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Chromosome numbers of invasive and potentially invasive species in the flora of the Republic of Altai. IV

E. Yu. Zykova^{1,2*}, T. V. An'kova^{1,3}, M. N. Lomonosova^{1,4}

¹ Federal State Institution of Science Central Siberian Botanical Garden of Siberian Branch of RAS,
Zolotodolinskaya str., 101, Novosibirsk, 630090, Russian Federation

² E-mail: elena.yu.zykova@gmail.com; ORCID iD: <https://orcid.org/0000-0002-1847-5835>

³ ORCID iD: <https://orcid.org/0000-0003-3661-0719>

⁴ ORCID iD: <https://orcid.org/0000-0003-0510-5349>

*Corresponding author

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Summary. Chromosome numbers ($2n$) in 10 invasive and potentially invasive plant species from the families Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Malvaceae, Oxalidaceae, Poaceae and Solanaceae are reported on the samples collected in the Republic of Altai. To determine the ploidy levels, we used methods of direct chromosome counting and flow cytometry in some cases. Among them, chromosome complements were first examined in Russia for *Eruca vesicaria* subsp. *sativa* ($2n = 22$). *Psammophiliella muralis* ($2n = 34$), *Medicago sativa* subsp. *varia* ($2n \approx 32$; $2C = 4.8$ pg) and *Solanum nigrum* ($2n = 48$) were first studied from Siberia. *Malva verticillata* ($2n = 42$), *Oxalis dillenii* ($2n = 24$), *Lolium perenne* ($2n = 14$) and *Setaria faberi* ($2n = 36$) were first studied from the Republic of Altai. Tetraploid cytotype and genome size of *Picris hieracioides* ($2n = 4x = 20$; $2C = 8.89$ pg) were discovered for the first time. Common distribution and the history of floristic findings of these species in the Republic of Altai are given. Previously published data on chromosome numbers from Russia are cited.

Числа хромосом инвазивных и потенциально инвазивных видов во флоре Республики Алтай: сообщение 4

Е. Ю. Зыкова, Т. В. Анькова, М. Н. Ломоносова

Федеральное государственное бюджетное учреждение науки «Центральный сибирский ботанический сад» Сибирского
отделения РАН, ул. Золотодолинская, 101, г. Новосибирск, 630090, Россия

Ключевые слова: кариологическое изучение, проточная цитометрия, размер генома, расселение видов, Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Malvaceae, Oxalidaceae, Poaceae, Solanaceae.

Аннотация. Приводятся данные о числах хромосом ($2n$) для 10 инвазивных и потенциально инвазивных видов из семейств Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Malvaceae, Oxalidaceae, Poaceae, Solanaceae, полученные на материале из Республики Алтай. Для определения уровня ploidy использован метод прямого подсчета хромосом, а для *Medicago sativa* subsp. *varia* и *Picris hieracioides* метод проточной цитометрии (FCM). Впервые для России определено число хромосом у *Eruca vesicaria* subsp. *sativa* ($2n = 22$), впервые для Сибири – у *Psammophiliella muralis* ($2n = 34$), *Medicago sativa* subsp. *varia* ($2n \approx 32$; $2C = 4.8$ пг), *Solanum*

nigrum ($2n = 48$), впервые для Республики Алтай – у *Malva verticillata* ($2n = 42$), *Oxalis dillenii* ($2n = 24$), *Lolium perenne* ($2n = 14$) и *Setaria faberi* ($2n = 36$). Впервые обнаружен тетраплоидный цитотип и определен размер генома у *Picris hieracioides* ($2n = 4x = 20$; $2C = 8.89$ пг). Для всех исследованных видов приводятся сведения по общему распространению, истории флористических находок и расселению на территории Республики Алтай, а также литературные данные по числам хромосом с территории России.

We continue the karyological study of invasive and potentially invasive species in the flora of Southern Siberia on the example of the Republic of Altai (Lomonosova et al., 2018; Zykova et al., 2018, 2020), one of the most popular touristic regions of Siberia. This paper provides information about 11 species that are active in the republic, four of which are included in the list of invasive or potentially invasive species of Siberia (Ebel et al., 2014). The information on the history of floristic studies of examined species in the territory of the Republic of Altai is provided here. Considering the distribution of species in the Republic of Altai, we refer to the northern regions: Gorno-Altaysk, Mayma, Choya, Turochak and Chemal; to the Central regions: Onguday, Ust'-Kan, Shebalino, Ust'-Koksa and to the South-Eastern regions: Ulagan and Kosh-Agach. For all species, the references on chromosome numbers revealed from the samples collected in Russia are given as well, as relevant information in the international database "The Chromosome Counts Database" (Rice et al., 2015) is reflected incompletely. Latin names of plants are given according to the "Catalog of Life" (Hassler, 2020).

