species has been indicated for the first time from Kosovo in 1990 (Siljak-Yakovlev & Wraber) and then in 2010 (Siljak-Yakovlev & al). Recently, Novaković & al. (2022) reported the same chromosome number $2n = 22$ from Serbia.

The karyotype of *C. melanocephala* is composed of ten metacentric (pairs 1, 2, 3, 5, 6, 7, 8, 9, 10 & 11) and one metacentric-submetacentric pair (pairs 4 bearing satellites on their short arms). Pair 5 also carries the satellites (Figs. 2, 2a, 3, 3a & 4). The karyotype formula was as follows: $2n = 20m + 2m-sm = 22$, revealing a very symmetrical karyotype

Table 2. Numerical data concerning the chromosome set of *Centaurea melanocephala*.

Chrom. pair	Long arm LA (µm)	Short arm (SA) in µm	Total length (TL) in µm	Relative length	Rapport LA/SA	Centromeric index	Chromosome type
1	1.29 (±0.04)*	1.17 (±0.03)*	2.46	110.01	1.11	47.42	m
2	1.29 (±0.04)	1.10 (±0.02)	2.40	107.29	1.17	46.09	m
3	1.25 (±0.03)	1.04 (±0.03)	2.29	102.29	1.20	45.45	m
4	1.35 (±0.05)	0.81 (±0.02)	2.17	97.03	1.67	37.50	m-sm-SAT
5	1.25 (±0.05)	0.83 (±0.06)	2.08	93.29	1.50	40.00	m-SAT
6	1.15 (±0.03)	0.90 (±0.03)	2.04	91.43	1.29	43.88	m
7	1.13 (±0.04)	0.85 (±0.02)	1.98	88.63	1.32	43.16	m
8	1.10 (±0.03)	0.81 (±0.08)	1.92	85.83	1.36	42.39	m
9	0.98 (±0.05)	0.83 (±0.12)	1.81	81.13	1.18	45.95	m
10	0.96 (±0.04)	0.71 (±0.09)	1.67	74.64	1.36	42.50	m
11	0.81 (±0.02)	0.71 (±0.02)	1.52	68.10	1.15	46.58	m

* standard error $\Sigma TL=44.68 \mu m$ $R=1.62$ $IAs\%=56.22$



Figs. 2-4. Mitotic metaphase plates of *Centaurea melanocephala* (2 & 3), corresponding karyograms (2a & 3a) and idiogram (4).

(IAS% = 56.22). Numerical data concerning this karyotype are presented in the Table 2. Total karyotype length (Σ TL) was 44.68 μ m indicating that the chromosomes of *C. melanocephala* are smaller than those of *C. kosaninii*. The ratio (R) between the longest and the shortest chromosome pair was $R = 1.62$.

Despite the same chromosome number, karyotype analyses show important differences between the two investigated species, suggesting their ancient differentiation.

Acknowledgment

Our colleague Dr. Tone Wraber passed away in 2010, we started this work on *Centaurea melanocephala* with him, and we now want to dedicate it to his memory.

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