ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 11: 49–69 (2025)
doi: 10.5281/zenodo.14784067
http://journal.asu.ru

RESEARCH ARTICLE

Nuclear DNA content in some *Chondrilla* taxa (Asteraceae) of European Russia and Western Kazakhstan

Alena S. Parkhomenko[1], Mikhail V. Skaptsov[2], Anna O. Kondratieva[1], Saveliy F. Efimenko[1], Aleksandr S. Kashin[1]

1 *Saratov State University, 83 Astrakhanskaya st., Saratov, Russian Federation*
2 *Altai State University, 61 Lenina av., Barnaul, Russian Federation*

Corresponding author: Aleksandr S. Kashin (kashinas2@yandex.ru)

Academic editor: R. Yakovlev | Received 10 December 2024 | Accepted 17 December 2024 | Published 3 February 2025

http://zoobank.org/870D250D-0FCF-4893-AF04-5E81D15AD6F6

Citation: Parkhomenko AS, Skaptsov MV, Kondratieva AO, Efimenko SF, Kashin AS (2025) Nuclear DNA content in some *Chondrilla* taxa (Asteraceae) of European Russia and Western Kazakhstan. Acta Biologica Sibirica 11: 49–69. https://doi.org/10.5281/zenodo.14784067

Abstract

The aim of study was to evaluate the nuclear DNA content in samples of *Chondrilla* taxa from European Russia and Western Kazakhstan using flow cytometry approach. The analysis was performed in 30 populations of 8 taxa from the genus (*C. brevirostris*, *C. laticoronata*, *C. juncea*, *C. latifolia*, *C. graminea*, *C. canescens*, *C. ambigua*, and *C. pauciflora*). It was revealed that *C. juncea*, *C. graminea*, *C. canescens* and *C. latifolia* have the smallest monoploid genome size (1Cx) (1.078–1.098 pg), *C. laticoronata* and *C. brevirostris* have the intermediate values (1.190–1.203 pg), and *C. pauciflora* and *C. ambigua* have the largest (1.309–1.449 pg), i.e. the DNA content consistently increases by approximately 10% between these groups of taxa. The obtained results confirm the opinion that *C. juncea*, *C. graminea*, *C. latifolia* and *C. canescens* are synonymous with the priority name *C. juncea*. *C. ambigua* is the only one diploid species among the studied taxa. *C. pauciflora* is most likely its triploid cytotype. The position of *C. laticoronata* and *C. brevirostris* DNA contents between the *C. juncea* subspecies group, and the group including *C. pauciflora* and *C. ambigua* can be explained by distant hybridization that took place in the past, when *C. ambigua* or *C. pauciflora* acted as a maternal parent, and two species from the subgenus *Chondrilla*, different for each combination, acted as a paternal ones. The obtained results indicate that within the studied range the DNA content in *Chondrilla* at the interpopulation level changes regularly along the latitudinal gradient. From the south to about 50°N, the genome size increases. From 50°N to the north, the nuclear DNA content decreases.

Keywords
Chondrilla, chromosome number, DNA content, flow cytometry

Introduction

The genus *Chondrilla* L. (Asteraceae) includes about 30 species (Leonova 1989; The World Flora Online). The genus is widespread in steppe and desert regions of Eurasia and North Africa. Most of the species have extensive areas, including secondary ones. *C. juncea* L. was introduced into Australia, Argentina, Canada and the USA, where it currently causes significant damage to crops on fields and pastures, demonstrating an extremely high invasive potential (Gaskin et al. 2013). Conversely, endemic species of the genus with a narrow range and a critically vulnerable state of small populations are known, such as *C. chondrilloides* (Ard.) H. Karst (Woellner et al. 2019, 2022) – a diploid, obligately sexual species as is *C. ambigua* Fisch. (Orsenigo et al. 2019).

At least seven taxa occur naturally in European Russia. Six of them belong to *Chondrilla* section of *Chondrilla* subgenus (*C. acantholepis* Boiss., *C. brevirostris* Fisch. Et Mey, *C. canescens* Kat. Et Kir., *C. graminea* Bieb., *C. juncea* L. and *C. latifolia* Bieb.) and one (*C. ambigua* Fisch.) to *Brachyrynchus* subgenus (Leonova 1989). In addition, populations of invasive *C. laticoronata* Leonova, belonging to *Arthrorhinchus* section of *Chondrilla* subgenus, have been increasingly detected. The species has been repeatedly noted in Astrakhan Region and Stavropol Territory (SARBG). In particular, there are herbarium collections from the surroundings of Verblyuzhiy, Akhtubinsky District, as well as from the surroundings of Sasykoli and Tambovka, Kharabalinsky District, Astrakhan Region. Long-term monitoring has shown that these populations are quite numerous and exist stably, showing no tendency to decrease in numbers. In Stavropol Territory the species is numerous in the surroundings of Arzgir, Arzgir District. There are herbarium collections from the surroundings of Tersky, Budyonovsky District (100 km south of the first one), as well as from the surroundings of Kurskaya, Kursk region (150 km south of the first one). According to literature data (Maevskii 2014), the species is also noted in the Chuvash Republic.

C. pauciflora Ledeb. was also indicated in the southeast of the Lower Volga region (Leonova 1989). However, this occurrence probably refers to the western regions of Kazakhstan, since in the zoning adopted in "Flora of the European Part of USSR", the western regions of Kazakhstan were included in the Lower Volga region. We have not found the species in this territory within European Russia. The nearest herbarium collections were from the surroundings of Khan Ordasy, Bokeyordinskiy district, Western Kazakhstan (Herbarium of the Botanical Garden of Saratov State University, SARBG). The collection of Moscow State University contains a herbarium collection from the Volgograd region (Volgograd city, Krasnoarmeysky district, near the Sudoverf station 5.09.1997. A. Sukhorukov – MW0551534) (Depository

of Live Systems, 2024). However, it was collected on a railway line, which probably could results from an accidental introduction by transport, and subsequently the species did not naturalized in this territory. Despite frequent visits to the Lower Volga region by A. P. Sukhorukov, in subsequent years (after 1997) this species was not noted by him.

The origin of genus *Chondrilla* is associated with Eastern Europe, Mediterranean or Caucasus, dating back to the late Miocene about 7.7 million years ago. It is assumed that it subsequently spread to Central Asia, where it underwent a process of secondary speciation (Tremetsberger et al. 2013). In this regard, the currently observed secondary expansion of *C. laticoronata* into Eastern Europe is of interest. There is still no clear understanding of the genus taxonomic structure. For example, *C. juncea* and *C. graminea* are considered by some authors as independent species in *Chondrilla* section of *Chondrilla* subgenus (Leonova 1989), while the others combine them into one species *C. juncea* (Global Compositae Checklist). Several authors also combine *C. canescens* (Tutin et al. 1976), *C. latifolia* (Tutin et al. 1976; Nasseh 2010), *C. acantholepis* (Tutin et al. 1976; Nasseh 2010) and *C. brevirostris* (Tutin et al. 1976) into one species under the priority name of *C. juncea*. The studies of morphological and molecular genetic variability confirm the validity of such conclusions, with the exception of *C. brevirostris* (Kashin et al. 2024).

Identification of some *Chondrilla* taxa is problematic due to the lack of reliable diagnostic traits, high morphological variation and phenotypic plasticity. According to the results of morphological (Kashin et al. 2018) and molecular genetics (Kashin et al. 2017, 2019) analyses, *C. ambigua* is the only one morphologically well-defined among the taxa listed for Eastern Europe and must be considered as species. Considering *C. brevirostris* and *C. laticoronata* as taxa of species rank is less obvious. According to revealed morphological variation and results of genetic analysis using ISSR markers, markers of trnT–trnF region of plastid DNA and the intergenic transcribed ribosomal spacer (ITS) of nuclear DNA, *C. juncea*, *C. latifolia*, *C. graminea*, *C. acantholepis* and *C. canescens* was not separated from each other and should be considered as synonyms of *C. juncea*.

