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Karyotypes of two endemic species of *Astrothamnus* Novopokr. (Asteraceae) from South Siberia (Tuva)

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Key words: *Astrothamnus*, chromosome number, karyotype.

Summary. The karyotypes of two endemic species such as *Astrothamnus heteropappoides* and *A. polifolius* from South Siberia were studied. Both species were diploids with the chromosome numbers of $2n = 2x = 18$. The polyploidy and satellites were not found. For the first time, the chromosome morphology has been studied and the idiograms have been plotted. The chromosome sizes ranged from 6.42 to 4.23 μm for *A. heteropappoides* and the karyotype formula of $8m + 1sm$ and that from 5.88 μm to 3.64 μm for *A. polifolius* and featuring the karyotype formula of $9m$.

Кариотипы двух эндемичных видов *Astrothamnus* Novopokr. (Asteraceae) из Южной Сибири (Тува)

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Ключевые слова: *Astrothamnus*, хромосомные числа, кариотип.

Аннотация. Приведены кариотипы двух эндемичных видов *Astrothamnus heteropappoides* и *A. polifolius* из Южной Сибири. Оба вида диплоидны с числом хромосом $2n = 2x = 18$. Полиплоидии и сателлитов не обнаружено. Впервые изучена морфология хромосом для этих видов и приведены идиограммы. Размер хромосом варьирует от 6.42 μm до 4.23 μm для *A. heteropappoides* с формулой кариотипа $8m + 1sm$ и от 5.88 μm до 3.64 μm для *A. polifolius* с формулой $9m$.

Introduction

The genus *Astrothamnus* Novopokr. belongs to the subtribe *Asterinae* (Cass.) Dumort. (the tribe *Astereae*, the family Asteraceae). According to Nesom & Robinson (2007), it includes 13 genera and approximately 270 species. The molecular-phylogenetic analysis of the subtribe *Asterinae* (Li et al., 2012; Korolyuk et al., 2015) states

the differentiation of Asiatic taxa into five well-supported clades. These are the *Galatella* group with the species of Asian genera *Galatella* Cass. s. l. and *Tripolium* Nees.; the groups of species of North American origin; and three groups of allied Eurasian species: (1) the group of typical Eurasian *Aster* L. (2) the *Heteropappus* group including *Heteropappus* Less. and *Kalimeris* Cass., as well as (3) *Astrothamnus* group. The monophyletic

clade *Asterothamnus* includes endemic Central Asian species of the genera *Asterothamnus* and *Rhinactinidia* Novopokr., several endemic species of *Aster* from China, and the monotypic genus *Arctogeron* DC. All the genera mentioned, except for *Asterothamnus*, contain polyploid taxa. According to the published data, a number of species of the Asteraceae featuring single chromosomes in a chromosome set that may differ morphologically from each other. This can serve as an additional taxonomic marker and reflect evolutionary relations of the allied taxa (Li, 2006). The species belonging to the *Asterothamnus* group have been less studied cytologically: the chromosome numbers of $2n= 18$, which have been reported in the two species of *Asterothamnus*, are known for two species of *Asterothamnus* (Krasnikov, Korolyuk, 1995) and those for *Arctogeron gramineum* (L.) DC. (Rostovtzeva, Ligus, 1978). However, the chromosome morphology has not been studied yet.

Asterothamnus includes seven xerophytic species which are restricted to semi-deserts or dry rocky steppes. Most of the species grow in the territories of Mongolia and China; two species are endemic to Middle Asia (Novopokrovskiy, 1950; Grubov, 1982; Chen et al., 2011). Two species occurring in Siberia, *A. heteropappoides* Novopokr. and *A. polifolius* Novopokr., are endemics to Tyva Republic (Korolyuk, 1997).

The aim of this work is to study karyotypic peculiarities of chromosomes of Siberian *A. heteropappoides* and *A. polifolius* as a part of

ongoing revision of the genera of the subtribe *Asterinae*.

Materials and methods

The plants for the study were collected in the territories of Ovyursky and Tes-Khemsky Regions of the Republic of Tyva. The herbarium samples are stored at the Herbarium of the Central Siberian Botanical Garden SB RAS (NS).

