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ORIGINAL ARTICLE

Cytogenetic characteristics of some *Trollius* L. species (Ranunculaceae) from Asian Russia

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The karyotypes (chromosome sets) of *Trollius austrosibiricus, T. kytmanovii*, and *T. riederianus* have been investigated for the first time. The karyotype formula has been obtained as $2n = 2x = 16$ = 2m + 2m/sm + 12sm for *Trollius austrosibiricus* and *T. riederianus*, and $2n = 2x = 16$ = 4m + 12sm for *T. kytmanovii.* A comparative analysis of the chromosome sets was conducted for these three species and the previously studied *Trollius altaicus, T. asiaticus, T. ledebourii*, and *T. lilacinus* (*Hegemone lilacina*) for several karyological parameters. The average absolute nuclear DNA content (2C-value) was originally determined for *Trollius austrosibiricus*, *T. kytmanovii*, *T. ledebourii*, and *T. riederianus* by flow cytometry.
Keywords: chromosomes, C-value, globe-flower, karyotype, nuclear DNA content, Ranunculaceae, *Trollius* L.

Introduction

A cytogenetic approach is widely used to solve the problems of plant systematics and phylogeny, including the family Ranunculaceae Juss. (Yuan & Yang, 2006; Mlinarec, 2012; Baltisberger & Hörandl, 2016). Karyotypes (chromosome sets) of some taxa have not yet been studied despite a wide range of current chromosome research methods (Badaeva & Salina, 2013; Sharma & Sen, 2019). However, chromosome number, size, and morphology are important characteristics of the species.

Genus *Trollius* L. (fam. Ranunculaceae), or globe-flower, includes 30–35 species that are perennial herbs distributed in the extra-tropical regions of the Northern Hemisphere (Doroszewska, 1974; Kadota, 2016; Luferov et al., 2018). Based on the modern molecular-genetic and morphological data, the genus *Trollius* has been assigned to the tribe Adonideae Kunth, together with *Adonis* L., *Calathodes* Hook.f. & Thomson and *Megaleranthis* Ohwi (Wang et al., 2010). In Russia, about 19–20 *Trollius* species are recognized (Luferov et al., 2018; Erst et al., 2019). These plants are used for decorative and medical purposes (Witkowska-Banaszczak, 2015; Jiang et al., 2020). The *Hegemone* Bunge ex Ledeb. is similar to the *Trollius,* and these taxons are sometimes treated as congeneric (e.g., Czerepanov, 1995; Li & Tamura, 2001). However, *Hegemone* can readily be distinguished from the yellow-flowered *Trollius* by its white, pink, or pale violet flowers, glandular stigma, sepals which are persistent in fruit, and leaf shape (Schipczinsky, 1937; Butkov, 1953; Siplivinsky, 1972; Friesen, 2003; Erst et al., 2020a). However, similar to the case of *Anemone-Pulsatilla*, when some authors consider that they belong to one genus, *Anemone* L. (Hoot et al., 2012), or two separate genera *Anemone* and *Pulsatilla* Mill. (Sramkó et al., 2019), the question with *Trollius-Hegemone* remains open to debate, and an integrative approach is required for a more in-depth insight into the status of the taxa (Erst et al., 2020b).

The study of *Trollius* chromosome sets started in the 1950–the 60s (Kurita, 1955; 1957; 1959; 1960; Doroszewska, 1967). The somatic chromosome number is known for most Trollius species, and it is $2n = 2x = 16$, which is extremely stable in the genus, with few exceptions (Rice et al., 2015). Previously, we presented data on chromosome sets of four species: *Trollius altaicus* C.A. Mey*, T. asiaticus* L.*, T. ledebourii* Rchb *T. lilacinus* Bunge (*Hegemone lilacina* Bunge), and nuclear DNA content of nine *Trollius* species (Mitrenina et al., 2020). The present work is a follow-up to this study, and it focuses on karyotypes of three species of Asian Russia: *Trollius ausrtosibiricus* Erst et Luferov (Erst et al., 2019)*, T. kytmanovii* Reverd., and *T. riederianus* Fisch. & C.A.Mey.

