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Nuclear genome size analysis in *Krascheninnikovia ceratoides* s. l. (Amaranthaceae)

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ABSTRACT

The total nuclear DNA amount (2C value) of 75 individuals of *Krascheninnikovia ceratoides* s. l. was assessed by flow cytometry in 16 populations. The mean 2C value in all samples ranged from 2.59 to 6.99 pg. The relationship between total 2C values and ploidy level was calibrated using chromosome counting in four populations. Diploids ($2n$ = 18) and tetraploids ($2n$ = 36) were revealed. In diploid plants, mean 2C value varied between 2.59 to 3.72 pg, in tetraploids − from 6.04 to 6.99 pg. The GS of diploids is approximately half the GS of tetraploids. Diploids were observed in seven populations from the Orenburg Region and the Republic of Tyva (Russia), Kyzylorda and Almaty Regions (Kazakhstan). Tetraploids were recorded in eight populations from Kazakhstan, Orenburg and Novosibirsk Regions, from the Republics of Altai and Sakha (Yakutia). One mixed population was found in the Republic of Khakassia represented by di- and tetraploids. We did not find any regularity in the geographical distribution of the two cytotypes. The studied plants showed no significant morphological features of taxonomic value, therefore we consider all the samples belong to *Krascheninnikovia ceratoides* s. str.

Keywords: chromosome number, cytotype, flow cytometry, genome size variation, 2C nuclear DNA content, ploidy level

РЕЗЮМЕ

Ломоносова М.Н., Панкова Т.В., Королюк Е.А., Шаулo Д.А., Осмонали Б., Николин Е.Г. Анализ размера генома *Krascheninnikovia ceratoides* s.l. (Amaranthaceae). Общее количество ядерной ДНК (2C-value) 75 особей *Krascheninnikovia ceratoides* s. l. из 16 популяций оценивали методом проточной цитометрии. Среднее значение 2C во всех образцах колебалось от 2,59 до 6,99 пг. Взаимосвязь между общими значениями 2C и уровнем плоидности была откалибрована с использованием подсчета хромосом в четырех популяциях. Были выявлены диплоиды ($2n$ = 18) и тетраплоиды ($2n$ = 36). У диплоидных растений среднее значение 2C варьировало от 2,59 до 3,72 пг, у тетраплоидов − от 6,04 до 6,99 пг. Показано, что размер генома диплоидов составляет примерно половину размера генома тетраплоидов. Диплоиды выявлены в семи популяциях из Оренбургской области, Республики Тыва (Россия), Кызылординской и Алматинской областей (Казахстан). Тетраплоиды − в восьми популяциях из Казахстана, Оренбургской и Новосибирской областей, из республик Алтай и Саха (Якутии). В Республике Хакасия была обнаружена одна смешанная популяция, представленная ди- и тетраплоидами. Мы не обнаружили какой-либо закономерности в географическом распределении двух цитотипов. Существенных морфологических различий между ними также выявлено не было, что указывает на принадлежность всех исследованных образцов к *Krascheninnikovia ceratoides* s. str.

Ключевые слова: проточная цитометрия, число хромосом, уровень плоидности, изменчивость размера генома, 2C содержание ядерной ДНК, цитотип

Krascheninnikovia ceratoides (L.) Gueldenst. s. l. (*Eurotia ceratoides* (L.) C.A. Mey., nom. illegit., *Ceratoides papposa* Botsch. et Ikonn., nom. illegit.) − a shrub, distributed mainly in Middle and Central Asia. To the north, this species occurs in the steppes of Eurasia from Romania, steppe islands of Siberia and Eastern Mongolia (Kamelin 2011). Outside of this area, there are separate isolated refugia in Western Asia (Turkey), in Western Europe (Spain and Austria), and in North Africa (Egypt and Morocco). In some isolated areas in the northern part of the range, *K. ceratoides* s. l. is protected as a steppe relic. In a number of Western European countries, this species is indicated as a subject to extinction (Pérez-Collazos & Catalán 2007).