The karyological analysis was made by using root meristem squash preparations from seedlings. Seeds were sprouted in petri dishes on wet sterile sand at 27 °C and light regime providing 16 hours of daylight and eight hours of darkness. The germs were treated in 0.2 % colchicine for two hours and then fixed in a mixture of ethanol-acetic acid (3:1). The chromosomes were stained with aceto-haematoxyline according to Smirnov (1968). Slides were examined under Axioscop-40 microscope. Chromosome analyses were made on 5–10 slides under 100X magnification. The study of karyotypes and their descriptions were made according to Levan et al. (1964). A1 was estimated according to Romero Zarco (1986), and TF % followed Huziwara (1962). Chromosomes and chromosome arm lengths measurement were made by MicroMeasure software environment (Aaron Reeves, USA).

Results and discussion

Morphological peculiarities of chromosomes of two rare species of *Asterothamnus* from South Siberia were studied.

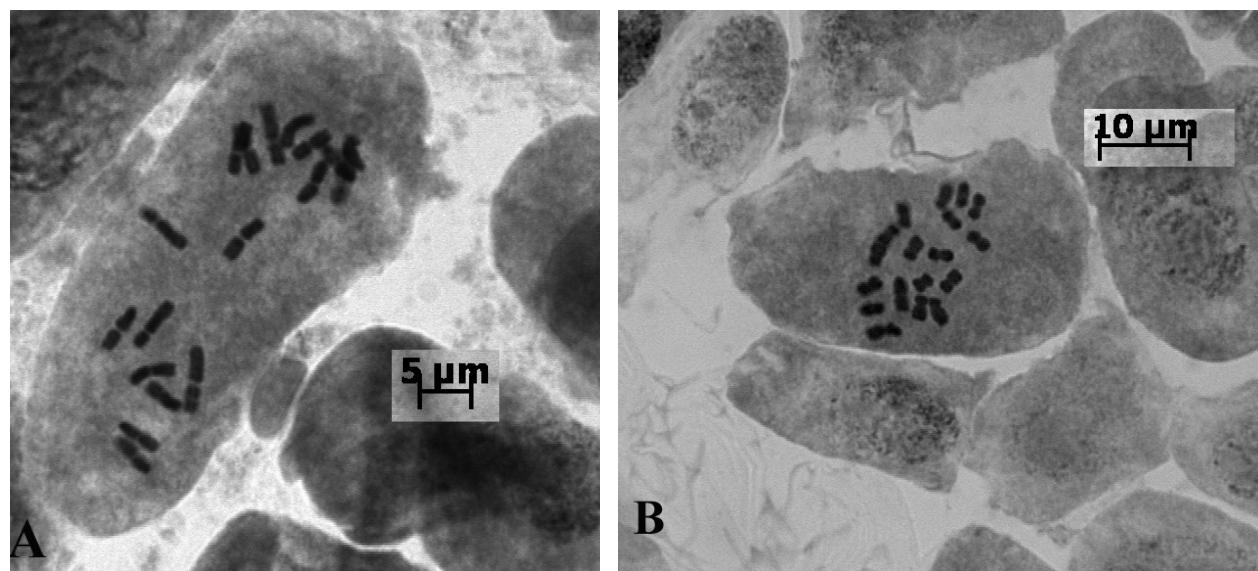


Fig. 1. Microphotograph of somatic metaphases: A – *Asterothamnus heteropappoides* ($2n = 18$). Scale bars: 5 μm ; B – *Asterothamnus polifolius* ($2n = 18$). Scale bars: 10 μm .

Table 1Parameters of mitotic metaphase chromosomes of *Asterothamnus heteropappoides*

Chromosome no	Total chromosome length (C) μm	Long arm length (L) μm	Short arm length (S) μm	Arm ratio R:L/S μm	Centromeric index I: (S/C).I00 μm	Relative size Mm	Centromeric position S.D
1	6.42	3.76	2.66	1.41	41.48	14.18	medium
2	5.59	3.55	2.04	1.74	36.46	12.34	smedium
3	5.30	3.08	2.22	1.39	41.91	11.70	medium
4	5.10	2.78	2.32	1.20	45.43	11.25	medium
5	4.90	2.78	2.12	1.31	43.21	10.82	medium
6	4.68	2.63	2.05	1.29	43.76	10.34	medium
7	4.61	2.48	2.13	1.16	46.22	10.16	medium
8	4.48	2.44	2.04	1.20	45.55	9.89	medium
9	4.23	2.23	2.00	1.12	47.20	9.33	medium
A1 = 0.23							
TF % = 42							