Trollius austrosibiricus is endemic to mountainous areas of the southern part of Western and Central Siberia – the Tuva Republic, Kemerovo Oblast, Krasnoyarsk Krai, Khakassia Republic (Erst et al., 2019), and the Altai Republic (new data). *Trollius austrosibiricus* is morphologically close to *T. chinensis* s. l. It is well distinguished by simple rhizomes (rather than by the multi-headed basal part of the plant, as in *T. chinensis*), shorter aerial shoots, sepals 8–14, petals 20–28 mm long, 1.2–1.5 times longer than, smaller flowers and shorter persistent styles. *Trollius chinensis* is an East Asian species occurring in Russia (Primorsky and Khabarovsk Krai, Sakhalin Island), in the north and northeast of China, on the Korean peninsula (Schipczinsky, 1937; Siplivinsky, 1972; Doroczewska, 1974; Kitagawa, 1979; Voroshilov, 1982; Luferov, 1991; 2004) and Mongolia (the extreme eastern border with China) (Serebryanyi, 2019). *Trollius austrosibiricus* is a cryptic species similar to *T. chinensis* in many morphological characteristics, but it has several differences, some of which will be described in this paper. It is likely that *T. austrosibiricus* is more widespread and can be found in the Mongolian Altai.

Trollius kytmanovii Reverd. is distributed in the southern part of Krasnoyarsk Krai (the forest part, near the Kansk forest-steppe – Shitkina, Mokhovaya, Bayronovka, Nikolskaya, Tagashi Villages), Evenkiysky District (valley of the Lower Tunguska River and its tributaries: Khuricha, Kananda, Kochechumo, Vivi, Yambukan, Tuba Rivers and Agatha Lake) (Reverdatto, 1943). According to Flora of Siberia, this species occurs in Eastern Siberia (Angara-Sayan and Prilensko-Katangsky floristic regions of Irkutsk Oblast, North and South Buryat floristic regions of the Buryatia Republic, Shilko-Argunsky Region of Trans-Baikal Territory) and Khubsugul Region of Mongolia (Friesen, 2003). *Trollius kytmanovii* is characterized by linear petals 1.5–2 times longer than stamens and exhibits intermediate features between *T. sibiricus* and *T. asiaticus*. The species is of hybrid origin; however, it should be proved.

Trollius riederianus is distributed in Eastern Siberia (Amur Oblast, Kamchatka Peninsula, Commander Islands, Sakhalin Oblast (Kurile Islands), Khabarovsk Krai, Primorsky Krai); North America (the Aleutian Islands) (Luferov et al., 2018). *T. riederianus* is characterized by round-pentagonal leaf blades, dentate with triangular acute and sharp teeth, 3–4 cm in diameter flowers, yellow-orange or yellow sepals, reddish-orange, equal to stamens or 1–3 mm longer petals, leaflets with arcuate, unbreakable 2–3 mm long persistent styles.

Materials and Methods

All the studied plants have been collected in their natural localities. The vouchers are listed in Table 1. *Trollius* rhizomes were stored in wet moss before having young leaves.

Karyotyping

Newly formed 0.3–0.5 cm long leaves were excised and pretreated in 0.5 % aqueous colchicine solution for 3–4 h at room temperature. After that, they were fixed in a mixture of 96 % ethanol and glacial acetic acid (3:1). Leaves were stained with 1 % aceto-hematoxylin, and the squash method was employed for the investigation of the karyotype (Smirnov, 1968). Chromosomes were counted in 20–30 mitotic cells for each species. Mitotic metaphase chromosome plates were studied using an Axio Star microscope (Carl Zeiss, Munich, Germany) and photographed using an Axio Imager A.1 microscope (Carl Zeiss, Germany) with AxioVision 4.7 software (Carl Zeiss, Germany) and AxioCam MRc5 CCD–camera (Carl Zeiss, Germany) at 1000× magnification in the Laboratory for Ecology, Genetics and Environmental Protection (Ecogene) of National Research Tomsk State University. KaryoType software (Altinordu et al., 2016) was used for karyotyping, and Adobe Photoshop CS5 (Adobe Systems, USA) and Inkscape 0.92 (USA) were used for image editing. Karyotype formulas were based on measurements of mitotic metaphase chromosomes taken from photographs. We studied metaphase plates with the most condensed chromosomes.