Occupying a vast area in various climatic conditions, *K ceratoides* s. l. is characterized by high morphological variability. Some forms from this group were described as separate species from the territory of Eurasia. Along with *K. ceratoides*, these are such as *K. ewersmanniana* (Stschegl. ex Losinsk.) Grubov, *K. arborescens* (Losinsk.) Cherep., *K. pungens* (Paziy) Podlech, *K. compacta* (Losinsk.) Grubov, *K. lenensis* (Kumin.) Tzvelev, *K. intramongolica* (H.C. Fu, J.Y. Yang, S.Y. Zhao) Z.Y. Zhu, C.Z. Liang, W. Wang. In America, a related species *K. lanata* (Pers) A. Meeusen et A. Smith is common. Here and further, the names of species are given as part of the genus *Krascheninnikovia*, even if they were referred in a number of publications to the genera *Eurotia* or *Ceratoides*.

Polyploidy is widespread among many groups of flowering plants and plays an important role in their evolution (Tate et al. 2005). Despite the commonness of this pheno-

menon in many plant species, the intraspecific ploidy variations are unknown for most species (Kolář et al. 2017). *K. ceratoides* s. l. forms a polyploid complex in which three cytotypes are known given in the literature under different names. The diploid cytotype ($2n = 18$) was noted in the Republic of Tyva (An'kova & Korolyuk 2017), Kazakhstan (An'kova et al. 2020; Zakharieva & Soskov 1981, given as *K. ewersmanniana*), China (Kurban 1984, as *K. arborescens* and *K. ewersmanniana*). The tetraploid cytotype ($2n = 36$) is known from the Republic of Altai (Lomonosova & Krasnikov 1993), Kazakhstan (An'kova et al. 2020) and China (Kurban 1984, as *K. latens* J.F. Gmel. nom. illeg.). In Central Europe and the Mediterranean, only tetraploid populations have been found living in Austria (Dobeš et al. 1997) and Spain (Seidl et al. 2020). The rarer hexaploid cytotype ($2n = 54$) is known from the Pamir highlands (Zakharieva & Soskov 1981, given under the name *K. latens*). However, the vast majority of these data was based on the analysis of single or few samples from one population. Thus, the question of intraspecific variability of the ploidy level in *K. ceratoides* remains far from fully studied, as well as the cytogeography of this polyploid complex.

In recent years, the interest in the study of *Krascheninnikovia* has increased based on the use of molecular methods. Thus, Heklau & Röser (2008), based on the analysis of ITS1-5.8 S-ITS2 genes and a review of some morphological features on the material previously attributed to *K. ceratoides*, *K. ewersmanniana*, *K. lenensis*, *K. arborescens* and *K. lanata* did not confirm the separation of these taxa in the rank of species. These authors combined all Eurasian species into one subspecies *K. ceratoides* subsp. *ceratoides*, and American species recognized as *K. ceratoides* subsp. *lanata* (Pursh) H. Heklau. Later, a detailed phylogenetic and biogeographic analysis was performed by Seidl et al. (2020, 2021) based on the use of several sections of nuclear and chloroplast DNA, as well as measurements of genome size (GS hereafter) and ploidy level by flow cytometry on the material from Eurasia and N America. The authors revealed low genetic variability of the studied populations and confirmed the conclusions of Heklau & Röser (2008) and also recognized the separation of the two subspecies mentioned above. At the same time, Pérez-Collazos & Catalán (2007) showed a sufficiently high genetic variability of two tetraploid populations of *K. ceratoides* from Spain by studying more variable DNA sections by ISSR analysis. This analysis was also used by Wang et al. (2015) for *K. arborescens* in China. It was found that *K. arborescens* possesses an unexpectedly high rate of genetic diversity in the studied area. Six populations were clustered into two main groups, a desert steppe group and a typical steppe group.