Karyological analysis of *Chondrilla* representatives revealed that its chromosome number varied from 2n=x=5 to 2n=5x=25. A high proportion of aneu- and mixoploids was observed among *C. brevirostris*, *C. laticoronata*, *C. juncea*, *C. latifolia*, *C. graminea* and *C. canescens*. A stable chromosome number was noted only for *C. ambigua* (2n=2x=10) and *C. pauciflora* (2n=3x=15) (Parkhomenko and Kashin 2018).

A relatively large number of *Chondrilla* taxa are characterized by apomictic reproduction (van Dijk 2003; Kashin et al. 2015). This feature is associated with interspecific hybridization, polyploidy, aneuploidy and other changes in genome structure, which complicates the species identification. In this context, the genome size can be an additional diagnostic trait useful for explaining the relationships between species and may act as potential tool for revealing their evolution aspects (Gregory 2001; Leitch et al. 2007; Bhadra et al. 2023).

The genome size has not been determined for 97 (Bennett and Leitch 2012; Pellicer and Leitch 2019) – 95% (Bureš et al. 2024) of angiosperm species. In genus *Chondrilla* the DNA content was determined only for *C. juncea* (Bennett and Leitch 2012; Garcia et al. 2013) and equaled 2C=3.01 pg. The genome size of other *Chondrilla* taxa has not been defined previously and any information on this issue is very important.

The aim of this study was to estimate the nuclear DNA content in plant samples of *Chondrilla* taxa from European Russia and Western Kazakhstan using flow cytometry methods.

Materials and methods

The studies were carried out on young plants grown in laboratory conditions from achenes collected in 2022 from 30 populations of 8 *Chondrilla* taxa (*C. brevirostris*, *C. laticoronata*, *C. juncea*, *C. latifolia*, *C. graminea*, *C. canescens*, *C. ambigua* and *C. pauciflora*) (Table 1; Figs 1, 2).

Table 1. Location of studied *Chondrilla* populations

Population code	Geographical coordinates		Locality
	Latitude	Longitude	
			C. ambigua
Dos-a	46°54.55'N	47°55.36'E	Russia, Astrakhan Region, Krasnoyarskiy District, near Dosang
			C. pauciflora
Kaz-p	48°49.18'N	47°30.29'E	Kazakhstan, Western-Kazakhstan Region, Bokeyordinskiy District, near Khan Ordasy
			C. laticoronata
Kaz-l	48°49.18'N	47°30.29'E	Kazakhstan, Western-Kazakhstan Region, Bokeyordinskiy District, near Khan Ordasy
Wer-l	47°43.13'N	46°53.18'E	Russia, Astrakhan Region, Akhtubinskiy District, near Verblyuzhiy
Tam	47°19.73'N	47°23.56'E	Russia, Astrakhan Region, Kharabalinskiy District, near Tambovka
Sas	47°33.35'N	46°58.14'E	Russia, Astrakhan Region, Kharabalinskiy District, near Sasykoli
			C. brevirostris
Kaz-b	48°49.18'N	47°30.29'E	Kazakhstan, Western-Kazakhstan Region, Bokeyordinskiy District, near Khan Ordasy
Wer-b	47°43.13'N	46°53.18'E	Russia, Astrakhan Region, Akhtubinskiy District, near Verblyuzhiy
Nor	46°32.40'N	47°55.57'E	Russia, Astrakhan Region, Narimanovskiy District, near Volzhskoe

Population code	Geographical coordinates		Locality
	Latitude	Longitude	
Bug-b	47°34.15'N	46°54.87'E	Russia, Astrakhan Region, Kharabalinskiy District, near Bugor
Vln	47°8.22'N	47°40.35'E	Russia, Astrakhan Region, Kharabalinskiy District, near Vol'noe
Bol	47°59.38'N	46°33.05'E	Russia, Astrakhan Region, Akhtubinskiy District, near Bolkhuny
Dos-b	46°17.39'N	46°41.56'E	Russia, Astrakhan Region, Narimanovskiy District, near Saygachniy
			C. juncea
Hvl-j	52°28.74'N	48°3.54'E	Russia, Saratov Region, Khvalynskiy District, near Khvalynsk
Al-j	52°15.01'N	46°20.23'E	Russia, Saratov Region, Bazarno-Karabulakskiy District, near Alekseevka
Baz-j	47°48.21'N	41°3.45'E	Russia, Rostov Region, Konstantinovskiy District, near Bazki
Kmh-j	50°8.08'N	45°26.25'E	Russia, Volgograd Region, Kamyshinskiy District, near Kamyshin
Ppv-j	51°23.50'N	45°36.54'E	Russia, Saratov Region, Gagarinskiy District, near Popovka
Vol	51°55.39'N	47°19.58'E	Russia, Saratov Region, Marksovskiy District, near Volkovo
Bots	51°33.95'N	46°0.7'E	Russia, Saratov Region, Saratov, Botanical Garden
Mel-j	50°48.19'N	45°34.54'E	Russia, Saratov Region, Krasnoarmeyskiy District, near Melovoe
Pri	51°43.65'N	44°56.52'E	Russia, Saratov Region, Atkarskiy District, near Prirechnoe
			C. graminea
Hvl-gr	52°28.74'N	48°3.54'E	Russia, Saratov Region, Khvalynskiy District, near Khvalynsk
Al-gr	52°15.22'N	46°19.96'E	Russia, Saratov Region, Bazarno-Karabulakskiy District, near Alekseevka
			C. latifolia
Kmh-l	50°8.08'N	45°26.25'E	Russia, Volgograd Region, Kamyshinskiy District, near Kamyshin
Baz-l	47°48.21'N	41°3.45'E	Russia, Rostov Region, Konstantinovskiy District, near Bazki
Rost	48°2.57'N	41°18.1'E	Russia, Rostov Region, Tatsinskiy District, near Verkhnekol'tsov
			C. canescens
Kap	48°32.17'N	45°51.12'E	Russia, Astrakhan Region, Akhtubinskiy District, near Kapustin Yar
Al-can	52°15.01'N	46°20.23'E	Russia, Saratov Region, Bazarno-Karabulakskiy District, near Alekseevka
Hvl-can	52°28.74'N	48°3.54'E	Russia, Saratov Region, Khvalynskiy District, near Khvalynsk