The measurements were performed on 3–5 metaphase plates. The symbols used to describe the karyotypes corresponded to those of Levan et al. (1964): m = median centromeric chromosome with an arm ratio of 1.0–1.7 (metacentric chromosome); sm = submedian centromeric chromosome with an arm ratio of 1.7–3.0 (submetacentric chromosome); st = subterminal centromeric chromosome with an arm ratio of 3.0–7.0 (subtelocentric chromosome); t = terminal centromeric chromosome with an arm ratio of 7.0–∞ (acrocentric or telocentric chromosome); T = chromosome without an obvious short arm, i.e., with an arm ratio of ∞. Additionally, we classified chromosomes with the intermediate value of the mean arm ratio: m/sm = meta–submetacentric chromosome and sm/st = submeta–subtelocentric chromosome.

Mean values of the centromeric indices (CI), mean chromosome length (CL), and relative chromosome length (RL) for each chromosome pair, total haploid length (THL), and mean chromosome length of the set (MCL) were determined. Besides, we calculated the Coefficient of Variation of Chromosome Length (CV_{CL}; Paszko, 2006), Coefficient of Variation of Centromeric Index (CV_{CI}; Paszko, 2006), Mean Centromeric Asymmetry (M_{CA}; Peruzzi & Eroğlu, 2013), and determined Stebbins asymmetry index (Stebbins, 1971).

Flow cytometry

Flow cytometry with propidium iodide (PI) staining was used to determine the absolute nuclear DNA content. Silica gel-dried leaf material was chopped with a sharp razor blade in a 1 ml cold nuclei extraction buffer composed of 50 mM Hepes, 10 mM sodium metabisulphite, 10 mM $MgCl_2$, 0.5 % polyvinylpyrrolidone, 0.1 % bovine serum albumin, 0.3 % Tween 20, 0.2 % Triton X-100, 50 µg/ml RNase, 1 µg/ml β-mercaptoethanol, and 50 µg/ml propidium iodide (PI). The samples were filtered through 50 µm nylon membranes into sample tubes and incubated in the dark at 4 °C for 15 min. The samples were measured using a Partec CyFlow PA flow cytometer equipped with a green laser at 532 nm wavelength. The absolute nuclear DNA content, the 2C–value according to Greilhuber et al. (2005), was calculated as the ratio of the mean fluorescence intensity of the sample nuclei to that of an internal multiplied by the total nuclear DNA content of the standard. A possible effect of secondary metabolites on the binding of the intercalating dye was evaluated by measuring the fluorescence of *Allium fistulosum* L. leaf samples prepared as described above, but with the addition of the supernatant from *Trollius,* samples centrifuged without PI (Erst et al., 2020b; Mitrenina et al., 2020). The samples were measured three times at 10 min intervals. If the *A. fistulosum* peak

showed no variation in the average values of the detection channels, the effect of secondary metabolites was considered negligible. *Allium fistulosum* L., 2C = 23.50 pg was used as an internal standard (Doležel et al., 1992; Ricroch et al., 2005; Smirnov et al., 2017). We used the Statistica 8.0 software (StatSoft, Inc.), Flowing Software 2.5.1 (Turku Centre for Biotechnology), and CyView software (Partec, GmbH) for data analyses. Flow cytometry was performed at the South-Siberian Botanical Garden, Altai State University (Barnaul, Russia).

Results and Discussion

Karyotypes

The karyotypes of *Trollius austrosibiricus, T. kytmanovii*, and *T. riederianus* have been investigated for the first time. All the plants studied were diploid, with $2n = 2x = 16$ (Table 1; Fig. 1). This somatic chromosome number is generally specific to the genus *Trollius* (Rice et al., 2015). Metacentric and submetacentric types of chromosomes were found via morphometric chromosome analysis. The karyotype formula was $2n = 2x = 16$ = 2m + 2m/sm + 12sm for *Trollius austrosibiricus* and *T. riederianus*, and for *T. kytmanovii*, it was $2n = 2x = 16$ = 4m + 12sm.

Table 1. Chromosome numbers ($2n$) and 2C–values (2C ± SD) in the seven studied *Trollius* species.