The chromosome numbers were determined by direct counting in metaphase on root meristem squash preparations. Seeds were germinated on sterile sand. The root tips were pretreated for two hours with 0.2 % colchicine water solution, fixed in 3 : 1 absolute ethanol-glacial acetic acid and stained with 1 % acetic hematoxylin. Metaphase plates were observed under 100× magnification by the Axioscope 40 (Karl Zeiss, Axio Lab) and photographed by the AxiCam MRc 5 digital camera.

The absolute nuclear DNA amount was assessed by flow cytometry using Cy Flow Space cytometer (manufactured by Sysmex Partec, Germany) equipped with a green laser. The sample preparation from dry leaves and FCM procedure followed that of Doležel et al. (2007). As internal standards were used *Solanum lycopersicum* Stupicke ($2C$ DNA content = 1.96 pg) (Doležel et al., 1992) for *Picris hieracioides* and *Pisum sativum* L. 'Ctirad' ($2C = 9.09$ pg) (Doležel et al., 1998) for *Medicago varia*.

The herbarium specimens (vouchers) are deposited in the Herbarium of the Central Siberian Botanical Garden SB RAS (NS).

ASTERACEAE

Inula helenium L., $2n = 20$

"Russian Federation, the Republic of Altai, Turochak district, Altai State Reserve, Yaylu village, along the roads and the streets, 51°46'N, 87°36'E. 14 VIII 2017. E. Zykova", Z443–5317 (fig. 1A).

– Perennial. European-Mediterranean-West Asian species (Gubanov, 1994), found in some regions of Siberia. In the Republic of Altai, most likely it is invasive. According to P. N. Krylov (1904), at the beginning of the 20th century the species was not known in Altai. The first localities were recorded in the middle of the 20th century in Mayma, Chemal, Shebalino and Ust'-Kan districts (Krylov, 1949). To date, the species is quite common in the northern regions of the republic; it does not settle in central regions. Occurs in roadsides, wastelands, along the banks of reservoirs.

The same chromosome number was determined from Chechen Republic (Magulaev, 1974), Choya district of the Republic of Altai (Rostovtseva, 1983), Primorye Territory (Probatova, 2014).

Diploid ($2x$), $x = 10$.

Picris hieracioides L., $2n = 20$; $2C = 8.89 \pm 0.32$ pg

"Russian Federation, the Republic of Altai, Mayma district, vicinity of Kysyl-Ozek village, quarry at a closed landfill, 51°58'N, 85°55'E. 15 VIII 2018. E. Zykova", Z691–2418 (fig. 1B).

– Biennial. European-Mediterranean-Central Asian species with a cosmopolitan secondary range (Geltman, 1989). It is common in most regions of Western Siberia, where it is a native species (Lomonosova, 1997). In the Republic of Altai, as invasive species, it was first discovered in the Mayma district in 2008 (Zykova, 2014). Currently, it is rapidly settling in the Mayma and Chemal districts (Zykova, 2015; 2019).

The chromosome number is given for the first time for the Republic of Altai, $2n = 20$ is a new cytotype for the species. $2n = 10$ was determined for the Novosibirsk Region (Krasnikov, Lomonosova, 1990), Irkutsk Region (Probatova et al., 2014a), Primorye Territory (Probatova, 2014).

We have confirmed ploidy level of this species on the base of flow cytometric data. According to

comprehensive study of *Picris hieracioides* on the material from 54 populations from Europe (Slovák et al., 2009), the genome size of this species ($2C$ -values) at the diploid level varied from 2.26 pg to 3.11 pg. Additionally, the authors have discovered one population with plants having $2C = 4.72$ pg. The authors assumed that these plants are triploids. Thus, using the direct chromosome count and flow cytometry, we have confirmed tetraploid ploidy level of Altai plants. Siberian populations are needed in further cytogenetic studies.

Tetraploid ($4x$), $x = 5$.

BRASSICACEAE

Eruca vesicaria subsp. *sativa* (Mill.) Thell.
(*E. sativa* Mill.), $2n = 22$

“Russian Federation, the Republic of Altai, Gorno-Altaysk city, brick factory area, outside the flowerbeds, along the fences, $51^{\circ}58'N$, $85^{\circ}55'E$. 12 VIII 2018. E. Zykova”, Z675–1818.