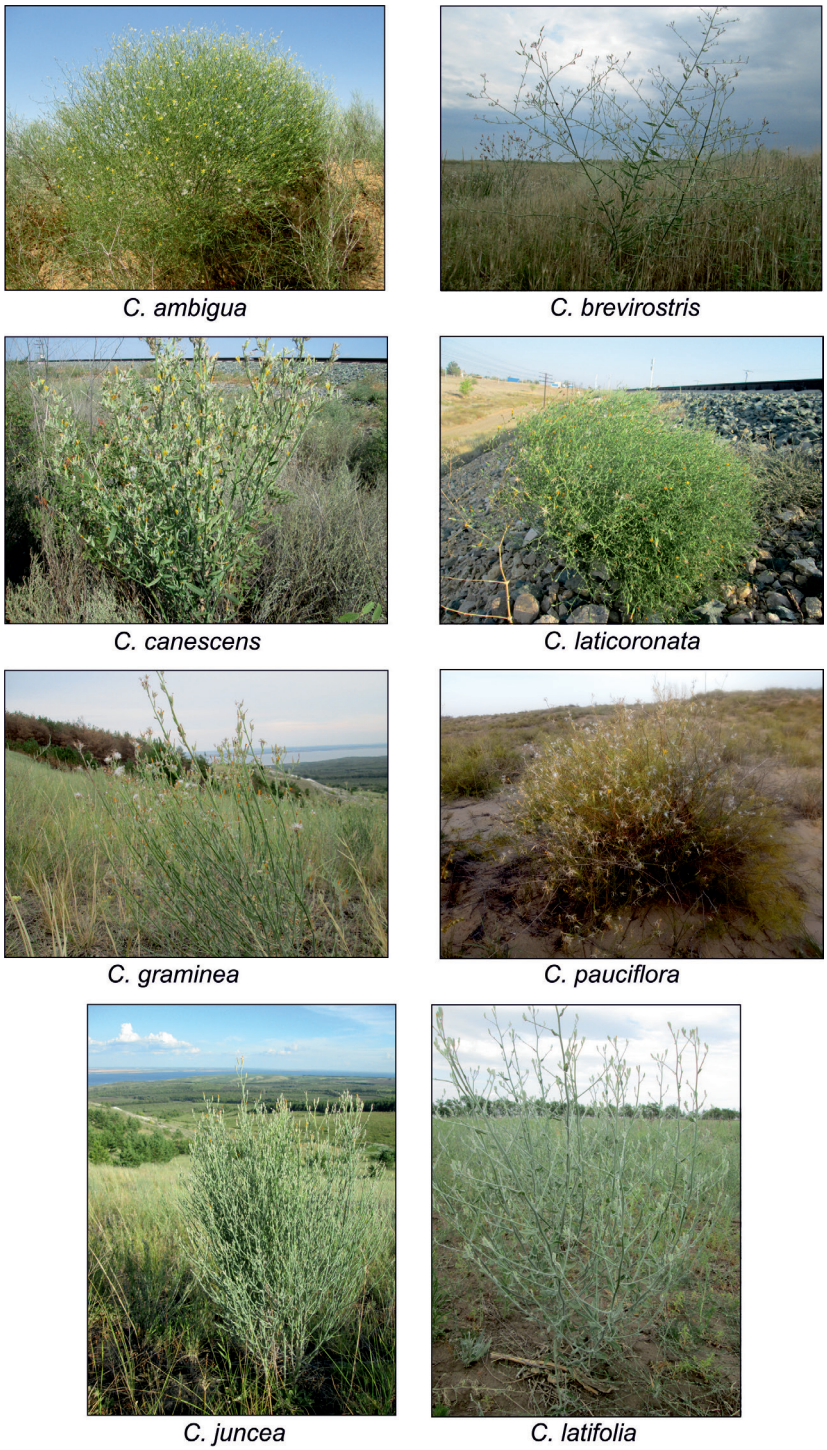

Figure 1. Representatives of genus *Chondrilla* from European Russia and Western Kazakhstan under study. Photos by A.S. Kashin.

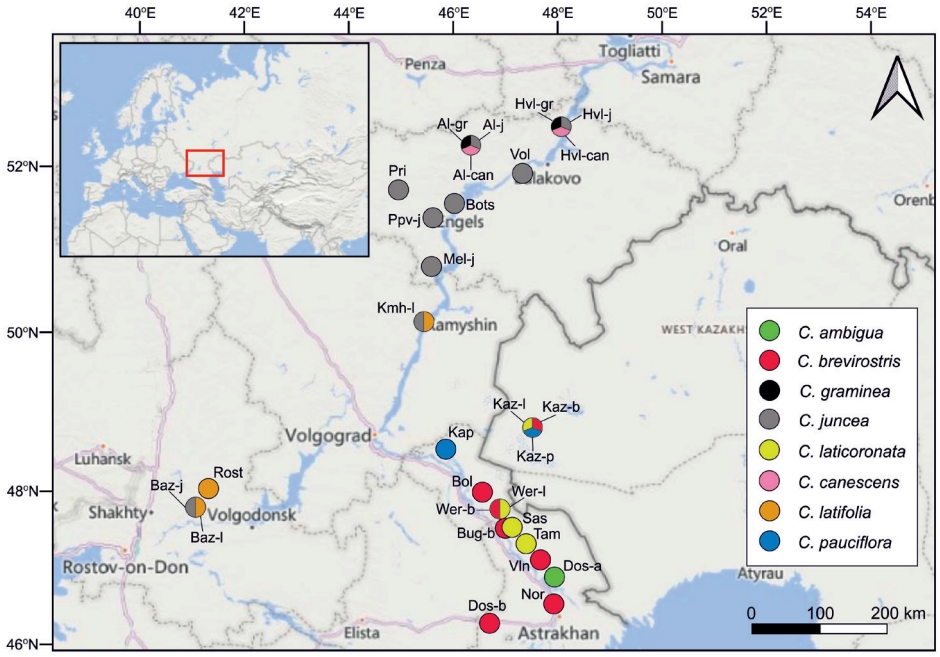

Figure 2. Location of studied *Chondrilla* populations.

The achenes were germinated on wet filter paper in Petri dishes in thermostat at +28°C, then transplanted into pots with soil and grown in a greenhouse until the leaf blade size reached 0.5×0.5 cm.

The DNA content was determined using flow cytometry by staining of nuclei with propidium iodide. The fresh leaves were crushed using a blade in 1 ml of Tris-$MgCl_2$ buffer (Pfosser et al. 1995) with following composition changes: 0.2 M Tris base, 4 mM $MgCl_2$, 0.5% Triton X-100, 50 µg/ml RNase, 10 mM sodium metabisulfite, 50 µg/ml propidium iodide (pH 7.5). Samples were filtered through a 50 µm nylon membrane. *Pisum sativum* cv. 'Ctirad' characterized by a constant genome size across the entire diversity of varieties (Doležel and Greilhuber 2010) was used as an internal standard, 2C=9.09 pg (Doležel et al. 1998). The DNA content (2C, pg) was calculated using formula:

$$2C, pg = (\text{Average peak value of sample}/\text{Average peak value of standard}) \times 2C \text{ of standard, pg}$$

For the calculation, peaks with at least 1000 nuclei and CV less than 3% were used (Doležel and Greilhuber 2010).

The fluorescence data of isolated nuclei were detected using Cytoflex flow cytometer (Beckman Coulter, Inc.) with a laser radiation source with a wavelength of 488 nm. Visualization and processing of histograms were performed in CytExpert

software (Beckman Coulter, Inc.). Statistical data were calculated in XLStat (Addinsoft).

Chromosome counting was performed on the same samples used for DNA analysis by flow cytometry. Actively growing root tips were collected and pretreated with an aqueous solution of bromonaphthalene at +4°C for 3.5 hours, and then were fixed in solution of 96% ethanol and glacial acetic acid (3:1) for at least 24 hours at +4°C. The root tips were stained by acetohematoxylin. The temporary squash preparations were prepared in 80% chloral hydrate. The material was analyzed under oil immersion using Carl Zeiss Axio Scope A1 microscope (×1600). Microphotography was performed using AxioCam MRc 5 (D) digital camera and 60N-C 1" 1.0x adapter. Based on the chromosome number (the basic chromosome number for *Chondrilla* is 5), plants were assigned to one or another ploidy level (Kubešová et al. 2010).

Results

Most of the nuclei isolated from studied *Chondrilla* taxa and *Pisum sativum* samples were in G_0/G_1 stage of cell cycle. The nuclei in G_2 stage, either were not found or were present at low frequency.

DNA content reached 2C=2.898±0.015 pg in the samples of sexual *C. ambigua* and 3.918±0.032 pg in the apomictic *C. pauciflora*. Therefore, the DNA content in *C. pauciflora* was 1.35 times higher than in *C. ambigua*. The direct counting of chromosomes established that *C. ambigua* is diploid (2n=2x=10) and *C. pauciflora* is triploid (2n=3x=15) (Fig. 3, Table 2).

The DNA contents in triploid samples of *C. laticoronata* (2C=3.570±0.026 pg) and *C. brevirostris* (2C=3.609±0.035 pg) were relatively close (Fig. 3). In the samples of *C. laticoronata* from two populations growing near each other about 40 km apart (the surroundings of Tambovka and Sasykoli) the DNA contents did not differ significantly (2C=3.557±0.006 and 3.564±0.030 pg, respectively) (Table 2). However, in contrast with *C. ambigua* and *C. pauciflora*, aneu- and mixoploidy were found at low frequency in these taxa's root meristems. The number of chromosomes in root meristems of the same sample differed in individual cells, deviating from the basic ploidy level in the range from 10 to 15 chromosomes.

