Vavilovskii Zhurnal Genetiki i Selektsii
Vavilov Journal of Genetics and Breeding. 2026;30(1):146-156

doi 10.18699/vjgb-26-16

Morphological and molecular genetic analysis of the genus *Iris* L. polymorphism in the Republic of Bashkortostan and the Orenburg Region

E.A. Aukhadieva[1], A.G. Blinov[2], E.I. Vshivtseva[2, 4] ✉, Z.Kh. Shigapov[3], A.R. Ishbirdin[5], Ya.M. Golovanov[3], A.V. Kryukova[3]

[1] Ufa Research Institute of Occupational Health and Human Ecology, Ufa, Russia
[2] Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
[3] South-Ural Botanical Garden-Institute – Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences, Ufa, Russia
[4] Novosibirsk State University, Novosibirsk, Russia
[5] Ufa University of Science and Technology, Ufa, Russia
✉ evsivceva4@gmail.com

Abstract. *Iris* is a cosmopolitan genus comprising 200 to 340 species distributed throughout the Northern Hemisphere. Although *Iris* is the most diverse group in the family Iridaceae, there are many uncertainties regarding its taxonomic composition and systematics. The aim of this study was to search for taxonomically significant morphological characters of the generative and vegetative spheres and molecular markers with subsequent assessment of their informativeness in identifying phylogenetic relationships and compliance with the most relevant modern classification systems of the genus *Iris*. As a result of constructing the structure of variability of morphometric parameters of 11 species, 10 taxonomic indicators were identified that were common to the analyzed taxa and were characterized by relatively low total and coordinated variability: length and width of the outer perianth lobes, length and width of the inner perianth lobes, length of the filament, anther and pistil, fruit width, as well as seed length and width. Nucleotide sequences of *trnL-trnF* fragments of chloroplast DNA were established for 13 samples of four species of wild flora of the Republic of Bashkortostan and the Orenburg Region: *Iris pumila* L., *I. scariosa* Willd. ex Link., *I. pseudacorus* L., *I. sibirica* L. The obtained sequences were used to construct a phylogenetic tree together with *trnL-trnF* sequences of seven more iris species extracted from the database. The tree contained five clusters: (1) *I. pumila*, *I. scariosa*; (2) *I. pseudacorus*, *I. setosa* Pall. ex Link; (3) *I. lactea* Pall.; (4) *I. sibirica*, *I. sanguinea* Hornem.; (5) *I. spuria* L., *I. xanthospuria* Mathew & Baytop., *I. foetidissima* L., *I. sintenisii* Janka. By the composition of their species, the identified clusters almost completely coincided with the clusters found during the morphological analysis. To confirm the obtained results, a phylogenetic analysis of the species of interest was performed on two more chloroplast sequences available in the database: *matK* and *trnS-trnG*. Clustering of the studied species on *trnS-trnG* and *matK* completely coincided with clustering on *trnL-trnF*. Thus, we can state that the morphological features identified for the *Iris* generic complex work in the taxonomic direction. The analysis also showed that *I. scariosa* from natural populations of the Republic of Bashkortostan and the Orenburg Region were identified correctly.

Key words: *Iris*; taxonomy; taxonomic indicators; nucleotide sequences; *trnL-trnF* chloroplast DNA sequence; phylogenetic tree

For citation: Aukhadieva E.A., Blinov A.G., Vshivtseva E.I., Shigapov Z.Kh., Ishbirdin A.R., Golovanov Ya.M., Kryukova A.V. Morphological and molecular genetic analysis of the genus *Iris* L. polymorphism in the Republic of Bashkortostan and the Orenburg Region. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov J Genet Breed*. 2026;30(1):146-156. doi 10.18699/vjgb-26-16

Funding. The work was carried out with the financial support of the state budget project FWNR-2022-0016.

Морфологический и молекулярно-генетический анализ полиморфизма рода *Iris* L. в Республике Башкортостан и Оренбургской области

Э.А. Аухадиева[1], А.Г. Блинов[2], Е.И. Вшивцева[2, 4] ✉, З.Х. Шигапов[3], А.Р. Ишбирдин[5], Я.М. Голованов[3], А.В. Крюкова[3]

[1] Уфимский научно-исследовательский институт медицины труда и экологии человека, Уфа, Россия
[2] Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия
[3] Южно-Уральский ботанический сад-институт – обособленное структурное подразделение Уфимского федерального исследовательского центра Российской академии наук, Уфа, Россия
[4] Новосибирский национальный исследовательский государственный университет, Новосибирск, Россия
[5] Уфимский университет науки и технологий, Уфа, Россия
✉ evsivceva4@gmail.com

Аннотация. *Iris* L. (Iridaceae Juss.) – это космополитный род, включающий от 200 до 340 видов, распространенных по всему Северному полушарию. Хотя *Iris* является самой разнообразной группой в семействе Iridaceae, существует множество неопределенностей относительно его таксономического состава и систематики. Цель