Species	Voucher information	$2n$	2C ± SD, pg
Trollius altaicus	Russia, Altai Republic, Ust-Kansky Raion, Ust-Kan – Tuecta Road, descent from the Yabogansky Pass, around the Tahoy River, 50°51′07″ N, 85°14′31″ E, alt. 1320 m, Erst A.S., Erst T.V., Boltenkov E.V., No.15, 05.06.2020 (NS).	16	8.45 ± 0.62
	Russia, Altai Republic, Ongudaisky Raion, R-256 Tashanta – Gorno-Altaysk Road, Seminsky Pass, 51°2′45″ N, 85°36′17″ E, alt. 1720 m, Erst A.S., Erst T.V., Boltenkov E.V., No. 46, 09.06.2020 (NS).	16	8.40 ± 0.19
Trollius asiaticus s.l.	Russia, Altai Republic, Ongudaisky Raion, R-256 Tashanta – Gorno-Altaysk road, climbing to the Seminsky Pass, 50°56′41″ N, 85°44′28″ E, alt. 1230 m, Erst A.S., Erst T.V., Boltenkov E.V., No. 40, 09.06.2020 (NS)	16	8.64 ± 0.02
Trollius austrosibiricus	Russia, Altai Republic, Kosh-Agacinsky Raion, near upper Boguty Lake, 49°42′53″ N, 89°29′45″ E, alt. 2475 m, Erst A.S., Erst T.V., Boltenkov E.V., No. 36, 08.06.2020 (NS)	16	9.36 ± 0.05
Trollius chinensis s.l.	Russia, Primorsky Krai, Khasansky Raion, near Kraskino Village, 42°42'23.8"N, 130°50'08.8"E, alt. 12 m, Koldaeva M.N., No. 001,10.06.2017 (NS)	16	8.78 ± 0.20
Trollius kytmanovii	Russia, Irkutskaya Oblast, Cheremkhovsky Raion, left bank of the Onot River, 0.5 km south-west of Onot Village, riverbank with willow, 52°42.211′N, 102°56.761′E, 13 May 2020, Chernysheva O., OC09 (NS)	16	9.09 ± 0.32
Trollius ledebourii	Russia, Amur Oblast, Tambovsky District, near Nikolaevka Village, 50°01'0,57" N, 127°40'0,39" E, alt. 129 m, Veklich T.N., 06.06.2020 (NS)	16	9.08 ± 0.17
Trollius riederianus	Russia, Amur Oblast, Zejsky District, Zejsky State Nature Reserve, "Bol'shaya Erakingra" River valley, 54°03'21.3"N, 126°22'45.0"E, alt. 666 m, Veklich T.N., No.002, 20.06.2020 (NS)	16	8.92 ± 0.16

Chromosome sets insignificantly varied in chromosome lengths and centromere positions. Chromosome relative length (RL) ranged from 5.17 to 7.32 % in *T. riederianus*. The degree of variability RL in *T. austrosibiricus* and *T. kytmanovii* was slightly

lower (Table 2). It should be mentioned that *T. chinensis*, being morphologically similar to *T. austrosibiricus* (Erst et al., 2019), appeared to differ from *T. austrosibiricus* in the karyotype formula, which was $2n = 2x = 16 = 8sm + 8st$ (Doroszewska, 1967). *Trollius* species have chromosome sets consisting of 1–2 pairs of metacentric chromosomes or chromosomes with an arm ratio of about 1.7 – borderline value between metacentric and submetacentric chromosome types. The rest of the chromosomes are heterobrachial (submetacentric and subtelocentric) with an arm ratio of less than 4 (Doroszewska, 1967; Yang, 2002; Mitrenina et al., 2020). Chromosomes of more asymmetric shape are not typical to *Trollius.* Total haploid chromosome length (THL) varied from 26.48±0.28 μm in *T. austrosibiricus* to 35.32±0.83 μm in *T. lilacinus*. The mean chromosome length of the set varied from 3.30±0.30 μm to 4.42±0.52 μm, respectively.

Table 2. Karyomorphological parameters of *Trollius ausrtosibiricus, T. kytmanovii*, and *T. riederianus* (Ranunculaceae).