In *C. brevirostris* populations, the minimum DNA content was revealed in the samples from southernmost habitat (the surroundings of Saygachny) (46°17.39'N) (2C=3.578±0.024 pg), and the maximum content was from the northernmost one (the surroundings of Bolkhuny) (47°59.38'N) at the boundary of this taxa's area (2C=3.657±0.040 pg). The direct chromosome counting in metaphase plates of the researched samples from these two populations determined that in population from Bolkhuny surroundings, the chromosome number within every sample varied in a wider range (from 10 to 20, often more than 15) than in population from Saygachny surroundings (from 12 to 15) but remaining equal to 15 in most metaphase plates.

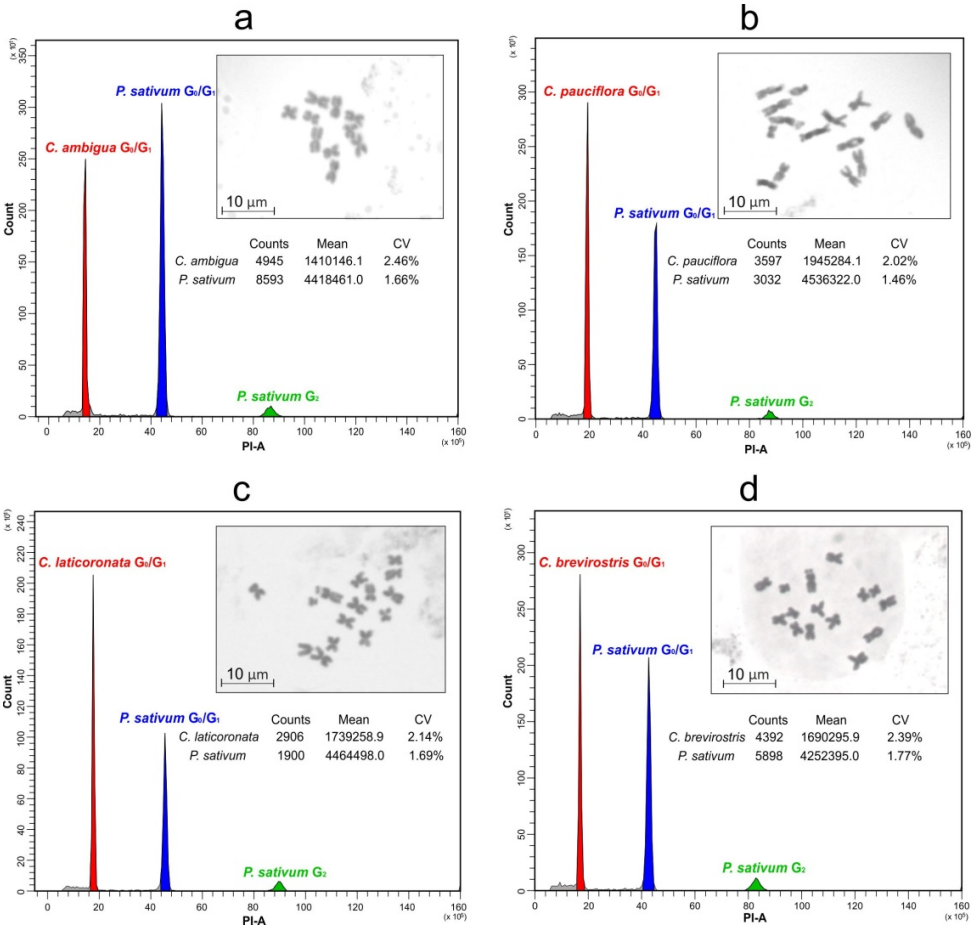

Figure 3. The examples of histograms of studied species and their karyotypes: a – *C. ambigua*; b – *C. pauciflora*; c – *C. laticoronata*; d – *C. brevirostris*. Here and later *Pisum sativum* cv. 'Ctirad' used as a standard. There are fluorescence intensity on the abscissa axis (PI-A) and number of nuclei on the ordinate axis (Count).

Table 2. The DNA content in *Chondrilla* plants

Population code *	Ploidy level**	2C, pg	SD, pg	CV, %	1Cx, pg
			C. ambigua		
Dos-a	2x	2.898	0.015	0.52	1.449
			C. pauciflora		
Kaz-p	3x	3.918	0.032	0.82	1.306
			C. laticoronata		
Kaz-l	3x	3.551	0.010	0.29	1.184

Population code *	Ploidy level**	2C, pg	SD, pg	CV, %	1Cx, pg
Wer-l	3x	3.609	0.043	1.18	1.203
Tam	3x	3.557	0.006	0.18	1.186
Sas	3x	3.564	0.030	0.85	1.188
Mean values of 2C and 1Cx, pg		3.570	0.026	0.01	1.190
			C. brevirostris		
Kaz-b	3x	3.558	0.023	0.65	1.186
Wer-b	3x	3.604	0.024	0.67	1.201
Nor	3x	3.600	0.016	0.44	1.200
Bug-b	3x	3.623	0.035	0.95	1.208
Vln	3x	3.643	0.036	0.98	1.214
Bol	3x	3.657	0.040	1.10	1.219
Dos-b	3x	3.578	0.024	0.67	1.193
Mean values of 2C and 1Cx, pg		3.609	0.035	0.01	1.203
			C. juncea		
Hvl-j	3x	3.218	0.024	0.75	1.073
Al-j	3x	3.224	0.032	1.00	1.075
Baz-j	3x	3.244	0.083	2.57	1.081
Kmh-j	4x	4.312	0.063	1.46	1.078
Ppv-j	3x	3.251	0.072	1.97	1.084
Vol	3x	3.275	0.044	1.34	1.092
Bots	3x	3.324	0.042	1.28	1.108
Mel-j	3x	3.253	0.063	1.94	1.084
Pri	3x	3.205	0.063	2.20	1.068
Mean values of 2C and 1Cx for triploids, pg		3.249	0.038	0.01	1.083
Mean values of 2C and 1Cx for tetraploids, pg		4.312	0.063	1.46	1.078
			C. graminea		
Hvl-gr	3x	3.239	0.021	0.65	1.080
Al-gr	3x	3.239	0.041	1.27	1.080
Mean values of 2C and 1Cx, pg		3.239	0.000	0.00	1.080
			C. latifolia		
Kmh-l	3x	3.270	0.031	0.94	1.090
Baz-l	3x	3.320	0.036	1.07	1.107
Rost	3x	3.294	0.038	1.16	1.098
Mean values of 2C and 1Cx, pg		3.295	0.025	0.01	1.098
			C. canescens		
Kap	3x	3.318	0.046	1.38	1.106

Population code *	Ploidy level**	2C, pg	SD, pg	CV, %	1Cx, pg
Al-can	3x	3.249	0.024	0.75	1.083
Hvl-can	3x	3.210	0.030	0.92	1.070
Mean values of 2C and 1Cx, pg		3.259	0.055	0.02	1.086

Notes: * populations' locations and coordinates are given in Table 1 and Fig. 2; ** the sample's ploidy level was determined by the most often occurring chromosome number in its metaphase plates. 1pg of DNA=978 Mbp (Doležel et al. 2003); 2C is DNA content; SD is standard deviation; CV is coefficient of variation; 1Cx is monoploid genome size.

The DNA contents in plants of the other two Astrakhan populations of *C. brevirostris* placed between the two above populations (the surroundings of Bugor and Volzhskoye) were 3.623±0.035 and 3.600±0.016 pg, respectively, i. e. were ranged between the maximum and minimum DNA contents in samples of the first two populations of this taxa. The chromosome number in metaphase plates varied from 10 to 15 in every sample.