– Annual. European-Mediterranean-West Asian species with a secondary Eurasian area (Kotov, 1979). It is rare in Siberia; single localities were recorded in the Tomsk and Novosibirsk regions, the Republic of Buryatia (Nikiforova, 1994), and the Altai Territory (Silantjeva, 2013). It was recently discovered in the Republic of Altai (Ebel et al., 2018). The chromosome number was determined for the first time in Russia.

Diploid ($2x$), $x = 11$.

CARYOPHYLLACEAE

Psammodiella muralis (L.) Ikonn., $2n = 34$

“Russian Federation, the Republic of Altai, Turochak district, vicinity of Ust’-Lebed’ village, roadside, $52^{\circ}17'N$, $87^{\circ}20'E$. 8 VIII 2015. E. Zykova”, Z794–4015.

– Annual. Euro-Mediterranean species, adventive in Siberia (Ikonnikov, 2004). In the Republic of Altai for a long time it was known only from the vicinity of the village of Choya, Choya district (Kovtonyuk, 1993), by the end of the 20th–beginning of the 21st centuries it was found in Turochak and Mayma districts and in the city of Gorno-Altaysk (Studenikina, 1999; Zykova, Erst, 2012). At present, it is rarely found in these areas in the fields, along the banks of water reservoirs and along roads in settlements (Zykova, 2015).

The chromosome number was determined for the first time on the Siberian material. The same chromosome number was known for the Amur Region (Probatova et al., 2005) and Primorye Territory (Probatova et al., 2017a).

Diploid ($2x$), $x = 17$.

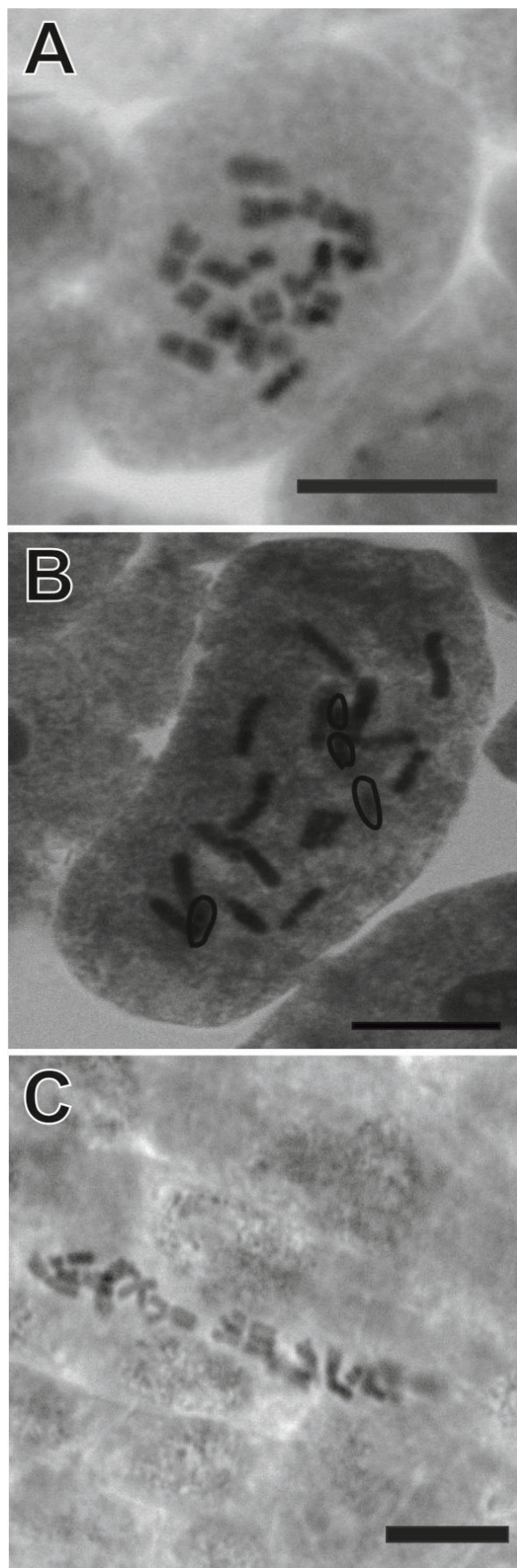


Fig. 1. Mitotic metaphases: A – *Inula helenium*, $2n = 20$; B – *Picris hieracioides*, $2n = 20$; C – *Lolium perenne*, $2n = 14$; Scale = 10 μ m.