Species	Chromosome pair	CL, μm	r	CI	RL, %	Chromosome type
Trollius austrosibiricus	I	3.73 (0.09)	2.13 (0.05)	0.32	7.05	sm
	II	3.60 (0.26)	1.72 (0.08)	0.37	6.80	m/sm
	III	3.45 (0.16)	1.52 (0.09)	0.40	6.52	m
	IV	3.35 (0.07)	2.30 (0.10)	0.30	6.33	sm
	V	3.26 (0.06)	2.65 (0.11)	0.27	6.16	sm
	VI	3.17 (0.07)	2.17 (0.17)	0.32	5.99	sm
	VII	2.99 (0.17)	2.10 (0.15)	0.32	5.65	sm
	VIII	2.93 (0.09)	1.83 (0.07)	0.35	5.53	sm
Trollius kytmanovii	I	4.59 (0.48)	1.94 (0.09)	0.34	7.26	sm
	II	4.50 (0.43)	1.57 (0.09)	0.39	7.12	m
	III	3.98 (0.19)	2.47 (0.17)	0.40	6.30	sm
	IV	3.90 (0.32)	2.80 (0.08)	0.26	6.17	sm
	V	3.89 (0.14)	1.53 (0.14)	0.40	6.16	m
	VI	3.88 (0.24)	2.24 (0.13)	0.31	6.14	sm
	VII	3.44 (0.29)	1.94 (0.13)	0.34	5.44	sm
	VIII	3.43 (0.09)	2.54 (0.16)	0.28	5.43	sm
Trollius riederianus	I	4.23 (0.14)	1.88 (0.08)	0.35	7.32	sm
	II	4.01 (0.17)	1.72 (0.06)	0.37	6.94	m/sm
	III	3.66 (0.08)	2.33 (0.03)	0.30	6.33	sm
	IV	3.65 (0.13)	2.68 (0.20)	0.27	6.32	sm
	V	3.63 (0.14)	1.41 (0.07)	0.42	6.28	m
	VI	3.49 (0.07)	1.99 (0.19)	0.34	6.04	sm
	VII	3.25 (0.11)	2.13 (0.17)	0.32	5.62	sm
	VIII	2.99 (0.23)	2.02 (0.09)	0.33	5.17	sm

Notes. CL – chromosome length, mean value (standard deviation); r – arm ratio, mean value (standard deviation); CI – centromeric index; RL – relative chromosome length; m – metacentric chromosome; sm – submetacentric chromosome.

Megaleranthis saniculifolia Ohwi, a representative of the tribe Adonidae, has a karyotype structure similar to *Trollius.* The karyotype formula of the species are $2n = 2x = 16 = 2m + 12sm + 2st$ (Lee, Yeau, 1985). Another related species, *Calathodes oxycarpa* Sprague, has a more asymmetric karyotype without metacentric chromosomes (Yang, 2002). Q.-E. Yang noted that chromosomes of *C. oxycarpa* were larger than those of *Trollius.* Based on these data, he concluded that *Calathodes* was an independent genus in the tribe. Nevertheless, it was demonstrated in several studies that karyotypes of some *Trollius* consisted of heterobrachial chromosomes only. These include *T. chinensis* Bunge, *T. europaeus* L., and *T. altaicus* (Doroszewska, 1967; Mitrenina et al., 2020). W. Wang et al. (2010) used molecular and morphological analysis data to conclude that *Calathodes* is a segregate genus of the tribe Adonideae, and *Megaleranthis* is a member of the amended "*Trollius*".

On the contrary, karyotypes of *Adonis* equally consist of isobrachial and heterobrachial chromosomes. For instance, the karyotype formula for *A. vernalis* L. (Schrager & Malakhova, 1981) and *A. amurensis* Regel & Radde (Volkova et al., 2020) was $2n = 2x = 16 = 8m + 8sm$, for *A. brevistyla* Franch. was $2n = 2x = 16 = 8m + 2sm + 6st$ (Yang, 2001). Similar formulas were obtained for *A. distorta* Ten. (Del Grosso & Pogliani, 1971) and *A. multiflora* Nishikawa & Koji Ito (Ikeda et al., 2006). Consequently, the genus *Adonis* is clearly distinguished from *Calathodes*, *Trollius,* and *Megaleranthis* by chromosome complement.

We estimated some karyotype asymmetry indices for seven *Trollius* species (Table 3). The quali-quantitative Stebbins asymmetry index (1971) was 3A for all karyotypes. It indicates that the proportion of chromosomes with an arm ratio <2 was equal or less than 0.5, as well as the ratio between the largest and smallest chromosomes, was less than 2. Additionally, we calculated three parameters detecting interchromosomal and intrachromosomal karyotype asymmetries: CV_{CL} – Coefficient of Variation of Chromosome Length, CV_{CI} – Coefficient of Variation of Centromeric Index (Paszko, 2006), M_{CA} – Mean Centromeric Asymmetry (Peruzzi & Eroğlu, 2013). CV_{CL} is an index for estimating interchromosomal asymmetry, i.e., the degree of the

difference between the chromosome lengths of a complement. It is a statistically correct parameter to show even a small variation among chromosome sizes in the complement (Peruzzi & Eroğlu, 2013).