Conspicuous is the fact, that the samples of *C. laticoronata* and *C. brevirostris* from the same habitat (the surroundings of Verblyuzhiy, Astrakhan Region), despite the differences in species membership, have similar values of DNA content (3.609±0.043 and 3.604±0.024 pg, respectively). The range of variation in sample's chromosome number was also close between these two taxa (from 12 to 15 for *C. laticoronata* and from 10 to 15 for *C. brevirostris*). In another common habitat (the surroundings of Khan Ordasy, Kazakhstan), the samples of these two taxa also have similar DNA contents, lower than the samples from other localities (3.551±0.010 and 3.558±0.023 pg, respectively). Interestingly, the variation of chromosome number within every sample (from 10 to 20) in this habitat was wider than in previous one, regardless of sample's species membership.

The samples from eight studied populations of *C. juncea* (Hvl-j, Al-j, Baz-j, Ppv-j, Vol, Pri, Bots and Mel-j) were triploids with the DNA content of 3.249±0.038 pg. The direct chromosome counting revealed wide variation within every analyzed sample, without any pattern. The chromosome number was 15 in most metaphase plates. The DNA contents in studied samples did not differ significantly at the inter-population level, despite the wide geographical range of their localities (Table 2, Fig. 4a).

Using the direct chromosome counting, it was found that the samples of *C. juncea* from Kmh-j population were tetraploids (2n=4x=20), but metaphase plates with 14–15 and 17–18 chromosomes were also observed. The DNA content in samples from this population was 4.312±0.063 pg (Table 2, Fig. 4b), that is 1.3 times higher than in triploid populations of this species.

The triploid samples from two *C. graminea* populations had identical DNA content values equaled 3.239 pg (Table 2, Fig. 5).

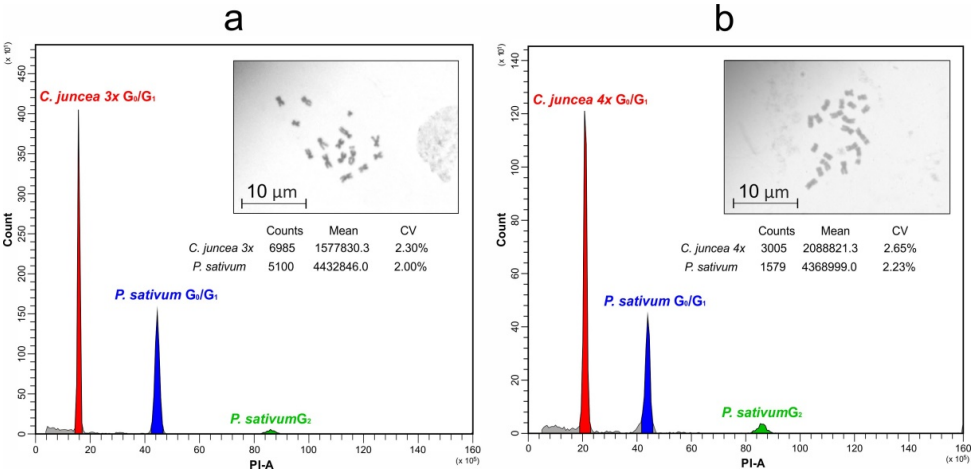

Figure 4. The examples of histograms of *C. juncea* samples and their karyotypes: a – triploid; b – tetraploid. There are fluorescence intensity on the abscissa axis (PI-A) and number of nuclei on the ordinate axis (Count).

The chromosome number in samples of *C. graminea* population from Khvalynsk surroundings varied from 10 to 15 and from 12 to 15 in population from the Alekseevka surroundings. These two populations are located about 120 km apart.

In all studied samples from three *C. latifolia* populations, the DNA content values did not differ significantly. The mean value of 2C was 3.295±0.025 pg (Table 2, Fig. 5). The direct chromosome counting found out that all studied samples of *C. latifolia* were triploids with variation in chromosome number from 10 (Baz-l) – 12 (Kmh-l and Rost) to 15.

Among three studied *C. canescens* populations two (Hvl-can and Al-can) was located about 120 km apart. The chromosome number in meristem cells varied from 10 (Hvl-can) – 13 (Al-can) to 15. The DNA contents in samples from these populations did not differ significantly and equaled 3.210±0.030 and 3.249±0.024 pg, respectively (Table 2). The third studied *C. canescens* population was located 400–500 km south of the first two. In samples of this population the chromosome number varied from 12 to 16. The DNA content value was higher than in first two populations placed relatively close to each other in terms of latitude, and equaled 3.318±0.046 pg (Table 2). The samples of all three *C. canescens* populations were triploids (Fig. 5b).

Discussion

It was established that *C. ambigua* is a sexual diploid species, and *C. pauciflora* is an apomictic triploid taxa. The stability of ploidy level and chromosome number in

plants of these taxa was confirmed earlier on large number of samples. Aneu- and mixoploidy were not observed in samples of these taxa (Parkhomenko and Kashin 2018). The DNA content growth which non-multiple to chromosome set with increasing (compared with *C. ambigua*) ploidy in *C. pauciflora* can be explained by a decrease in chromosome size and DNA content after polyploidization round (genome downsizing), i.e. implementation of a correction mechanism against excessive genetic information in evolutionary past (Leitch and Bennett 2004). Therefore, the separation of these taxa occurred in the relatively distant past, but their chromosomes probably did not undergo any significant morphological changes during this time. This follows from the fact that all chromosomes of two taxa are metacentric, varying only in size, and in a similar way within groups of homological chromosomes (Parkhomenko et al. 2019).

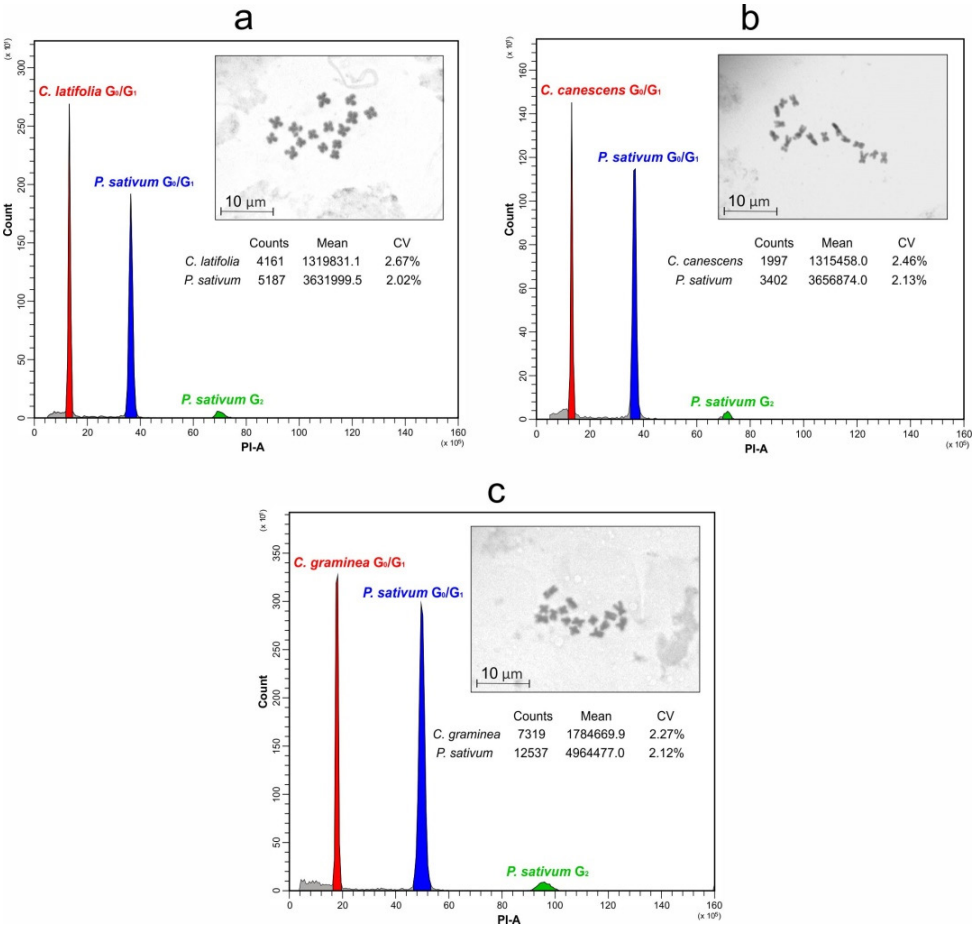

Figure 5. The examples of histograms of studied species and their karyotypes: a – *C. latifolia*; b – *C. canescens*; c – *C. graminea*. There are fluorescence intensity on the abscissa axis (PI-A) and number of nuclei on the ordinate axis (Count).