настоящего исследования – поиск молекулярных маркеров и таксономически значимых морфологических признаков генеративной и вегетативной сферы и оценка их информативности для выявления филогенетического родства представителей рода *Iris*. Образцы ирисов взяты из природных популяций Республики Башкортостан и Оренбургской области, а также получены из ботанических садов Санкт-Петербурга, Бонна, Байройта и Брно. В результате построения структуры изменчивости морфометрических показателей 11 видов найдено 10 таксономических индикаторов, общих для анализируемых таксонов и характеризующихся относительно низкими общей и согласованной изменчивостью: длина и ширина наружных долей околоцветника, длина и ширина внутренних долей околоцветника, длина тычиночной нити, пыльника и пестика, ширина плода, а также длина и ширина семени. Установлены нуклеотидные последовательности *trnL-trnF* фрагментов хлоропластной ДНК для 13 образцов четырех видов дикорастущей флоры Республики Башкортостан и Оренбургской области: *Iris pumila* L., *I. scariosa* Willd. ex Link., *I. pseudacorus* L., *I. sibirica* L. Полученные последовательности были использованы для построения филогенетического дерева совместно с *trnL-trnF* последовательностями еще семи видов ирисов, извлеченных из базы данных. На филогенетическом древе формируется пять групп (кластеров): 1) *I. pumila* L., *I. scariosa* Willd. ex Link; 2) *I. pseudacorus* L., *I. setosa* Pall. ex Link; 3) *I. lactea* Pall.; 4) *I. sibirica* L., *I. sanguinea* Hornem.; 5) *I. spuria* L., *I. xanthospuria* Mathew & Baytop., *I. foetidissima* L., *I. sintenisii* Janka. Выявленные кластеры по составу входящих в них видов практически полностью совпадают с кластерами, обнаруженными при морфологическом анализе. Для подтверждения полученных результатов проведен филогенетический анализ интересующих видов еще на двух хлоропластных последовательностях, доступных в базе данных: *matK* и *trnS-trnG*. Кластеризация исследованных видов на *trnS-trnG* и *matK* полностью совпадает с кластеризацией на *trnL-trnF*. Таким образом, можно констатировать, что выявленные для родового комплекса *Iris* морфологические признаки демонстрируют значимую диагностическую ценность при проведении таксономических исследований.
Ключевые слова: *Iris*; таксономия; таксономические индикаторы; нуклеотидные последовательности; *trnL-trnF* последовательность хлоропластной ДНК; филогенетическое древо

Introduction

The genus *Iris* L. is the largest and most cosmopolitan in the family Iridaceae, distributed mainly in the temperate zones of the Northern Hemisphere, and includes from 200 to 340 species (Dorofeeva, Zhurbenko, 2020). Despite significant progress in the study of the genus *Iris*, there are still many uncertainties regarding its taxonomic composition and systematics. The genera boundaries of irises are controversial, and recent data appear to favor much narrower boundaries (Crespo et al., 2015; Boltenkov et al., 2020). In addition, its composition is periodically supplemented with new described species (Zhao, 1992; Mitic, 2002; Alexeeva, 2013a), which is often associated with morphological variability and, as a consequence, repeated descriptions of species with a wide range (Boltenkov et al., 2018a).

Classifications of the genus *Iris* are most often based on anatomical, morphological and cytogenetic characteristics (Doronkin, Krasnikov, 1984; Mathew, 1989; Makarevich et al., 2001), as well as on the results of molecular biological and biochemical studies (Dorogina et al., 2012; Weber et al., 2020). The difficulty is that systematics have both, broad and narrow understandings of the genus *Iris*. There are no commonly accepted classification, and the most popular classification schemes (Rodionenko, 1961; Mathew, 1989; Doronkin, 2006) have differences not only in position of individual species, but also higher taxonomical units – subgenera and sections. Attempts at resolving those contradictions using modern methods often provide ambiguous results. Molecular RAPD-analysis by I.F. Makarevich and colleagues (2001) showed greater agreement with the system of G.I. Rodionenko (1961) in establishing phylogenetic relationships.

Studies of Siberian species have revealed unexpected groupings: species from *Xyridion* and *I. sibirica* (*Limniris*) formed one group, while *I. lactea* and *I. setosa* (*Limniris*) formed another group with species from subgenus *Iris*. These data are contradicting with the existing schemes, especially in regard to polymorphic subgenus *Limniris*. The lack of consensus on the composition of the genus *Iris* among modern researchers requires further comprehensive studies to clarify phylogenetic relationships of the species included in this genus.

There are publications in the literature on how assessment of the structure of morphological variability can be used in biology for classifying morphological characteristics of some plant and animal species according to the ratio of general and coordinated variability. The authors identify four groups of indicators: ecological-biological systemic, biological, genetic (taxonomic), and ecological indicators (Rostova, 2002; Ishbirdin et al., 2005).

Ecological and biological systemic indicators are characterized by high general and coordinated variability, strong dependence on environmental conditions, and the ability to induce coordinated changes in the entire morphosystem of an organism (number of buds, shoot length in *C. rubra*, leaf blade length in *Triticum aestivum* L.).

Biological indicators have moderate environmental dependence, low total and high coordinated variability. They also determine the morphostructure of the plant (e. g., shoot height, leaf parameters in *Cephalanthera rubra*, the number of metameres in the vegetative part of the annual shoot in *Helianthus annuus*).

Genetic (taxonomic) indicators are characterized by low general and coordinated variability, high autonomy and weak dependence on external conditions (for example, the number of leaves in *C. rubra*), and are the most informative for taxonomic studies.

Ecological indicators exhibit strong, relatively independent variation, and their changes are only mildly correlated with the overall system of the organism, but they are sensitive

even to minor external influences (for example, the number of immature flowers, signs of branching in *Rhinanthus* L.). For species such as *H. annuus* L., *C. rubra* L., *Panicum miliaceum* L., and a number of other flowering plants, these traits have already been identified. For the *Iris* genus complex, a correlation analysis of morphological traits to identify these indicators was conducted for the first time.

DNA polymorphisms in noncoding regions of chloroplast genome are successfully used to establish phylogenetic relationships between species of the genus *Iris* (Pleines et al., 2009). Among the most often used regions of chloroplast DNA are the *trnL* intron and the *trnL-trnF* intergenic spacer, as well as other variable regions of chloroplast DNA including: *atpB-rbcL*, *trnS-trnG*, and *trnH-psbA* intergenic spacers (Kozyrenko et al., 2009; Boltenkov et al., 2016).

The aim of the current study is to search for taxonomically significant morphological features of the generative and vegetative spheres and molecular markers, followed by an assessment of their informativeness in identifying phylogenetic relationships and compliance with the most relevant modern classification systems of the genus *Iris*.