With the development of flow cytometry methods (FCM hereafter), this approach has become widely used to determine the level of ploidy in plants (Pellicer et al. 2021). The popularity of the method is associated with the high speed and simplicity of the analysis of the material compared to the direct counting of chromosomes in metaphase plates on squashed preparations conducing more analyses in a short time. Furthermore, this method can be used on dried plant material (herbarium samples) in some species (Suda & Trávníček 2006). This technique has been successfully used in the analysis of 100 samples of the genus *Krascheninnikovia* from various parts of the range (Seidl et al. 2020). Two levels of ploidy were identified, diploids (with an average DNA content of 2C = 2.9 ± 0.2 pg) and tetraploids (2C = 5.6 ± 0.2 pg).). Similar values of GS were given by Seidl et al. (2021) – 2.8± 0.3 pg and 5.8± 0.2 pg, correspondingly. However, particular data on the DNA content in separate populations were not provided in these papers. Only the mean values of GS for all diploids and tetraploids were indicated. Single data on the GS are available for samples from the Novosibirsk Region and the Republic of Altai (Lomonosova et al. 2020). But the cytogeography of the genus *Krascheninnikovia* has not been fully studied, as well as the independence of the taxa already described in this polyploid complex. So, Aktayeva (1973), based on the analysis of morphology and karyology of the two most widely distributed and often recognized species in the rank, *K. ceratoides* and *K. ewersmanniana*, did not confirm the independence of the latter species. At the same time, in the modern Flora of China (Zhu et al. 2003), four species of this genus are given, such as *K. ceratoides* s. str., *K. ewersmanniana*, *K. arborescens*, and *K. compacta*.

In this paper we set out (1) to obtain a novel information about plant nuclear DNA amount in 16 populations of the genus *Krascheninnikovia* from Russia and Kazakhstan based on flow cytometry; (2) to complement, in some cases, these genome size estimates with determination of the chromosome number in the same populations; (3) to show the possibility of using herbarium samples to determine the ploidy level.

MATERIAL AND METHODS

The material for the study was collected in nature in 2017–2021 in 16 populations in Kazakhstan and Russia (Table 1). The studied plants were prepared according to the standard method of drying herbarium samples. Seeds from some plants were grown in the laboratory to produce seedlings and subsequent direct counting of chromosomes. Herbarium vouchers are saved in the Herbarium of the Central Siberian Botanical Garden SB RAS (NS).

The total content of DNA in nuclei (2C value) of the tested samples were estimated using Cy Flow Space cytometer (Sysmex Partec, Germany) fitted with 532 nm green laser. *Solanum lycopersicum* ´Stupicke polni rane´ (2C = 1.96 pg) was used as internal standard (Doležel et al. 2007). The seeds of the standard were obtained from the Center for Structural and Functional Plant Genomics of the Institute of Experimental Botany of the Academy of Sciences of the Czech Republic, Olomouc-Holice. Sample preparation followed the two-step procedure using Otto buffers (Doležel et al. 2007). Leaf tissue of the studied plant and standard was chopped with 500 µl of modified ice-cold Otto I buffer (Otto 1992) complemented with 0.1 m citric acid plus 0.5 % Triton X-100. The nuclear suspension was filtered in a nylon membrane with a pore size of 42 µm and mixed with a staining solution consisting of 1 ml Tris–$MgCl_2$ buffer (0.4 m Tris-base, 4 mm $MgCl_2 \times 6H_2O$) with propidium iodide (50 µg/ml), RNase (50 µg/ml) and

Table 1. The studied populations, nuclear DNA content (2C, pg), DNA ploidy level and chromosome number of *Krascheninnikovia ceratoides* s. l. (No, number of analyzed plants)