The other studied *Chondrilla* taxa are facultatively apomictic. They have the basic chromosome number equals to 2n=3x=15, and characterized by aneu- and mixoploidy (Parkhomenko and Kashin 2018; Parkhomenko et al. 2019). The direct chromosomes counting in roots of the studied samples revealed that metaphase plates with different chromosome number are found even in neighbouring cells within one root meristem, which certainly makes it difficult to analyze ploidy. As previously discovered (Parkhomenko and Kashin 2018) and confirmed in this work, the absolute majority of root meristem's cells have the dominant ploidy level (2n=3x=15), and the proportion of cells with other ploidy levels or with aneuploid chromosome number is insignificant (not more than 3%, i.e. lays beyond the flow cytometer resolution).

Therefore, revealing aneu- and mixoploidy in the samples of studied taxa by flow cytometry is beyond the sensitivity of this method because the DNA content in the sample genome is averaged over all nuclei passed through the detector, and the proportion of aneu- and mixoploid nuclei in the sample is low. In addition, the chromosome sizes in karyotypes of studied species have a high variation level that also complicates the finding aneu- and myxoploidy by flow cytometry.

In samples of all studied *Chondrilla* taxa, except for *C. ambigua* and *C. pauciflora*, the chromosome morphology was also variable (different combinations of meta-, submeta- and acrocentric chromosomes were found in the same group of homological chromosomes), which indicates large rearrangements that occurred earlier (Parkhomenko and Kashin 2018; Parkhomenko et al. 2019). In general, the chromosome sizes in *Chondrilla* samples karyotypes also varied widely – from 10 to 3μm. Thus, it is very difficult, most likely impossible to estimate per chromosome DNA content for plants of this genus.

Increase in DNA content of *C. laticoronata* and *C. brevirostris* triploids compared with the diploid of *C. ambigua* not 1.5 but 1.2 times can also be explained by reduction in size and morphological changes in chromosomes related to implementation of the correction mechanism against excessive genetic information in the evolutionary past (Leitch and Bennett 2004).

In *C. laticoronata* populations growing close to each other, the DNA content did not differ significantly. At the same time, in populations of *C. brevirostris*, significantly distant from each other, it varied greatly. The minimum DNA content was found in the samples from southernmost habitat, and the maximum content was from the northernmost one. The intermediate DNA content values were detected in plants from the other two *C. brevirostris* populations placed between the two abovementioned populations.

Therefore, spatial distribution of nuclear DNA content in samples of studied *C. brevirostris* populations changes along latitudinal gradient: the DNA content decreases from north to south. This pattern is consistent with the ideas of some researchers about global latitudinal gradient, when increasing in angiosperm genome size, probably mediated by climatic mechanisms, is observes from the equator to 40–50° north latitude (Bureš et al. 2024). The authors concluded that climate has

a strong influence on genome size distribution along the global latitude gradient while the effect of polyploidy frequency is insignificant. Our data on DNA content in *C. brevirostris* genomes support the validity of such ideas at the interpopulation level of variation of single taxa. The obtained results indicate that there is a correlation between the latitudinal change in genome size of this taxa and the range of chromosome number variability of karyotype.

The samples of *C. laticoronata* and *C. brevirostris* growing together in the same habitat have close DNA content values but differ from 2C samples of these taxa growing separately in other localities. This fact shows that such proximity of genome sizes may due to hybridization between plants of two taxa accompanied by different types and intensity of chromosome rearrangements in different localities. This is also supported by the fact that in joint habitats plants have morphological characteristics of both *C. laticoronata* and *C. brevirostris*.

Therefore, the recent invasion of *C. laticoronata* in Russian Astrakhan Region and Western Kazakhstan from Central Asia is of interest (Parkhomenko et al. 2023). The taxa was not previously noted for the flora of Eastern Europe (Leonova 1989). Hybridization processes started in recently emerged common habitats of *C. laticoronata* and *C. brevirostris* can lead to the same effects as in taxa from *C. juncea* group (*C. acantholepis* Boiss., *C. canescens*, *C. graminea*, *C. juncea* and *C. latifolia*). It was revealed that these taxa are not separated by any of the morphological characteristics and must be treated as subspecies with priority name *C. juncea* (Parkhomenko et al. 2023; Kashin et al. 2024). The genetic polymorphism also has the similar pattern. It was shown by using ISSR markers, plastid DNA trnT–trnF region markers and nuclear DNA intergenic transcribed ribosomal spacer (ITS) (Kashin et al. 2017, 2019).

C. laticoronata and *C. brevirostris* are well separated in the term of morphology and molecular genetics. The existence time of such separation will depend on the extent of *C. laticoronata* expansion into Eastern Europe and how far it invades into the ranges of other *Chondrilla* taxa. To date the intergrades observed only between *C. laticoronata* and *C. brevirostris* in common habitats in Astrakhan Region and Western Kazakhstan. However, as mentioned above, the species is numerous in several places of Stavropol Territory. According to literature data (Maevskii 2014), it is also found in the Chuvash Republic. The area of *C. brevirostris* does not extend to these regions, but *C. juncea* populations are widely represented there. It remains unclear whether *C. laticoronata* and *C. juncea* are capable of occupying common localities and forming hybrids between each other. In terms of systematics these taxa are more distant from each other than *C. laticoronata* and *C. brevirostris* (Leonova 1964; Kashin et al. 2024).

In this regard, it is interesting that in the surroundings of Khan Ordasy, Republic of Kazakhstan, besides *C. laticoronata* and *C. brevirostris*, *C. pauciflora* differs in DNA content from both taxa, also grows. The 8.8% decrease in DNA content of *C. laticoronata* and *C. brevirostris* compared to *C. pauciflora* is most likely due to differences in size and chromosome morphology of these taxa (Fig. 3). We have not found

plants with morphological features transient between *C. pauciflora* and these two taxa. From this it follows that there is no hybridization between *C. pauciflora* and *C. laticoronata* or *C. brevirostris*. This can be explained by its more distant phylogenetic relationship since they belong to different subgenera of *Chondrilla* (Leonova 1989). Although, in evolutionary significant period of time, *C. pauciflora* or its close relative *C. ambigua* most likely participated as a maternal form in speciation of *C. laticoronata* and *C. brevirostris* by synthesogenesis (Kashin et al. 2019, 2024).

In eight studied triploid populations of *C. juncea*, the DNA contents did not differ significantly, despite the wide geographical range of its localities. As noted, *C. juncea* is the only one species from genus *Chondrilla* the DNA content of which has been previously determined (Bennett and Leitch 2012). The size of its 2C genome amounted to 3.01 pg. In present study, the 2C value close to the previously determined was obtained. Probably, the 7.5% difference is due to actual differences in DNA content of *C. juncea* samples from different localities. However, it is most likely an inter-laboratory error related to the use of different flow cytometers and protocols for nuclei isolation and staining.

Previously, a preliminary assessment of DNA content in *Chondrilla* plants was carried out (Parkhomenko et al. 2023). However, firstly, the study was carried out only on single specimens (one from each population). Secondly, sample preparation included not one, as in this study, but two stages: isolation of nuclei in Otto I buffer with modifications, then filtration of nuclei through a nylon membrane, and then staining of nuclei using Tris-$MgCl_2$ buffer with PI, RNase and β-mercaptoethanol. During the two-stage sample preparation protocol, it was possible to isolate much fewer nuclei than by one-step protocol. Thirdly, the assessment of DNA content in that study was not entirely correct. Namely, the isolation and staining of nuclei in samples and standard were carried out independently and flow cytometry was carried out using its mixture in one working solution. It was resulted in a pseudo-internal standardization that underestimated the final DNA 2C values.