Materials and methods

Plant material. The objects of morphological studies were wild-growing representatives of species of the genus *Iris* (*I. pseudacorus* L., *I. sibirica* L., *I. pumila* L., *I. scariosa* Willd. ex Link), collected from natural populations of Bashkortostan Republic and Orenburg Region, and introduced to the collection area of the South-Ural Botanical Garden-Institute of the Ufa Federal Research Center of the Russian Academy of Sciences in 2019–2021. As well as from seed material grown in obtained from delectuses from the botanical gardens of St. Petersburg (*I. lactea* Pall., *I. setosa* Pall., *I. halophila* Pall.), Bonn (*I. sanguinea* Hornem., *I. spuria* L.), Bayreuth (*I. carthaliniae* Fomin) and Brno (*I. graminea* L.). Details on species used, collection locations and geographical coordinates are listed in Table 1.

Due to the identification of a large number of controversial issues regarding the taxonomy of species of the genus *Iris* L., in this work we focused on the classifications of three authors: B. Mathew (1989), G.I. Rodionenko (1961, 2013), and V.M. Doronkin (2006).

Morphological analysis. Morphometric parameters were recorded for 25 plants of each species. To assess variability,

Table 1. Iris samples used in this work, places and time of their collection

Species	Material collection location	Geographical coordinates
I. pseudacorus	Russia, Republic of Bashkortostan, Kushnarenkovsky district, Ilmurzino village	54.952323 N, 55.811026 E
	Russia, Republic of Bashkortostan, Kushnarenkovsky district, Taraberdino village	55.092243 N, 55.418299 E
	Russia, Republic of Bashkortostan, Demsky district of Ufa, Romanovka village	54.736672 N, 55.834274 E
I. sibirica	Russia, Republic of Bashkortostan, Birsk city	55.454523 N, 55.537686 E
	Russia, Republic of Bashkortostan, Agidel city	55.844276 N, 53.935868 E
I. scariosa	Russia, Republic of Bashkortostan, Khaibullinsky district, Tashtugai mountains	51.910798 N, 58.494056 E
	Russia, Orenburg Region, Kuvandyk district, Ramazanovo village	51.526943 N, 57.446162 E
	Russia, Orenburg Region, Svetlinsky district, Tobolsky settlement	51.430173 N, 61.164358 E
I. pumila (yellow flowers)	Russia, Republic of Bashkortostan, Kuyurgazinsky district, Lena village	52.802508 N, 55.603231 E
I. pumila (blue flowers)	Russia, Republic of Bashkortostan, Kuyurgazinsky district, Lena village	52.802508 N, 55.603231 E
I. pumila (yellow flowers)	Russia, Republic of Bashkortostan, Kuyurgazinsky district, Yakshimbetovo village	52.578021 N, 55.654848 E
I. pumila (blue flowers)	Russia, Republic of Bashkortostan, Kuyurgazinsky district, Yakshimbetovo village	52.578021 N, 55.654848 E
I. pumila (yellow flowers)	Russia, Orenburg Region, Perevolotsky district, village of Rychkovka	51.721765 N, 54.523130 E

the following parameters were analyzed: generative shoot height, leaf length and width, perianth segment length and width, reproductive element length (pistil and stamen) during the flowering phase, and fruit and seed length and width after full ripening. Measurements of morphometric parameters of shoots, flowers and fruits were carried out using a ruler, and those of seeds, using a Levenhuk DTX 90 microscope (Golubev, 1962). Standard statistical processing of the obtained data was carried out using the programs MS Excel and IBM SPSS Statistics 21 (Zaitsev, 1984; Dospekhov, 1985). The arithmetic mean, standard error of the mean, and standard deviation were calculated. To assess intrapopulation variability of morphological traits, the coefficient of variation (CV, %) was calculated (Zaitsev, 1990).

A comparison of biometric indicators was conducted to determine the statistical significance of their differences across 2019–2021 years. The values of the studied indicators were tested for normal distribution using the Kolmogorov–Smirnov test. To compare independent samples with a normal distribution, a one-way analysis of variance was used, and for samples that do not obey the law of normal distribution, the Kruskal–Wallis test was used. The analysis showed that for all the parameters studied, the difference is insignificant at a significance level of W = 5 %, which makes it possible to assess the structure of variability of morphological characteristics and classify them into groups.

The structure of trait variability is constructed using the method of N.S. Rostova (2002), using the programs MS Excel and IBM SPSS Statistics 21. Morphological traits are coordinated in the space of total (the trait's coefficient of variation) and consistent (the squared correlation coefficient averaged over the trait) variability. According to their indicator role, traits are divided into three groups: taxonomic (genetic), biological, and ecological-biological systemic.

Isolation of total DNA, PCR amplification and determination of nucleotide sequences and phylogenetic analysis. Total DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol. 50–100 mg of dried leaves from each plant were used for DNA extraction. The quantity and quality of the isolated DNA were determined using a NanoDrop2000 spectrophotometer (Thermo Scientific, USA) and electrophoretic separation in a 1 % agarose gel containing ethidium bromide (0.5 mg/ml) in 1xTAE.

PCR amplification was performed as described in (Makarevich et al., 2003) with primers specific for the *trnL/trnF* chloroplast genes:

trnL (5′-CGAAATCGGTAGACGCTACG-3′);

trnF (5′-ATTTGAACTGGTGACACGAG-3′).

The reaction was performed in a 20 µl volume using BioMaster LR HS-PCR-Color (Biolabmix, Novosibirsk, Russia) with 10 pmol of each primer and 30 ng of total DNA. The resulting PCR fragments were separated on a 1 % agarose gel and isolated from the gel using the QIAquick Gel Extraction Kit (QIAGEN, Germany), followed by sequencing. Sequencing reactions were performed using 200 ng of DNA fragment and the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific, USA) on an ABI 3130XL genetic analyzer (Applied Biosystems, USA) at the Genomics Center of the Siberian Branch of the Russian Academy of Sciences (http://www.niboch.nsc.ru/doku.php/corefacility). The nucleotide sequences of the *trnL-trnF* fragments of chloroplast DNA are presented in GenBank (No. PV335670–PV335682).