Nr pop	Voucher information	No	2C DNA (mean ± SD, pg)	2C DNA range (min-max (CV%)	DNA ploidy level /chromosome number
1	Russia, Orenburg Region, Krasnoshchekovo, 51.4175°N 57.14348°E, 7.10.2020, T.An'kova 219	5	3.25±0.02	3.23–3.28(0.77)	~ 2*x*
2	Russia, Orenburg Region, Orenburg nature reserve, 51.114806°N 57.665917°E, 8.10.2020, T.An'kova 220	4	3.26±0.06	3.17–3.31 (1.96)	~ 2*x*
3	Kazakhstan, Kyzylorda Region, Aktan Batyr vil., 46.008333°N 62.04944°E, 6.09.2019, B. Osmonali et al. 932a	3	2.94±0.15	2.79–3.08 (4.97)	~ 2*x*
4	Kazakhstan, Almaty Region, Kopa vil., 43.297083°N 76.230194°E, 3.11.2019, T. An'kova 210	5	3.72±0.07	3.62–3.78 (1.79)	2*x* / 2*n* = 18
5	Kazakhstan, Almaty Region, Zailisk Alatau, 43.334667°N 75.880556°E, 3.11.2019, T. An'kova 213	5	3.44±0.07	3.33–3.50 (1.92)	2*x* / 2*n* = 18
6	Russia, Republic of Tyva, "Arshaan", 51.627583°N 94.435472°E, 22.08.2019, D. Shaulo 82	5	3.58±0.09	3.47–3.71 (2.46)	~ 2*x*
7	Russia, Republic of Tyva, Begreda, 51.968611°N 94.333333°E, 20.08.2019, D. Shaulo 84	5	3.31±0.05	3.25–3.37 (1.61)	~ 2*x*
8	Russia, Republic of Khakassia, Abakan city, 54.960278°N 92.4625°E, 24.08.2020, M. Lomonosova 1428	3	2,59±0,05	2,53–2.62 (1.91)	~ 2*x*
	the same population	3	5.83±0.22	5.58–5.97 (3.77)	~ 4*x*
9	Kazakhstan, Kyzylorda Region, Ayteke bi vil., 46.035833°N 62.226111°E, 6.09.2019, B. Osmonali et al. 931a	4	5,83±0,07	5,77–5.92 (0.26)	~ 4*x*
10	Russia, Orenburg Region, Donguz river valley, 51.534444°N 55.106944°E, 5.10.2020, T.An'kova 216	5	6.07±0.07	5.99–6.16 (0.36)	~ 4*x*
11	Kazakhstan, Almaty Region, Kapshagay, 43.879917°N 77.060389°E, 2.11.2019, T. An'kova 207	5	6.99±0.10	6.82–7.10 (0.62)	4*x* / 2*n* = 36
12	Russia. Novosibirsk Region, Antonovo, 54.091458°N 81.370428°E, 7.08.2019, T. An'kova s. n.	5	6.74±0.08	6.68–6.87 (0.37)	~ 4*x*
13	Russia. Novosibirsk Region, 54.23071°N 82.81569°E, Kurilovka, 24.07.2020, A. Korolyuk, E. Korolyuk 427	3	6.68±0.15	6.54–6.84 (0.74)	~ 4*x*
14	Russia. Novosibirsk Region, Mayak, 54.658825°N 83.100060°E, 21.07.2020, N. Lashchinsky s. n.	5	6.56±0.04	6.53–6.62 (0.09)	~ 4*x*
15	Russia, Altai Republic, Chegan-Usun, 50.07379°N 88.42228°E, 2.09.2019, I. Smelyansky s. n.	5	6.57±0.17	6.37–6.82 (1.86)	4*x* / 2*n* = 36
16	Russia, the Republic of Sakha (Yakutia), Orto-Doydu, 61.775556°N 129.394722°E, 26.08.2019, E. Nikolin s. n.	5	6.04±0.10	5.91–6.18 (0.71)	~ 4*x*

β-mercaptoethanol (1 µl/ml) (Doležel et al. 1998, Pfosser et al. 1995, Skaptsov et al. 2017). For each sample at least 10 000 nuclei were analyzed. Only histograms with coefficients of variation (CV) for the G0/G1 peak of the analyzed *Krascheninnikovia* sample below 5 % were considered. The sample 2C DNA (pg) content was calculated based on the values of the G1 peak as follows: [(Sample G1peak mean) / (Standard G1peak mean)]×Standard G1 peak mean value)] (Doležel & Bartoš 2005).

The results obtained were processed using the program STATISTICA 64. For evaluating variance in 2C DNA content of the whole dataset, we used one-way ANOVA to analyze designs with a single categorical independent variable (factor). The nonparametric Kruskal-Wallis test was performed to illustrate variability of GS among the studied populations.