The DNA content in triploid *C. juncea* samples was 1.12 times higher than in diploid *C. ambigua* samples. By comparison of triploid *C. juncea* samples with *C. laticoronata* and *C. brevirostris* samples, the DNA content of the latter exceeds the one of *C. juncea* by 10%. The DNA content in *C. juncea* samples was 20.3% lower than in *C. pauciflora* samples. Consequently, a decrease in nuclear DNA content of approximately 10% is observed in direction from *C. pauciflora* to *C. brevirostris* and *C. laticoronata* and then to *C. juncea*. These differences confirm that, in the taxa genesis, *C. brevirostris* and *C. laticoronata* take an intermediate position between *C. juncea* on the one side, and *C. pauciflora* and *C. ambigua* – on another. Previous studies by target sequencing methods using plastid DNA trnT–trnF region markers and nuclear DNA intergenic transcribed ribosomal spacer (ITS) shown that *C. laticoronata* and *C. brevirostris* are much closer to each other than to the other taxa (Kashin et al. 2017, 2019).

The DNA content in tetraploid *C. juncea* samples was 1.3 times higher than in triploid samples. This is entirely consistent with the proportional change in DNA

content in tri- and tetraploids of the same taxa. This circumstance speaks in favor of the fact that plants of this taxa with higher and lower ploidy levels appeared recently and represent a consequence of transitions in ploidy levels in real time without any evolutionary history and associated with dynamics of apo-amphimixis implementation in taxa's seed reproduction system.

The 2C contents in samples of all *C. graminea* populations and *C. juncea* triploids did not differ significantly, which indicates the validity of classifying these two taxa as one species and treated them as subspecies with the priority name *C. juncea* (Kashin et al. 2024).

In all studied samples from three *C. latifolia* populations, the DNA content values did not differ significantly from each other and from *C. graminea* samples. The DNA content in *C. latifolia* samples was 9% lower than in *C. laticoronata* and *C. brevirostris* samples, and 19% lower than in *C. pauciflora* samples. DNA content in *C. latifolia* samples from all three studied populations did not differ significantly from the contents in *C. juncea* and *C. graminea* samples. This suggests that *C. latifolia* also treated as subspecies of *C. juncea*.

Samples from two *C. canescens* populations growing relatively close to each other (at a distance of about 120 km) had similar DNA content values and did not differ significantly from 2C values in samples from *C. juncea*, *C. graminea* and *C. latifolia* populations. The localities of the latter were close in latitude to the growth sites of these two populations of *C. canescens*. This confirms that *C. canescens* is not an independent species and represents only a subspecies of *C. juncea*.

It should be noted that, despite the close DNA content values in two *C. canescens* populations described above, the samples from northern population were characterized by slightly lower DNA content than the samples from the southern one. In samples of third *C. canescens* population, located 400–500 km to the south from the first two populations, the DNA content was significantly higher than in the first two populations. The samples from all three studied *C. canescens* populations were triploids. Thus, *C. canescens* samples from northern populations had 2C values 3.4% lower than the samples from southern population. These changes of genome sizes in *C. canescens* can be explained by the same pattern discussed above. This pattern means that along a global latitudinal gradient, the genome size of angiosperms increases from the equator to 40–50° N and decreases from 40–50° N to more northern latitudes (Bureš et al. 2024).

In this review the authors collected the largest data set on angiosperm genome sizes covering more than 5% of known species. They analyzed the genome sizes distribution by using a complete data set of geographical distribution for all angiosperms, and primarily focusing on the differences in the genome sizes between species and higher taxonomic units. Our findings at least on two *Chondrilla* taxa indicate that such pattern also observed at the intraspecific level of variation. The absence of such DNA content pattern for samples from other studied taxa can be explained by its relatively narrow distribution along geographic latitude (within 0.6 degree for *C. laticoronata* and within 0.25 degree for *C. graminea*). Across studying

territory *C. latifolia* and *C. juncea* have a latitudinal gradient in nuclear DNA content distribution similar to observed for *C. canescens*, although it is less pronounced.

Conclusion

It was revealed that *C. juncea*, *C. graminea*, *C. canescens* and *C. latifolia* have the smallest monoploid genome sizes (1.078–1.098 pg), *C. laticoronata* and *C. brevirostris* have an intermediate values (1.190–1.203 pg), *C. pauciflora* and *C. ambigua* have the largest ones (1.309–1.449 pg).

The obtained results indicate that the DNA content in *Chondrilla* populations distributed along a latitudinal gradient varies regularly. From the south and up to about 50°N, the genome size increase, as it was shown for *C. brevirostris* populations. The nuclear DNA content decrease from 50°N to the north as it was shown for *C. canescens* populations. A less pronounced latitudinal gradient was also observed in distribution of genome sizes of *C. latifolia* and *C. juncea*. Such spatial pattern was not found for the remaining taxa, most likely because its studied populations grown in a very narrow latitudinal range.

The nuclear DNA content decreases in a row from *C. pauciflora* to *C. brevirostris* and *C. laticoronata*, and then to *C. juncea* subspecies complex by approximately 10% at each step. These differences point out the fact that, *C. brevirostris* and *C. laticoronata* take an intermediate position in taxa genesis between the *C. juncea* subspecies group on the one side, and *C. pauciflora* and *C. ambigua* on the other side. *C. laticoronata* and *C. brevirostris* much closer to each other than to the other taxa.

The nuclear DNA contents in samples of *Chondrilla* taxa populations from European Russia and Western Kazakhstan are consistent with the earlier findings on genus taxonomic structure within the studied part of the area. The results suggest that *C. juncea*, *C. graminea*, *C. latifolia* and *C. canescens* are synonymous with the priority name *C. juncea*. Its most likely have the status of subspecies (*C. juncea* ssp. *juncea*; *C. juncea* ssp. *graminea*; *C. juncea* ssp. *canescens*; *C. juncea* ssp. *latifolia*) and have no significant difference in DNA content. *C. ambigua* is the only one diploid species among studied taxa, and *C. pauciflora* is most likely its triploid cytotype. The location of *C. brevirostris* and *C. laticoronata* in terms of genome size between the group of subspecies of *C. juncea*, on the one hand, and *C. pauciflora* and *C. ambigua*, on the other, can be explained by distant hybridization that took place in the remote past, when *C. ambigua* or *C. pauciflora* acted as the maternal parent, and some of the species from subgenus *Chondrilla* – as paternal. Increase in DNA content, non-multiple to chromosome set, with increase in ploidy level can be explained by decrease in chromosome size and DNA content after polyploidization round, i.e. by correction mechanism against excessive genetic information in evolutionary significant period of time (Leitch and Bennett 2004).

The similarity of genome sizes in samples of different taxa growing in the same habitat can be explained by hybridization, accompanied by chromosomal rearrangements.

Acknowledgement

The study was supported by Russian Science Foundation grant No. 22-24-00340, https://rscf.ru/project/22-24-00340/.