For phylogenetic analysis, *trnL-trnF* iris chloroplast DNA sequences retrieved from the GenBank database and obtained in this study were used. Nucleotide sequence alignment was performed using MAFFT v7.312 (Katoh, Standley, 2013). Suitable nucleotide substitution model was selected using the Bayesian Information Criterion. The phylogenetic tree was constructed using the maximum likelihood method with the Jukes–Cantor model in the IQ-tree program (Trifinopoulos et al., 2016). The reliability of the constructed tree was tested using the Bootstrap method with a number of repetitions equal to 1,000.

Results

Analysis of morphometric parameters

Morphological data for three years of research were analyzed. Biometric indicators were compared for statistical significance of differences between the years. The analysis showed that for all studied parameters, the differences were insignificant at a significance level of W = 5 %. This allowed us to assess the structure of morphological trait variability based on the ratio of total and consistent variability and classify them into groups. Generalized morphometric parameters of studied species are listed in the Table 2.

An analysis of the variability of morphometric parameters of species of the genus *Iris* revealed the following patterns:

1. The highest variability level is typical for the length of a leaf blade (CV reaches 20.7 %) and the height of generative shoots (CV up to 16.7 %).
2. The most stable indicators – width of the inner perianth lobes (CV = 2.2–5.3 %) and pistil length (CV = 0.8–3.4 %), demonstrating the smallest spread of the variation coefficient values.
3. Fruit and seed parameters show higher variability in fruit length (CV up to 9.4 %), compared to its width (CV up to 5.8 %), and moderate variability in seed size (CV in the range of 0.9–5.9 %).

Morphological features are coordinated in the space of general (coefficient of variation of the feature) and consistent (squared correlation coefficient averaged for the feature) variability. Graphs were compiled showing the structure of morphological trait variability. As an example, Figure 1 shows the structure of variability for *I. pseudacorus*. Variability structure for *I. sibirica*, *I. sanguinea*, *I. setosa*, *I. halophila*, *I. graminea*, *I. carthaliniae*, *I. spuria*, *I. pumila*, *I. scariosa*, *I. lactea* is given in the Supplementary Materials[1].

[1] Supplementary Materials are available at: https://vavilovj-icg.ru/download/pict-2026-30/appx10.pdf

Table 2. Generalized morphometric parameters of species from genus *Iris* (2019–2021)

Parameter	Value ranges (min–max)	Coefficients of variation (CV, %)
Aboveground shoots		
Height of generative shoot, cm	9.0–97.5	8.5–16.7
Leaf length, cm	7.1–97.2	6.8–20.7
Lead width, cm	0.5–1.9	4.1–10.9
Flower		
Length of outer lobes, cm	4.0–6.1	1.4–4.2
Width of outer lobes, cm	1.1–3.3	0.8–6.0
Length of inner lobes, cm	1.0–5.1	1.6–6.7
Width of inner lobes, cm	0.2–2.2	2.2–5.3
Length of stamen filament, cm	1.1–1.6	1.4–3.8
Anther length, cm	1.1–2.0	1.2–5.0
Pistil length, cm	2.8–4.4	0.8–3.4
Fruits and seeds		
Fruit length, cm	3.3–6.3	3.1–9.4
Fruit width, cm	1.0–2.1	0.7–5.8
Seed length, cm	0.4–0.8	0.9–4.5
Seed width, cm	0.2–0.8	1.2–5.9

In the current study, according to their indicator role, the features were divided into three groups: taxonomic (genetic), biological and ecological-biological (Fig. 1). As a result of constructing the variability structure, 10 taxonomic indicators were identified that are common to the analyzed taxa and characterized by relatively low total (CV = 0.8–6.0 %) and consistent (r_m^2 = 0.005–0.078) variability: the length and width of the outer perianth lobes, the length and width of the inner perianth lobes, the length of the filament, anther and pistil, the width of the fruit, and the length and width of the seed.

To establish the relationship of the studied representatives of the genus *Iris*, a cluster analysis (hierarchical classification, Ward's method) was carried out and a dendrogram of the differences and similarities between the species was constructed based on the identified diagnostic markers (Fig. 2). The species in the dendrogram can be grouped into six clusters:

1. *I. carthaliniae*, *I. halophila*, *I. spuria*, representatives of subgenus *Xyridion* (Rodionenko, 1961) or *Limniris* (Mathew, 1989).
2. *I. lactea*, subgenus *Limniris* (Rodionenko, 1961; Mathew, 1989) or *Eremiris* (Doronkin, 2006).
3. *I. sibirica*, *I. sanguinea*, subgenus *Limniris*.
4. *I. pumila*, *I. scariosa*, subgenus *Iris*.
5. *I. graminea*, subgenus *Xyridion* or *Limniris*.
6. *I. pseudacorus*, *I. setosa*, subgenus *Limniris*.

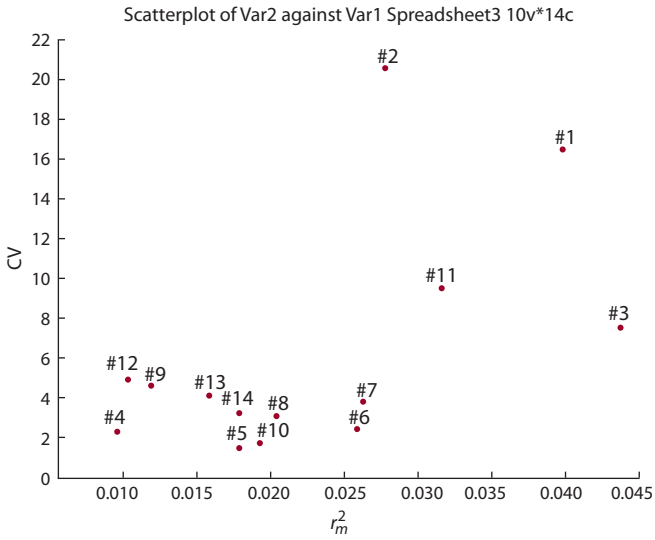

Fig. 1. Structure of variability of morphological characteristics of *I. pseudacorus*.