Table 2Parameters of mitotic metaphase chromosomes of *Asterothamnus polifolius*

Chromosome no	Total chromosome length (C) μm	Long arm length (L) μm	Short arm length (S) μm	Arm ratio R:L/S	Centromeric index I: (S/C).I00 μm	Relative size	Centromeric position S.D
1	5.88	3.25	2.63	1.24	44.71	14.96	medium
2	4.75	2.70	2.05	1.32	43.12	12.09	medium
3	4.58	2.84	1.75	1.62	38.10	11.66	medium
4	4.30	2.39	1.90	1.26	44.30	10.93	medium
5	4.21	2.44	1.76	1.38	41.95	10.70	medium
6	4.09	2.24	1.85	1.21	45.26	10.40	medium
7	4.02	2.23	1.79	1.25	44.52	10.23	medium
8	3.84	2.12	1.72	1.23	44.84	9.77	medium
9	3.64	2.04	1.60	1.27	44.02	9.25	medium
A1 = 0.23							
TF % = 42							

***Asterothamnus heteropappoides* Novopokr.; 2n = 2x = 18** (Fig. 1A).

Collection site: “The Republic of Tyva, Ovyur-sky Region, 3 km down the Khundurgun Pass towards Khandagaity settlement. Road-side by the Khandagaity River bank, 50°73'914"N, 92°17'339"E, 05 IX 2013. A. Yu. Korolyuk, E. A. Korolyuk s. n.”

The karyotype consisted of nine pairs chromosomes. The centromeres of the 1st and 3–9th pairs are located at the region (m), while that of the 2nd pairs at the region (sm). Satellites were not visualized. Chromosome sizes ranged from 6.42 to 4.23 μm . The longest arm was 3.76 μm , the shortest arm was 2.00 μm . Karyotype formula of the species was 2n = 8m + 1sm. (Table 1, Fig. 2A). The previously known number of chromosomes determined for the

material from Erzinsky Region of Tyva Republic was confirmed (Krasnikov, Korolyuk, 1995).

***Asterothamnus polifolius* Novopokr.; 2n = 2x = 18** (Fig. 1B).

Sampling location: “The Republic of Tyva, Tes-Khemsky Region, east of Ak-Chyraa settlement. The Khoohlu River Valley, pebble alluvium, 50°70'480"N, 93°34'650"E, Altitude = 816 m. 05 IX 2013. A. Yu. Korolyuk, E. A. Korolyuk s. n.”

The karyotype consisted of nine pairs of chromosome. Most of the centromeres were situated at the centric region (m). Satellites were not found. Chromosome sizes ranged from 5.88–3.64 μm . The longest arm was 3.25 μm , while the shortest one was 1.6 μm . Karyotype formula: 9m (Fig. 2B; Table 2).

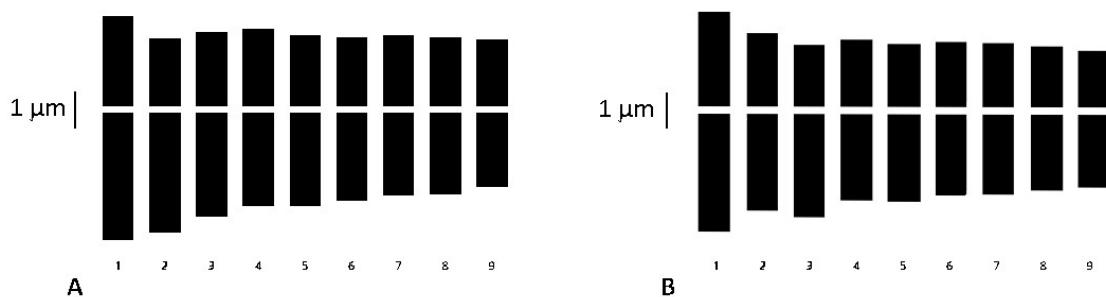


Fig. 2. Idiograms: A – of *A. heteropappoides*, B – of *A. polifolius*.

The chromosome number previously determined for the material from O-Shynaah settlement of Tes-Khemsky Region was confirmed (Krasnikov, Korolyuk, 1995).

Conclusively, the karyotypes of the species studied were similar in chromosome number and size, as well as no satellites and secondary constrictions. Minor differences were revealed for chromosome

morphology. Thus, karyotype formula of *A. heteropappoides* was $2n = 18 = 8m + 1sm$, while that of *A. polifolius* was $9m$.

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