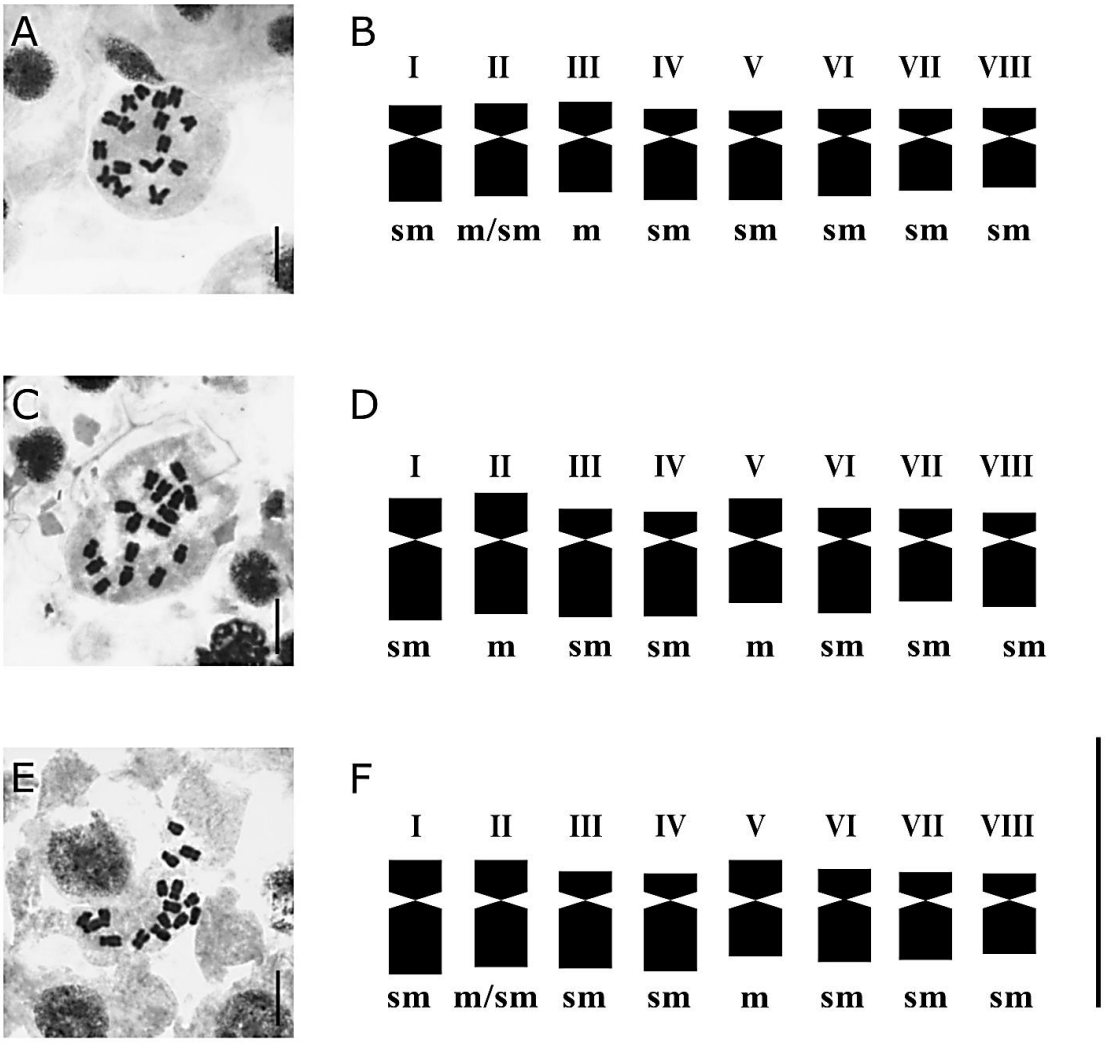

Fig. 1. Mitotic metaphase plates (A, C, E) and haploid idiograms (B, D, F): A, B – *Trollius austrosibiricus* (2n = 16); C, D – *Trollius kytmanovii* (2n = 16); E, F – *Trollius riederianus* (2n = 16). I–VIII – chromosome pairs; m – metacentric chromosome; sm – submetacentric chromosome. Scale bars = 10 μm.