The *X*-axis shows the consistent variability, the *Y*-axis shows the total variability. 1 – height of generative shoot, 2 – leaf length, 3 – leaf width, 4 – length of outer perianth lobes, 5 – width of outer perianth lobes, 6 – length of inner perianth lobes, 7 – width of inner perianth lobes, 8 – length of filament, 9 – length of anther, 10 – length of pistil, 11 – length of fruit, 12 – width of fruit, 13 – length of seed, 14 – width of seed.

Molecular phylogenetic analysis of species of the genus *Iris*

During the current project, the nucleotide sequences of *trnL-trnF* fragments of chloroplast DNA were established for 13 samples of 4 species of irises: *I. pumila*, *I. pseudacorus*, *I. sibirica*, *I. scariosa* (Table 1). The obtained sequences were used to construct a phylogenetic tree together with the *trnL-trnF* sequences of 7 more iris species retrieved from the database: *I. setosa*, *I. lactea*, *I. sanguinea*, *I. spuria*, *I. xanthospuria* Mathew & Baytop., *I. foetidissima* L., *I. sintenisii* Janka. As a

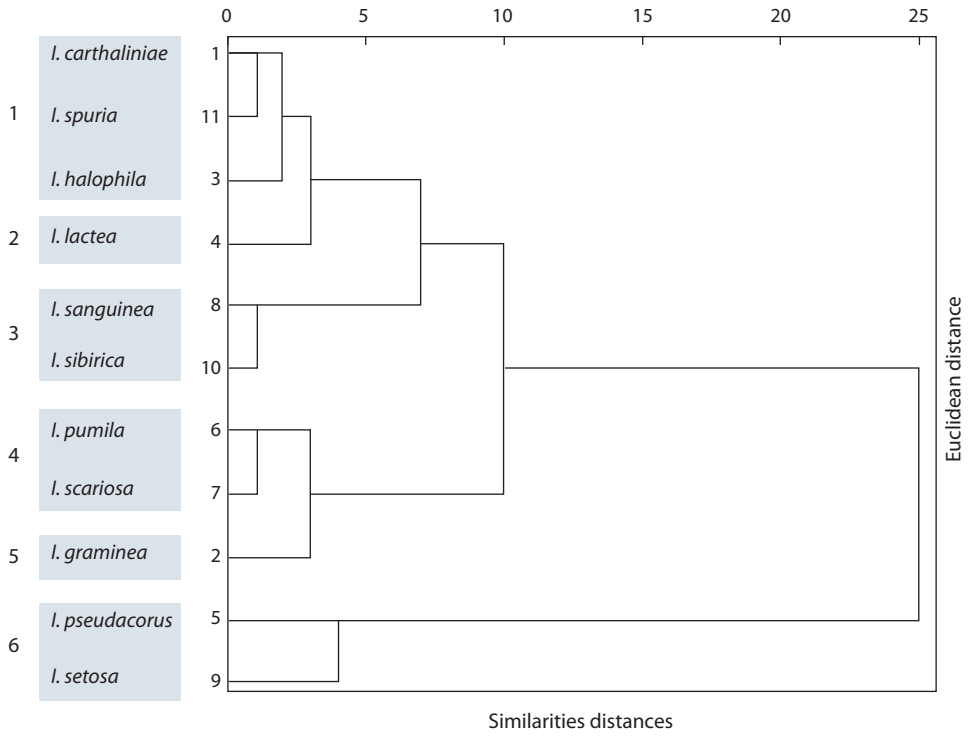

Fig. 2. Dendrogram of differences and similarities of species of the genus *Iris* by taxonomic indicators.

result of this study, *trnL-trnF* sequences from 63 accessions of 11 species were analyzed. The resulting phylogenetic tree is presented in Figure 3. The collection locations of the studied iris accessions are marked on the tree.

There are five clusters on the tree:

1. *I. spuria, I. xanthospuria, I. sintenisii, I. foetidissima*.
2. *I. lactea*.
3. *I. sibirica* and *I. sanguinea*.
4. *I. pumila* and *I. scariosa*.
5. *I. pseudacorus* and *I. setosa*.

These clusters, in terms of the composition of their species, almost completely coincide with the clusters found during morphological analysis (Fig. 2).

To confirm our results, we conducted a phylogenetic analysis of the *Iris* species of interest using two additional chloroplast sequences available in the NCBI database: *matK* and *trnS-trnG*. The dendrogram, obtained using *matK* sequences, showed that the samples split in five groups, similar to the grouping for *trnL-trnF* sequences (Fig. 4):

1. *I. spuria* and *I. halophila*.
2. *I. lactea*.
3. *I. sibirica* and *I. sanguinea*.
4. *I. pumila* and *I. scariosa*.
5. *I. pseudacorus* and *I. setosa*.

However, there are minor differences between the *trnL-trnF* and *matK* trees. In the pairs *I. pumila/I. scariosa, I. sibirica/I. sanguinea,* and *I. halophila/I. spuria,* the *matK* sequences do not confirm species differences. While the species *I. pseudacorus* and *I. setosa* are reliably different from each other. If interpopulation differences in the *trnL-trnF* sequences were found in only two species (*I. scariosa* and *I. setosa*), such differences in the *matK* sequences are present in a larger number of species: *I. setosa, I. pseudacorus, I. sibirica, I. sanguinea,* and *I. lactea*. They may also be present in *I. pumila* and *I. scariosa*, but we found only three *matK* gene sequences from these species in the database.