References

Bennett MD, Leitch IJ (2012) Plant DNA C-values database (release 6.0). Available from http://data.kew.org/cvalues/ [accessed 23 Desember 2022]

Bhadra S, Leitch IJ, Onstein RE (2023) From genome size to trait evolution during angiosperm radiation. Trends in Genetics 39(10): 728–735. https://doi.org/10.1016/j.tig.2023.07.006

Bureš P, Elliott TL, Veselý P, Šmarda P, Forest F, Leitch IJ, Lughadha EN, Gomez MS, Pironon S, Brown MJM, Šmerda Ja, Zedek F (2024) The global distribution of angiosperm genome size is shaped by climate. New Phytologist 242(2): 744–759. https://doi.org/10.1111/nph.19544

Depository of Live Systems (branch "Plants") (2024) Collection "Moscow University Herbarium". Available from: https://plant.depo.msu.ru/open/public/item/MW0551534 [Accessed on 10 December 2024]

van Dijk PJ (2003) Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. Philosophical Transactions of the Royal Society B: Biological Sciences 358(1434): 1113–1121. https://doi.org/10.1098/rstb.2003.1302

Doležel J, Greilhuber J (2010) Nuclear genome size: Are we getting closer? Cytometry A 77(7): 635–42. https://doi.org/10.1002/cyto.a.20915

Doležel J, Bartoš J, Voglmayr H, Greilhuber J (2003) Nuclear DNA content and genome size of trout and human. Cytometry 51: 127–128. https://doi.org/10.1002/cyto.a.10013

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysák MA, Nardi L, Obermayer R (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. Annals of Botany 82: 17–26. https://doi.org/10.1093/oxfordjournals.aob.a010312

Garcia S, Hidalgo O, Jakovljević I, Siljak-Yakovlev S, Vigo J, Garnatje T, Vallès J (2013) New data on genome size in 128 Asteraceae species and subspecies, with first assessments for 40 genera, 3 tribes and 2 subfamilies. Plant Biosystems 147: 1219–1227. https://doi.org/10.1080/11263504.2013.863811

Gaskin JF, Schwarzländer M, Kinter CL, Smith JF, Novak SJ (2013) Propagule pressure, genetic structure, and geographic origins of *Chondrilla juncea* (Asteraceae): an apomictic invader on three continents. American Journal of Botany 100: 1871–1882. https://doi.org/10.3732/ajb.1200621

Global Compositae Checklist (GCC) (2023) Int. Compos. Alliance. Checklist dataset. Available from: https://doi.org/10.15468/g7yhgt [Accessed on 27 April 2023]

Gregory TR (2001) Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biological Reviews 76: 65–101. https://doi.org/10.1111/j.1469-185X.2000.tb00059.x

Kashin AS, Kritskaya TA, Popova AO, Parkhomenko AS (2017) ISSR analysis of genetic diversity of *Chondrilla* species (Asteraceae) in European part of Russia. Bulletin of Moscow Society of Naturalists. Biological series 122(1): 60–70. [In Russian]

Kashin AS, Kritskaya TA, Parkhomenko AS, Schanzer IA (2019) Genetic polymorphism in *Chondrilla* (Asteraceae) in southern European Russia and the nature of *Chondrilla juncea* L. Nordic Journal of Botany 37(11): 402–420. https://doi.org/10.1111/njb.02420

Kashin AS, Popova AO, Kochanova IS, Ugolnikova EV, Polyakova YA (2015) Some parameters of the seed reproduction system in populations of *Chondrilla* (Asteraceae) species in the Lower Volga region. Botanicheskii zhurnal 100(8): 828–840. https://doi.org/10.1134/S0006813615080074 [In Russian]

Kashin AS, Parkhomenko AS, Kondratieva AO, Bogoslov AV, Shilova IV (2024) Diversity of the genus *Chondrilla* L. (Asteraceae) in Eastern Europe. Biodiversitas. Journal of Biological Diversity 25(5): 1901–1910. https://doi.org/10.13057/biodiv/d250506

Kashin AS, Petrova NA, Shanzer IA, Kondratyeva AO, Shilova IV, Parkhomenko AS (2018) Variability of morphological parameters of some *Chondrilla* (Asteraceae) taxa in European Russia in context of their taxonomy. Botanicheskii zhurnal 103(11): 1407–1436. https://doi.org/10.1134/S0006813618110030 [In Russian]

Kubešová M, Moravcová L, Suda J, Jarošík V, Pyšek P (2010) Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia 82: 81–96.

Leitch IJ, Bennett MD (2004) Genome downsizing in polyploid plants. Biological Journal of the Linnean Society 82(4): 651–663. https://doi.org/10.1111 /j.1095-8312.2004.00349.x

Leitch IJ, Beaulieu JM, Cheung K, Hanson L, Lysak MA, Fay MF (2007) Punctuated genome size evolution in Liliaceae. Journal of Evolutionary Biology 20: 2296–2308. https://doi.org/10.1111/j.1420-9101.2007.01416.x

Leonova TG (1964) The genus Chondrilla – *Chondrilla* L. In: Bobrov EG, Tsvelev NN (Eds) Flora of USSR. Nauka, Moscow-Leningrad, 560–586. [In Russian]

Leonova TG (1989) Chondrilla – *Chondrilla* L. In: Tsvelev NN (Ed.). Flora of European part of USSR. Vol. 8. Nauka, Leningrad, 57–61. [In Russian]

Maevskii PF (2014) Flora of middle zone of the European part of USSR. KMK Scientific Press, Moscow, 635 pp. [In Russian]

Nasseh Y (2010) Revision on the genera *Chondrilla* and *Heteroderis* (Asteraceae) in Iran. Iranian Journal of Botany 16(1): 91–95.

Orsenigo S, Adorni M, Alessandrini A, Armiraglio S, Castello M, Forte L, Gennai M, Magrini S, Medagli P, Montagnani C, Prosser F, Selvaggi A, Villani M, Viciani D, Wagensommer RP, Fenu G (2019) Global and regional IUCN red list assessments. Italian Botanist 7: 107–124. https://doi.org/10.3897/ italianbotanist.7.35467

Parkhomenko AS, Kashin AS (2018) Karyotypic variability in some species of the genus *Chondrilla* (Asteraceae). Botanicheskii zhurnal 103(6): 726–739. https://doi.org/10.1134/S0006813618060030 [In Russian]

Parkhomenko AS, Kashin AS, Grebenyuk LV (2019) Chromosome polymorphism in *Chondrilla* (Asteraceae) species in the European part of the range. Botanicheskii zhurnal 104(4): 137–154. https://doi.org/10.1134/S0006813619040069 [In Russian]

Parkhomenko AS, Kondratieva AO, Bogoslov AV, Shilova IV, Kashin AS (2023) Morphological variability in *Chondrilla* taxon populations in European Russia and adjacent territories. Povolzhskiy Journal of Ecology 3: 331–351. https://doi.org/10.35885/1684-7318-2023-3-331-351 [In Russian]

Parkhomenko AS, Kuzmina USh, Musin KhG, Efimenko SF, Epifanov VS, Kashin AS (2023) Preliminary assessment of nuclear DNA content in *Chondrilla* (Asteraceae) plants of European Russia and Western Kazakhstan using flow cytometry. Izvestiya of Saratov University. Chemistry. Biology. Ecology 23(2): 197–208. https://doi.org/10.18500/1816-9775-2023-23-2-197-208 [In Russian]

Pellicer J, Leitch IJ (2019) The Plant DNA C-values database (release 7.1): an updated online repository of plant genome size data for comparative studies. New Phytologist 222(2). https://doi.org/10.1111/nph.16261

Pfosser M, Amon A, Lelley T, Heberle-Bors E (1995) Evaluation of sensitivity of flow cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. Cytometry 21(4): 387–393.

The World Flora Online (2023) Available from: http://www.worldfloraonline.org/ [Accessed on 10 January 2023]

Tremetsberger K, Gemeinholzer B, Zetzsche H, Blackmore S, Kilian N, Talavera S (2013) Divergence time estimation in Cichorieae (Asteraceae) using a fossil-calibrated relaxed molecular clock. Organisms Diversity and Evolution 13: 1–13. https://doi.org/10.1007/s13127-012-0094-2

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1976) Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae). Vol. 4. Cambridge University Press, Cambridge, 505 pp.

Woellner R, Bräuchler C, Kollmann J, Wagner TC (2022) Biological flora of Central Europe: *Chondrilla chondrilloides* (Ard.) H. Karst. Perspectives in Plant Ecology, Evolution and Systematics 54: 125657. https://doi.org/10.1016/j.ppees.2021.125657

Woellner R, Müller N, Reich M, Wagner TC, Kollmann J (2019) Species conservation measures for endangered wild river species – a potential study on the Bavarian Alpine rivers using four example species. Natur und Landschaft 94: 509–516. https://doi.org/10.17433/12.2019.50153753.509-516 [In German]