Chromosome numbers were determined by direct counting in the metaphase stage on root meristem of squash preparations. Seeds were germinated in Petri dishes at 25°C (day) and 16°C (night) on sterile sand. For pre-fixation treatment, the seedlings were kept for 2 hours at room temperature in 0.2 % colchicine solution (Radzhabli & Rud' 1972), fixed in acetic acid alcohol (3 : 1) and stained with acetogematoxilin according to Smirnov (1968). The chromosome complements were examined at Axioscope 40 microscope using the AxioVision 4.8 software

RESULTS AND DISCUSSION

The total nuclear DNA amount (2C value) of 75 individuals of *Krascheninnikovia ceratoides* s. l., 3−5 per population, was assessed by flow cytometry according to the method described in Doležel et al. (2007) on the material from 16 populations from the north-eastern part of the area. GS estimates of the investigated samples are provided in Table 1. The mean 2C value or GS in *K. ceratoides* for all samples ranged from 2.59 pg in population 8 ("pop." here and further, the numbers of populations correspond to the table 1) from Khakassia, to 6.99 pg in pop. 11 from Kazakhstan. To prove that GS allows reliable inference of ploidy levels in Krascheninnikovia, we combined GS measurements with chromosome numbers estimation in four populations. Two studied accessions revealed diploids with $2n = 2x = 18$ in pop. 4 (mean GS (2C = 3.72 pg) and in pop. 5 (mean GS 2C = 3.44 pg), and two accessions were tetraploids with $2n = 4x = 36$ in pop. 11 (mean GS 2C = 6.99 pg) and in pop.15 (mean GS 2C = 6.57 pg). Thus, the GS of the diploids was approximately half the GS of the tetraploids. Considering this, the GS (2C values) of all studied individuals in 16 populations were divided in two groups. The first group was formed by diploids with 2C-value varied from 2.53 to 3.78 pg, and the second group − by tetraploids with 2C-value from 5.58 to 7.1 pg (Fig. 1A).

The analysis of variance (ANOVA) showed significant differences among ploidy level. ANOVA one-way current effect: $F(16, 60) = 990.35$, $p = 0.0000$. Effective hypothesis decomposition, vertical bars denote 0.95 confidence intervals.

The GS of the studied accessions in the most populations have one ploidy level, except for pop. 8 from Khakassia. This population consists of both diploids and tetraploids individuals growing side by side (Fig. 1A). Co-occurrence

of different cytotypes within populations of mixed-ploidy species is common in plants (Kolár et al. 2017).

The mean 2C content is 3.32±0.32 pg in all diploids, and 6.38±0.42 pg in all tetraploids. In diploid plants, mean 2C value varied between 2.59 pg (pop. 8 in Khakassia) to 3.72 pg (pop. 4 in Kazakhstan), in tetraploids from 6.04 pg (pop. 16 in Yakutia) to 6.99 pg (pop. 11 in Kazakhstan). Inter-population differences in GS may be due to various reasons including different geographical and environmental conditions (Greilhuber 2005). In order to find out the true causes of intra- and inter-population genome size variability in *K. ceratoides* s. l., it is necessary to conduct further analysis on a wider sample of populations. In general, the data we obtained shows that GS is a reliable indicator for determining the ploidy level in *K. ceratoides* using herbarium material (Fig. 1B).

The geographic distribution of two studied cytotypes of *K. ceratoides* is shown in Fig. 2. In general, diploids were observed in seven populations from Orenburg Region, Tyva, Kyzylorda and Almaty Regions. Tetraploids were observed in eight populations from Kazakhstan, Orenburg and Novosibirsk Regions, from Altai and Sakha (Yakutia) Republics. One mixed population was found in the Khakassia, represented by di- and tetraploids. We did not find any regularity in the geographical distribution of the two cytotypes. Our expert assessment of the morphological peculiarities of the samples did not reveal any significant differences between the studied populations. This is especially true for the shape and size of a leaf blade, one of the key taxonomic features in the genus *Krascheninnikovia*. A separate position of the tetraploid *K. lenensis*, far extending the main part of its distribution area, has also not been confirmed.