There is less information about the *trnS-trnG* sequences in the database than about the previous two. Figure 5 shows a tree constructed from the *trnS-trnG* sequences. It contains four out of the five clusters obtained in the previous trees: (1) *I. lactea*; (2) *I. sibirica* and *I. sanguinea*; (3) *I. pumila* and *I. scariosa*; (4) *I. pseudacorus* and *I. setosa*. Cluster five is missing because there are no *trnS-trnG* sequences of *I. spuria* and *I. halophile* in the database. We will not go into detail on the analysis of the *trnS-trnG* tree due to the small number of samples for some species. However, the main conclusion is clear: the clustering of the studied species on the *trnS-trnG* tree is completely consistent with that on the *trnL-trnF* and *matK* trees.

Discussion

Morphometric parameters

A comparative analysis of the morphometric parameters of the studied species with published data (Volkova, 2010) revealed a high degree of correspondence in the aboveground traits for most species, with minor deviations observed in some taxa. The detected differences are presumably determined by the specific cultivation conditions, which are supported, in particular, by the influence of temperature amplitude on the variability

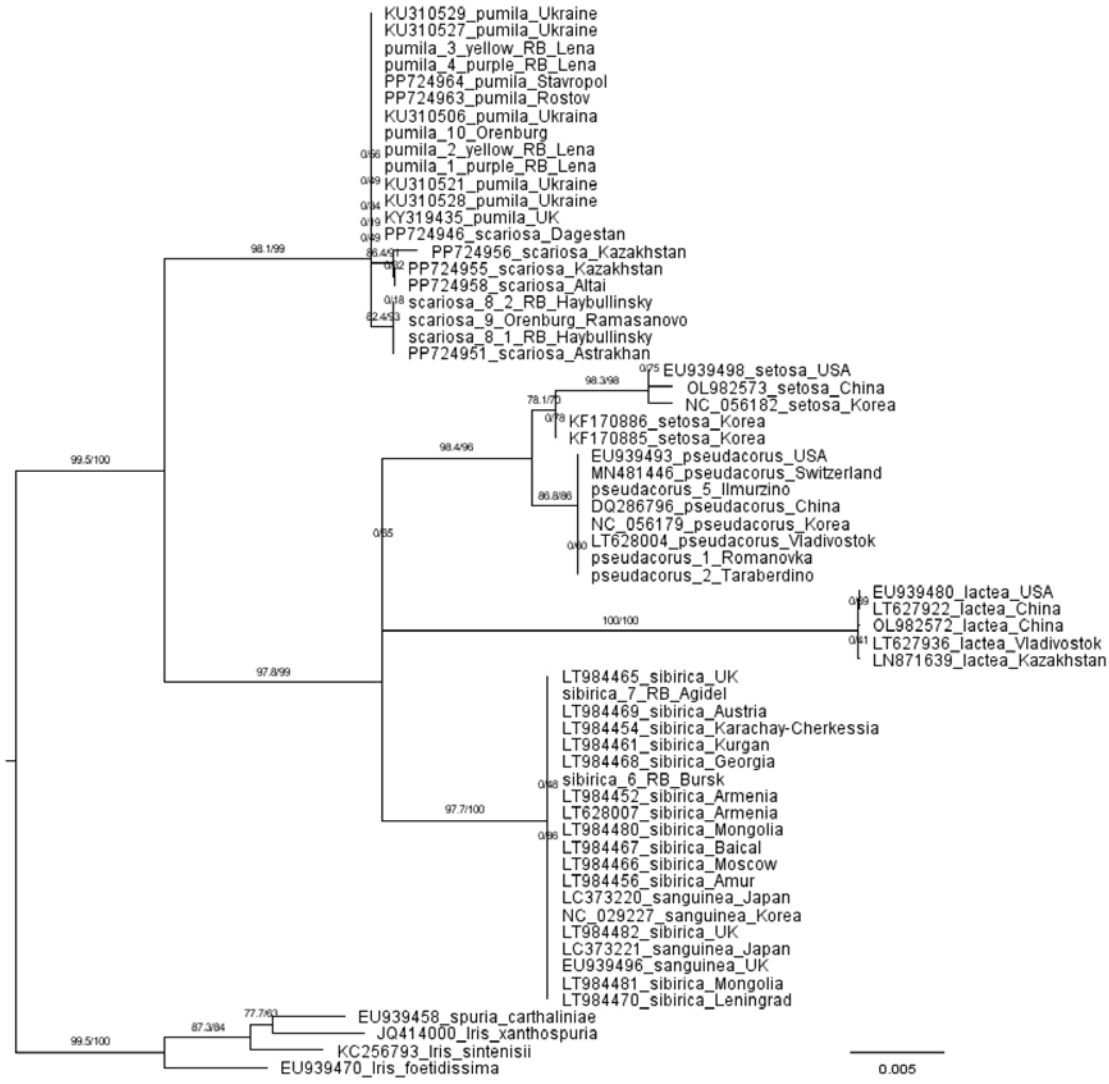

Fig. 3. Phylogenetic tree constructed from iris *trnL-trnF* cpDNA sequences using the ML method.
Collection locations and nucleotide sequence numbers from Genbank database are marked on the tree.

of morphometric parameters of the generative shoot and leaf in *I. pumila* from populations of the Lower Volga region and the Southern Urals (Kashin et al., 2022).

Based on the structure and morphometry of the perianth, the species were divided into conventional groups: (1) with long narrow segments (*I. carthaliniae*, *I. halophila*, *I. spuria*, *I. graminea*, *I. lactea*); (2) with large outer and reduced inner segments (*I. pseudacorus*, *I. setosa*); (3) with large broad segments (*I. sibirica*, *I. sanguinea*); (4) with medium-sized segments (*I. pumila*, *I. scariosa*), which indicates a high degree of reliability and validity of modern classifications of the genus.