Table 3. Karyotype parameters of the seven *Trollius* species.

Species	PL	2*n*	Karyotype formula	THL	MCL	CV_{CL}	CV_{CI}	M_{CA}	KA
T. altaicus	2*x*	16	14sm + 2st	27.31 (0.73)	3.41 (0.36)	10.61 (0.15)	12.21 (1.16)	39.43 (0.30)	3A
T. asiaticus	2*x*	16	2m + 10sm + 2sm/st + 2st	28.19 (0.50)	3.54 (0.41)	11.47 (1.81)	16.74 (2.00)	38.49 (1.38)	3A
T. austrosibiricus	2*x*	16	2m + 2m/sm + 12sm	26.48 (0.28)	3.30 (0.30)	8.99 (1.19)	11.57 (0.72)	33.77 (1.56)	3A
T. kytmanovii	2*x*	16	4m + 12sm	31.61 (1.81)	3.95 (0.43)	10.82 (2.00)	14.57 (1.77)	34.68 (0.93)	3A
T. ledebourii	2*x*	16	2m/sm + 10sm + 4st	31.72 (1.44)	3.96 (0.40)	10.10 (0.88)	18.50 (2.34)	39.21 (1.34)	3A
T. lilacinus (H. lilacina)	2*x*	16	4m + 8sm + 4st	35.32 (0.83)	4.42 (0.52)	11.92 (2.31)	21.62 (0.59)	38.08 (0.16)	3A
T. riederianus	2*x*	16	2m + 2m/sm + 12sm	28.91 (0.52)	3.59 (0.39)	10.88 (1.99)	12.95 (1.52)	32.82 (1.56)	3A

Notes. PL – ploidy level; 2*n* – somatic chromosome number; THL – total haploid length; MCL – mean chromosome length; CV_{CL} – Coefficient of Variation of Chromosome Length; CV_{CI} – Coefficient of Variation of Centromeric Index; M_{CA} – Mean Centromeric Asymmetry; KA – Stebbins asymmetry index; m – metacentric chromosome; sm – submetacentric chromosome; st – subtelocentric chromosome.

The index value varied from 8.99 to 11.92 among the seven studied *Trollius*, i.e., interchromosomal asymmetry levels were similar to each other. M_{CA} was used to estimate the intrachromosomal asymmetry. The lowest M_{CA} level was obtained for *T. riederianus* (32.82) and *T. austrosibiricus* (33.77), followed by *T. kytmanovii* (34.68), which lack subtelocentric chromosomes. The rest four *Trollius* species have 1–2 pairs of subtelocentric chromosomes and, consequently, have higher MCA values (38.08–39.43). CV_{CI} is an additional karyological parameter, not correctly referred to as the karyotype asymmetry. It is a measure of intrachromosomal heterogeneity. It can be used sometimes as an optional third parameter to reveal karyotype relationships among organisms, in addition to the asymmetry *sensu stricto* (Peruzzi & Eroğlu, 2013). The index value varied from 11.57 in *T. austrosibiricus* to 21.62 in *T. lilacinus*. The values of CV_{CI} did not correlate with M_{CA} in the studied *Trollius* species.

Genome size

The average absolute nuclear DNA content (2C–value) was initially determined for *Trollius austrosibiricus*, *T. kytmanovii*, *T. ledebourii*, and *T. riederianus* by flow cytometry. Specimens from other locations were additionally studied for *T. altaicus*, *T. asiaticus*, and *T. chinensis* (Table 1; Fig. 2). 2C–values for the three last species were close to our previous results (Mitrenina et al., 2020). The nuclear DNA content in *T. austrosibiricus*, *T. kytmanovii*, *T. ledebourii*, and *T. riederianus* was higher than that in other studied *Trollius* and attained 9.36±0.05 pg, 9,09±0.32 pg, 9.08 ± 0.17 pg, and 8.92±0.16 pg, respectively. It should be noted that morphologically similar species, *Trollius austrosibiricus,* and *T. chinensis*, differed in the karyotype structure and the 2C–value. The second species showed a lower 2C–value that reached 8.78±0.20 pg and 8.87±0.26 pg in specimens from different locations. In conclusion, it should be noted that the absolute nuclear DNA content varied from 8.20±0.24 pg in *Trollius farreri* to 9.80±0.29 pg in *T. lilacinus* (*Hegemone lilacina*) (Mitrenina et al., 2020). The 2C–value correlated with the total haploid length (THL) in all the studied *Trollius* except *T. austrosibiricus*, which can be due to the higher chromosome condensation in *T. austrosibiricus* or methodical aspects (Mártonfiová, 2013).