FABACEAE

Medicago sativa subsp. *varia* (Martyn) Arcang. (*M. varia* Martyn), $2n \approx 32$, $2C = 4.80 \pm 0.11$ pg

“Russian Federation, the Republic of Altai, Gorno-Altaysk city, Lenkina street, the territory of the Gorno-Altaysk University, wasteland, $51^{\circ}58'N$, $85^{\circ}55'E$. 25 VIII 2017. E. Zykova”, Z465–7117.

– Perennial. Distributed in Eastern Europe and Kazakhstan (Ebel et al., 2016). It occurs in many Siberian regions and is included in the list of invasive and potentially invasive species of Siberia (Ebel et al., 2014). The first localities in the republic were discovered in 2009 (Zykova, 2015). Now the species is very active in Gorno-Altaysk city and Mayma district, it was recently found in Aktash village of Ulagan district (Ebel et al., 2016).

Previously $2n = 32$ was determined from Khabarovsk Territory (Probatova et al., 2017b).

We ascertained ploidy level of this species using flow cytometric analysis. This data is consistent with previously published for *M. sativa* var. *varia* (Sakiroglu, Kaya, 2012).

Tetraploid ($4x$), $x = 8$.

MALVACEAE

Malva verticillata L. (*M. mohileviensis* Downar, *M. pulchella* Bernh.), $2n = 42$

“Russian Federation, the Republic of Altai, Gorno-Altaysk city, Shosseynaya street, wasteland, $51^{\circ}58'N$, $85^{\circ}55'E$. 22 VIII 2017. E. Zykova”, Z793–6717.

– Annual. An Asian species, settled in temperate and subtropical regions of the globe, including the most regions of Southern Siberia (Mikhaylova, Ebel, 2016), where it is invasive or potentially invasive species (Ebel et al., 2014). In the Republic of Altai, until the 1990s, it was known in several settlements of the Onguday district, near the mouth of the Bashkaus River in Ulagan district, as well as on the cordons of the Altai Nature Reserve (Zolotukhin, 1983; Vlasova, 1996; Pyak et al., 2000). Over two decades, the species settled in Chermal and Maima districts and in the city of Gorno-Altaysk (Shaulo et al., 2010). At present, it is common along roads, near buildings, on wastelands, in vegetable gardens in the Northern regions of the republic. It is occasionally found in the Central regions and now it penetrates into the South-Eastern ones, where it is a noxious weed of garden crops (Zykova, 2015).

The chromosome number is given for the first time for Western Siberia. The same number is indicated for the Trans-Baikal Territory, Amur Region (Probatova et al., 2012), Irkutsk Region

(Probatova et al., 2014a); $2n = 56$ – for the Irkutsk Region (Probatova et al., 2009); $2n = 84$ – for Primorye Territory (Probatova, 2014).

Hexaploid ($6x$), $x = 7$.

OXALIDACEAE

Oxalis dillenii Jacq. (*Xanthoxalis stricta* (L.) Small), $2n = 24$

“Russian Federation, the Republic of Altai, Turochak district, Altai State Reserve, cordon Karatash, weed in the yard, $51^{\circ}46'N$, $87^{\circ}23'E$. 14 VIII 2017. E. Zykova”, Z447–5117; “Russian Federation, the Republic of Altai, Mayma district, vicinity of Karlushka village, closed dump, $51^{\circ}58'N$, $85^{\circ}51'E$. 18 VIII 2017. E. Zykova”, Z345–6417; “Russian Federation, the Republic of Altai, Turochak district, 7 km from Artybash village, on the bank of the Biya River, near the road, $51^{\circ}46'N$, $87^{\circ}15'E$. 18 VIII 2017. E. Zykova”, Z343–5617.

– Annual. A North American species that has spread and naturalized in most parts of the world (Tzvelev, 1996). In Siberia, it has been found since the end of the 20th century (Tzvelev, 1996; Lomonosova, Sukhorukov, 2000). The first localities in the Republic of Altai were found in 2007 in Gorno-Altaysk city and Mayma district (Ebel, 2008), later in Chermal district (Shaulo et al., 2010). At present, it is common in most northern regions; as a weed it occurs along roads and on wastelands (Zykova, 2015).

The chromosome number is given for the first time for the Republic of Altai. The same number was determined from Primorye Territory (Probatova, 2014), Novosibirsk Region (An`kova et al., 2019).