A comparative analysis of the morphometric characteristics of fruits and seeds of *Iris* from natural populations of the Republic of Bashkortostan and the Orenburg Region with published data from other regions (Alexeeva, 2020) revealed moderate geographic polymorphism. In terms of seed size, the smallest seeds are characteristic of populations of *I. sibirica* (Saratov Region), *I. halophila* and *I. pumila* (Volgograd Region); a large-seeded form was recorded in *I. pseudacorus* (Primorsky Krai). Regarding fruit parameters, a slight reduction was observed in *I. setosa*, *I. graminea*, *I. spuria*, and *I. pumila* (Belgorod Region). The detected slight variability in size, as well as in the pigmentation of reproductive structures, reflects micropopulation differentiation of the species and local adaptive responses to growing conditions, ensuring their successful reproduction under diverse environmental conditions.

The coefficient of variation of morphological traits serves as a useful tool for the preliminary assessment of the stability of morphological characteristics, however, it is incorrect to judge the taxonomic significance of traits solely on the basis of the coefficient of variation. Taxonomic decisions must be based on a synthesis of morphological, genetic, and ecological data. For the genus *Iris*, this is particularly relevant due to its polymorphism and tendency toward hybridization. The method for identifying taxonomic characters proposed by N.S. Rostova (2002) is based on the analysis of correlation

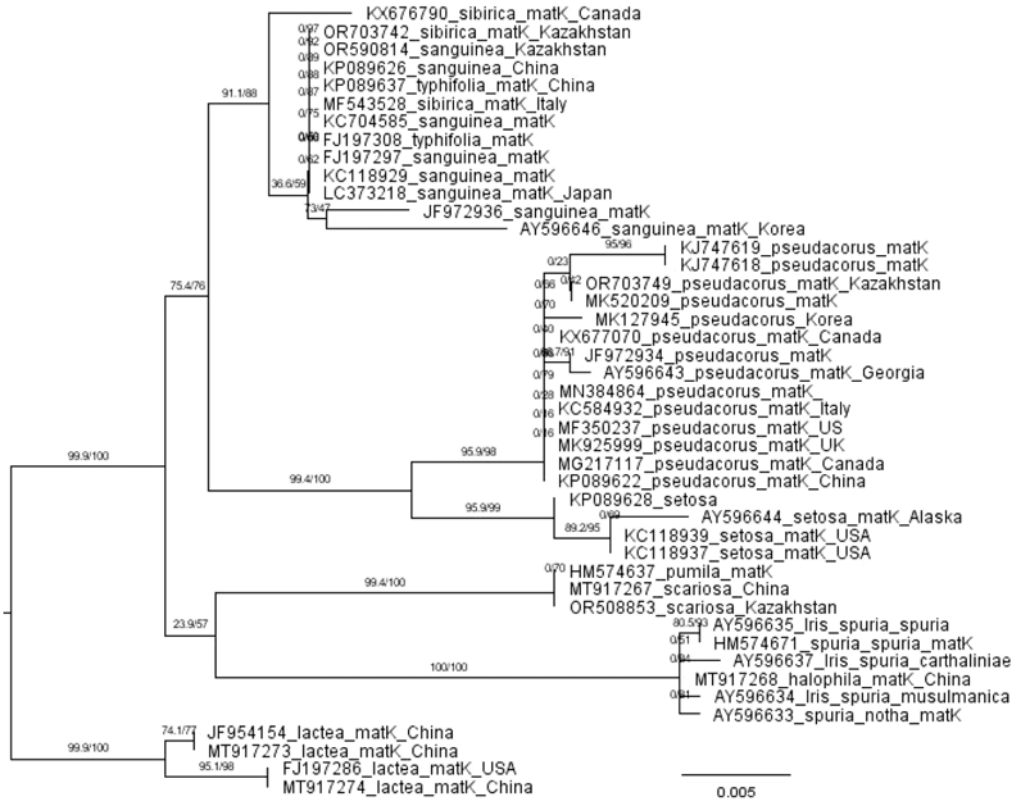

Fig. 4. Phylogenetic tree constructed from the *matK* nucleotide DNA sequences of irises using the ML method.
Collection locations and nucleotide sequence numbers are marked on the tree.