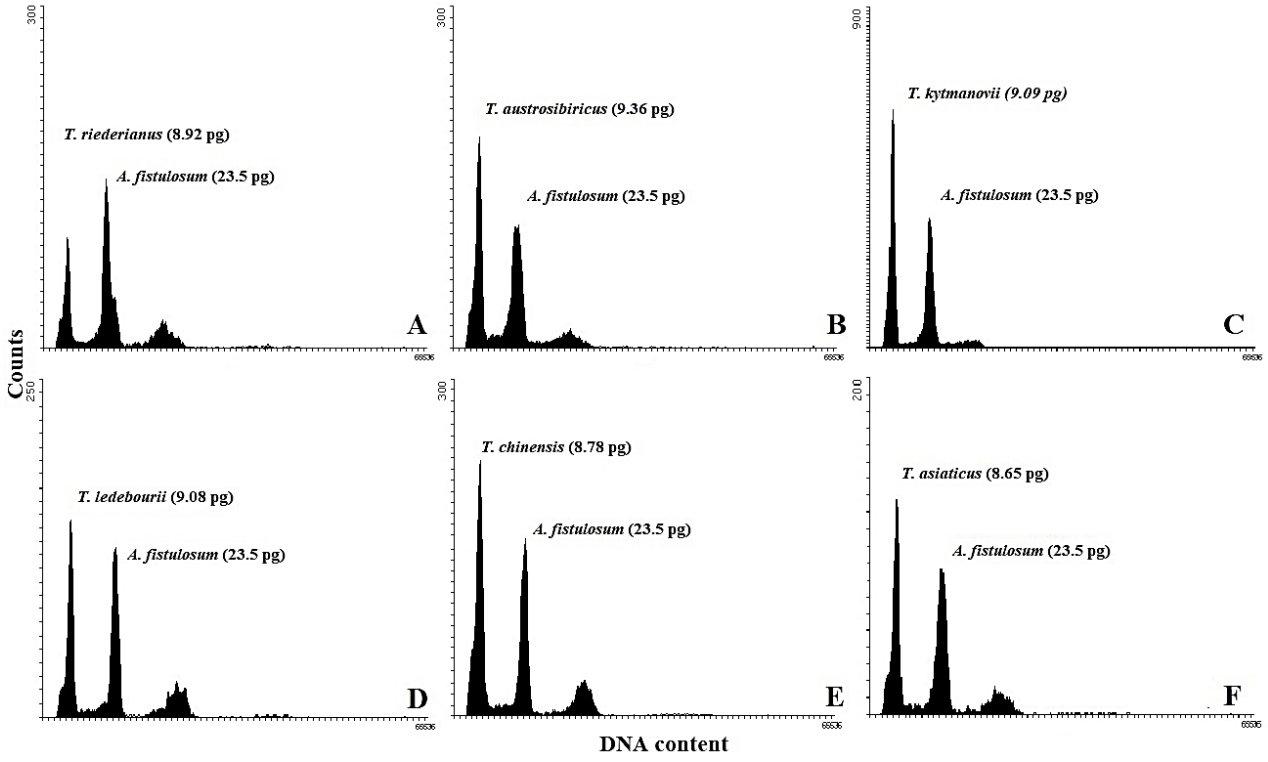

Fig. 2. Flow cytometric histograms of some studied *Trollius* species: A – *Trollius riederianus*; B – *Trollius austrosibiricus*; C – *Trollius kytmanovii*; D – *Trollius ledebourii*; E – *Trollius chinensis*; F – *Trollius asiaticus.* The *Allium fistulosum L.* was used as an internal standard.

Conclusion

Ultimately, a similarity of karyotypes is typical of the genus *Trollius*. They have equal chromosome numbers and similar karyomorphological traits. Nevertheless, we revealed some variability of the absolute nuclear DNA content in the genus. The karyotype evolution was probably associated with small chromosome mutations, which insignificantly changed chromosomes shape. The genome size, in the meanwhile, had been changing.

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