CONCLUSIONS

Nuclear DNA content (2C value) was correlated with ploidy level across 16 *Krascheninnikovia ceratoides* populations studied. Genome size and DNA ploidy level were reported for the first time from populations in the Republics of Khakassia and Sakha (Yakutia) and from Orenburg Region. We confirmed the chromosome numbers and ploidy levels for *K. ceratoides* previously reported in Altai. We identified two separate groups of DNA content and showed their correspondence to two cytotypes, diploid and tetraploid, by means of chromosome counts in four populations. The most studied populations were uniformly di- or tetraploid, beside one mixoploid population from the Republic of Khakassia in which two cytotypes occur side by side. We conclude that GS is a good tool for determining the ploidy level within the genus *Krascheninnikovia* and, most importantly, this method can be used in the analysis of herbarium samples. This makes possible the study in detail the geographical variability in polyploid complexes and understand their history and evolution.

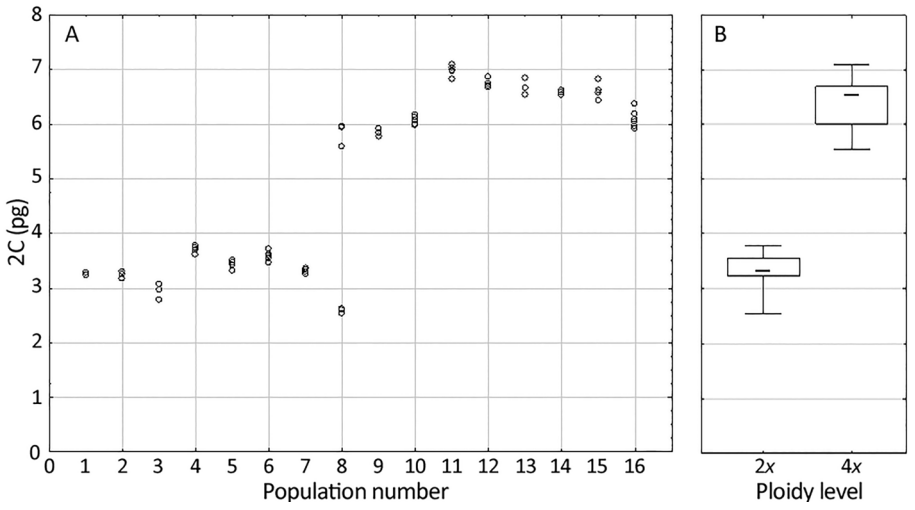

Figure 1 Genome size (2C) estimates for all individuals of 16 populations of *Krascheninnikovia ceratoides* (L.) Gueldenst. s. l. (A) and Box-and-whisker plots demonstrating variation in genome size (2C, pg) in diploids (n=35) and tetraploids (n=41) of *K. ceratoides* as inferred from Kruskal Wallis analysis (B). Boxes define the 25th and 75th percentiles, horizontal segments inside indicate medians. Whiskers indicate full range of GS variation. The population numbers correspond to the Table 1

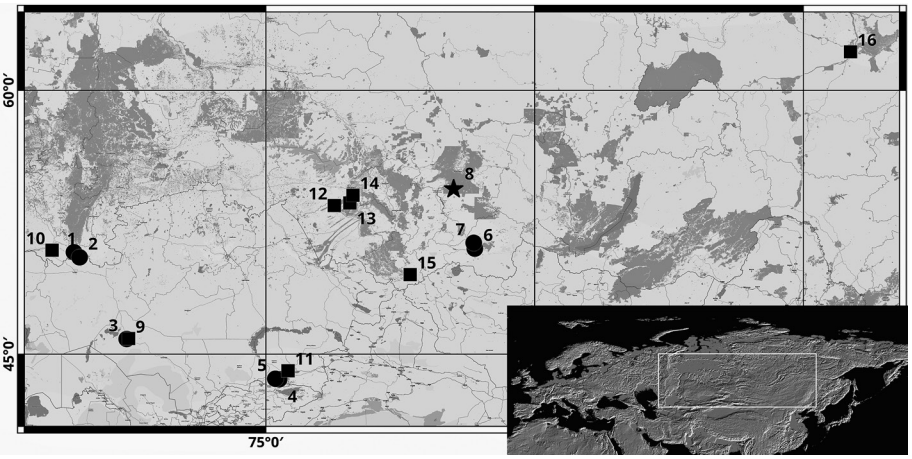

Figure 2 Cytotype distribution of the studied populations of *Krascheninnikovia ceratoides* (L.) Gueldenst. s. l. (circle – diploid, square – tetraploid). The asterisk marks the mixoploid population

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Ministry of Science and Higher Education of the Russian Federation, also under Agreement № ЕП/29-10-21-4 of October 29, 2021 between BIN RAN and CSBG SB RAS. In preparing the publication, materials from the bioresource scientific collections of the CSBG SB RAS UNU № USU 440537 (NS) were used.