Tetraploid ($4x$), $x = 6$.

POACEAE

Lolium perenne L., $2n = 14$

“Russian Federation, the Republic of Altai, Turochak district, Yogach village, stadium, $51^{\circ}46'N$, $87^{\circ}15'E$. 29 VI 2017. E. Zykova”, Z618–2617 (fig. 1C).

– Perennial. Euro-Mediterranean-West Asian species (Tzvelev, Probatova, 2019). In the beginning of 20th century the species was known in three localities of West Siberia (Krylov, 1928). Currently it is included in the list of invasive and potentially invasive species of Siberia (Ebel et al., 2014). It entered the Republic of Altai in the 2000s, and actively settles in Gorno-Altaysk city, Mayma, Choya and Turochak districts (Zykova et al., 2019). It occurs on wastelands, along roads, on pebbles, in the built-up areas.

The chromosome number is given for the first time for Western Siberia. The same number was determined from Irkutsk Region (Chepinoga et al., 2010), Republic of Crimea (Petrova, 1968), Krasnodar Territory (Probatova et al., 2009, 2012).

Diploid ($2x$), $x = 7$.

***Setaria faberi* R. A. W. Herrm., $2n = 36$**

“Russian Federation, the Republic of Altai, Mayma district, vicinity of Mayma village, as a weed near the customs post, 52°02'N, 85°54'E. 1 VIII 2015. E. Zykova”, Z433–3015; “Russian Federation, Altai Territory, Soviet district, Shulgin Log village, streets of the village, by the roads, 52°11'N, 85°50'E. 14 VIII 2016. E. Zykova”, Z499–3516.

– Annual. An East Asian species spreading across Eurasia and North America (Shouliang, Phillips, 2006). In Siberia, it was first recorded in the Altai Territory (Pyak et al., 2000). At about the same time, the first locality was found in the Republic of Altai: in the Ulagan district along the Chuisky tract between the mouths of the Boka and Belgebash rivers (Pyak, Ebel, 2001). At present, it is common in the Mayma district and in the city of Gorno-Altaysk (Shauro et al., 2010; Zykova, 2015); found in the village of Iogach, Turochak district and the village of Myyuta, Shebalino district (Zykova et al., 2019).

The chromosome number is given for the first time for the Republic of Altai. The same chromosome number is indicated for the species from Amur Region (Probatova, Sokolovskaya, 1983), Primorye (Probatova et al., 2014a), Khabarovsk (Probatova et al., 2014b) and Altai (Punina et al., 2013) Territories.

Tetraploid ($4x$), $x = 9$.

SOLANACEAE

***Solanum nigrum* L., $2n = 48$**

“Russian Federation, the Republic of Altai, Mayma district, vicinity of Karlushka village, closed dump, 51°58'N, 85°51'E. 18 VIII 2017. E. Zykova”,

Z501–6417; “Russian Federation, the Republic of Altai, Gorno-Altaysk city, pebbled riverbank of the Maima River, 51°58'N, 85°55'E. 18 VIII 2017. E. Zykova”, Z500–6117.

– Annual. Holarctic species (Poyarkova, 1981). In Siberia, it has been observed since the beginning of the 20th century (Krylov, 1907), in the Republic of Altai – since the forties of the century (Krylov, 1939; Zolotukhin, 1983). At present, the species is common in the northern and central regions of the republic; it is found near dwellings, in crops, on wastelands and fallow lands, along roads (Zykova, 2015).

This rare cytotype of the species was determined for the first time for Russia. Previously $2n = 60$ was revealed in the Stavropol Territory and Chechen Republic (Magulaev, 1984); $2n = 72$ in the Moscow Region, Sverdlovsk Region (Gerasimenko, Reznikova, 1968) and Primorye Territory (Probatova, 2014).

Tetraploid ($4x$), $x = 12$.

Conclusion

To date, the chromosome numbers of 49 invasive and potentially invasive plant species distributed in the Republic of Altai were examined. The data for 10 species are presented in this study. In the studied species, four are diploids. Six of them are polyploids, namely *Solanum nigrum*, *Setaria faberi*, *Picris hieracioides*, *Malva verticillata*, *Oxalis dillenii*. Among 10 investigated species, the most active on the territory of the Altai Republic are hexaploid *Malva verticillata*, tetraploid *Oxalis dillenii* and diploid *Lolium perenne*. New tetraploid cytotype for *Picris hieracioides* was identified.

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