LITERATURE CITED

Aktaeva, M.K. 1973. Karyology and morphological analysis of *Krascheninnikovia* Gueldenst. (*Eurotia* Adans.). *Izvestiya Akademii Nauk Kazahskoi SSR. Ser. Biol.* 6: 4−6 (in Russian). [Актаева М.К. 1973. Кариология и морфологический анализ рода *Krascheninnikovia* Gueldenst. (*Eurotia* Adans.) // Известия Академии Наук Казахской ССР, сер. биол. № 6. С. 4–6].

An'kova, T.V. & E.A. Korolyuk 2017. IAPT/IOPB chromosome data 26 (K. Marhold, ed.). *Taxon* 66(6):1487–1488, E2–4.

An'kova, T.V., M.N. Lomonosova, M.S. Voronkova, A.A. Petruk, B. Osmonali & P.V. Vesselova 2020. IAPT chromosome data 32/2 (K. Marhold ed.). *Taxon* 69(5): 1127, E3–E6.

Dobeš, Ch., B. Hahn & W. Morawetz 1997. Chromosomenzahlen zur Gefäßpflanzen-Flora Österreichs. *Linzer biologische Beiträge* 29(1):5–43.

Doležel, J. & J. Bartoš 2005. Plant flow cytometry and estimation of nuclear genome size. *Annals of Botany* 95:99–110.

Doležel, J., J. Greilhuber, S. Lucretti, A. Meister, M.A. Lysák, L. Nardi & R. Obermayer. 1998. Plant Genome size estimation by flow cytometry: Inter-laboratory comparison. *Annals of Botany* 82:17–26.

Doležel, J, J. Greilhuber & J. Suda 2007. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2:2233–2244.

Greilhuber, J 2005. Intraspecific variation in genome size in angiosperms: identifying its existence. *Annals of Botany* 95:91–98.

Heklau, H. & M. Röser 2008. Delineation, taxonomy and phylogenetic relationships of the genus *Krascheninnikovia* (Amaranthaceae subtribe Axyridinae). *Taxon* 57(2):563−576.

Kamelin, R.V. 2011. Ancient xerophilous family Chenopodiaceae in the Turanian and Central asian flora. *Botanicheskii Zhurnal* 96(4):441–464 (in Russian with English summary). [Древнексерофильное семейство Chenopodiaceae во флоре Турана и Центральной Азии // Ботанический журнал. Т. 96, № 4. С. 441–464].

Kolář, F, M. Čertner, J. Suda, P. Schönswetter & B.C. Husband 2017. Mixed ploidy species: progress and opportunities in polyploid research. *Trends in Plant Science* 22:1041–1055.

Kurban, N. 1984. Karyotype analysis of three species of *Ceratoides*. *Acta Phytotaxonomica Sinica* 22:466–468 (in Chinese with English summary).

Lomonosova, M.N. & A.A. Krasnikov 1993. Chromosome numbers in some members of the Chenopodiaceae. *Botanicheskii Zhurnal* 78(3):158–159 (in Russian). [Ломоносова М. Н., Красников А. А. 1993. Числа хромосом некоторых представителей семейства Chenopodiaceae // Ботанический журнал. Т. 78, № 3. С. 158–159.

Lomonosova, M.N., T.V. An'kova, M.S. Voronkova, E.A. Korolyuk, E.V. Banaev & M.V. Skaptsov 2020. Ploidy level in the representatives of Chenopodiaceae from North Asia as revealed by genome size and chromosome numbers. *Turczaninowia* 23(1):24.

Otto, F. 1992. Preparation and staining of cells for high-resolution DNA analysis. In: *Flow cytometry and cell sorting* (A. Radbruch, ed.), pp. 101–104, Springer, Berlin.

Pellicer, J., R.F. Powell & I.J. Leitch 2021. The application of flow cytometry for estimating genome size, ploidy level, endopolyploidy, and reproductive modes in plants. In: *Molecular Plant Taxonomy. 2nd edition* (P. Besse, ed.) pp. 329–361. Human Press, Springer, New York.

Pérez-Collazos, E. & P. Catalán 2007. Genetic diversity analysis and conservation implications for the Iberian threatened populations of the irano-turanian relict *Krascheninnikovia ceratoides* (Chenopodiaceae). *Biological Journal of the Linnean Society* 92:419–429.

Pfosser, M., A. Amon, T. Lelley & E. Heberle-Bors 1995. Evaluation of sensitivity of flow cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. *Cytometry* 21:387–393.

Radzhabli, E.P. & V.D. Rud'. 1972. *The formation and use of polyploid forms of plants*. Nauka, Novosibirsk, 132 pp. (in Russian). [Раджабли Е.П., Рудь В.Д. 1972. Получение и использование полиплоидных форм растений. Новосибирск: Наука. 32 с.].

Seidl, A., E. Pérez-Collazos, K. Tremetsberger, M. Carine, P. Catalán & K-G. Bernhardt 2020. Phylpogeny and biogeography of pleistocene holarctic steppe and semidesert goosefoot plant *Krascheninnikovia ceratoides*. *Flora* 262:151504.

Seidl, A., K. Tremetsberger, S. Pfanzelt, F.R. Blattner, B. Neuffer, N. Friesen, H. Hurka, A. Shmakov, O. Batlai, A.Ž. Čalasan, P.V. Vesselova & K-G. Bernhardt. 2021. The phylogeographic history of *Krascheninnikovia* reflects the development of dry steppes and semi-deserts in Eurasia. *Scientific Reports* 11:6645.

Skaptsov, M.V., M.N. Lomonosova, M.G. Kutsev, S.V. Smirnov & A.I. Shmakov. 2017. The phenomenon of endopolyploidy in some species of the Chenopodioideae (Amaranthaceae). *Botany Letters* 164(1):47–53.

Smirnov, Yu.A. 1968. Accelerated method for studying somatic chromosomes in fruit trees. *Tsitologiya* 10(12):1132–1134 (in Russian). [Смирнов Ю.А. 1968. Ускоренный метод исследования соматических хромосом плодовых // Цитология. Т. 10, № 12. С. 1132–1134].

Suda, J., & P. Trávníček 2006. Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry – new prospects for plant research. *Cytometry Part A* 69:273–280.

Tate, J.A, P.S. Soltis & D.E. Soltis 2005. Polyploidy in plants. In: *The evolution of the genome* (T. Gregory, ed.), pp. 371−426, Academic Press, Esevier Inc.

Wang, P.C., L.L. Zhao, B.T. Mo, Y. Zhang, J. Chen & L.B. Wang 2015. Genetic diversity of *Ceratoides arborescens*, a species endemic to China, detected by inter-simple sequence repeat (ISSR). *Genetics Molecular Research* 14(2):56585666.

Zakharjeva, O.I. & Yu.D. Soskov 1981. Chromosome number in desert herbage plants. *Byulleten' Vsesoyuznogo nauchno-issledovatel'skogo Instituta Rastenievodstva im. N.I. Vavilova* 108:57–60 (in Russian). [Захарьева О.И., Сосков Ю.Д. 1981. Хромосомные числа некоторых пустынных кормовых растений. // Бюллетень Всесоюзного научно-исследовательского Института Растениеводства им. Н.И. Вавилова. Т. 108. С. 57–60].

Zhu, G.-L., S.L. Mosyakin & S.E. Clemants 2003. Chenopodiaceae. In: *Flora of China, vol. 5* (Z.Y. Wu, P.H. Raven & D.-Y Hong, eds), pp. 351–414, Science Press, Beijing & Missouri Botanical Garden Press, St. Louis.