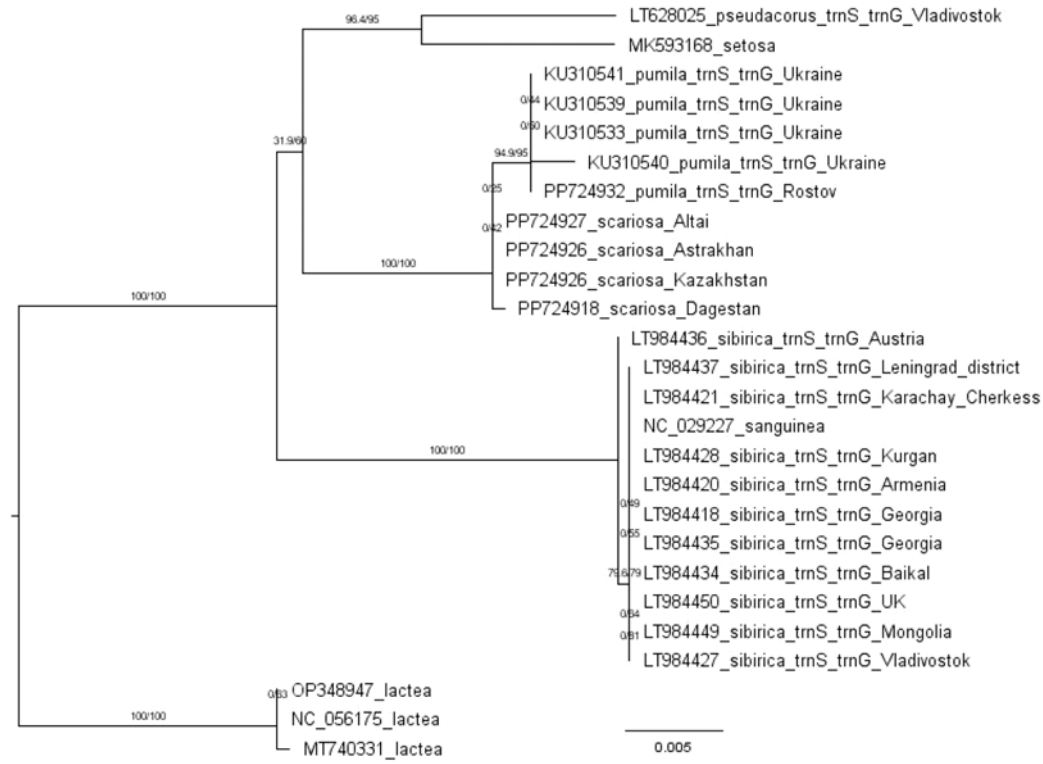

Fig. 5. Phylogenetic tree constructed from *Iris trnS-trnG* cpDNA sequences using the ML method.
Collection locations and nucleotide sequence numbers are marked on the tree.

pleiades – groups of interrelated morphological traits, which detects stable relationships among characters. The method helps distinguish conservative taxonomic traits from those dependent on environmental conditions.

It should be noted that *I. scariosa* is characterized by high variability of morphological traits, especially, like the closely related *I. pumila*, in flower coloration. The species was described from the westernmost margin of its main range based on a specimen collected by Pallas east of the Volga River. It occurs predominantly in the northwestern part of the Caspian Lowland and in the Eastern Ciscaucasia; it is an endemic Caspian European–Caucasian species (Red Book…, 2024).

The taxonomic position of the closely related species *I. scariosa* and *I. glaucescens* Bunge presents certain difficulties. Both species are either synonymized or considered separate (Tsvelev, 1979; Alexeeva, 2020), with *I. scariosa* being the preferred species. *I. glaucescens* was described in Central Asia. The species is represented by small populations found in Russia at the edge of its range in the south of Western Siberia. Outside of Russia, it is known in northeastern Kazakhstan, northwestern Mongolia, and China (Alexeeva, 2020). A similar trend is observed in the Southern Urals: in the Republic of Bashkortostan, *I. scariosa* is included in the regional Red Book (Red Book…, 2021–2024); in the neighboring Chelyabinsk Region, *I. glaucescens* is also listed (Kulikov, 2005). Thus, the taxonomic distinction between these closely related species is challenging. There is evidence in the literature, supported by molecular, morphological, and palynomorphological analysis, that *I. glaucescens*, as well as *I. timofejewii* and *I. curvifolia*, are synonyms of *I. scariosa* (Boltenkov, Artyukova, 2024).

As follows from the dendrogram of species similarities-differences based on the identified diagnostic markers, the closest phylogenetic relationships are observed between the following pairs of species: *I. carthaliniae* and *I. spuria*, *I. sibirica* and *I. sanguinea*, *I. pumila* and *I. scariosa*. Their affinity is also evident in the general habit of the plants. In this way, the pair *I. carthaliniae* and *I. spuria* consists of tall plants characterized by long rhizomes with thickened segments, robust, branched stems, broad sword-shaped leaves, and multi-flowered inflorescences (3–5 flowers). The pair *I. sibirica* and *I. sanguinea* comprises medium-sized irises with short rhizomes bearing narrow segments, hollow, weakly branched stems, narrow linear leaves arranged in basal clumps, and few-flowered inflorescences (2–3 flowers). The pair *I. pumila* and *I. scariosa* includes low-growing plants with thick creeping rhizomes, bearing very short stems, broad-linear or lanceolate leaves in a basal tuft, and large (relative to plant size), few (1–2) flowers positioned close to the ground.

Molecular phylogenetic analysis of species from the genus *Iris*

The results of morphological analysis showed that *I. sibirica* and *I. sanguinea* are very close to each other, and this fact was confirmed by the results of molecular phylogenetic analysis. Moreover, according to the obtained data, we can assume that *I. sibirica* and *I. sanguinea* are the same species, since the *trnL-trnF* sequences of all analyzed samples of these species are identical, regardless of the collection location. Other researchers had previously come to this conclusion by analyzing a number of morphological characteristics and using molecular phylogenetic data (Boltenkov et al., 2020).

A very similar situation is observed in the second pair of closely related species – *I. pumila* and *I. scariosa*. All analyzed *I. pumila* specimens, both those with yellow and those with purple flowers, have identical *trnL-trnF* sequences. The species is polychrome and is characterized by color polymorphism, which is determined by the normal reaction of individuals to environmental conditions (Kashin et al., 2022), and variations in the color of the perianth segments are not associated with molecular genetic polymorphism of populations.

Three sequence variants have been identified in *I. scariosa*. The first variant (from the Republic of Dagestan) is completely identical to the *trnL-trnF* sequence of *I. pumila*, which likely indicates that it is *I. pumila*. The other two variants differ from *I. pumila* by substitutions of several nucleotides. The second variant of *I. scariosa* was found in samples from the Republic of Kazakhstan and the Altai Republic, and the third variant was found in samples from the Republic of Bashkortostan and the Orenburg Region, and the last two variants differ from each other by a single nucleotide substitution. Thus, a comparative analysis of the noncoding sequences of the *trnL-trnF* region of the chloroplast DNA of *I. scariosa* from natural populations, as well as an analysis of literary data, shows that this species is correctly identified in the Republic of Bashkortostan and the Orenburg Region.

The situation is completely different in the third pair of related species – *I. setosa* and *I. pseudacorus*. First of all, these are evidently two different, albeit closely related, species, which is clearly visible on the phylogenetic tree and has been previously noted by other authors (Choi, Lee, 2024). However, these species differ from each other in terms of intraspecific variability. All *I. pseudacorus* samples analyzed showed identical *trnL-trnF* sequences, while five *I. setosa* samples analyzed showed the presence of two types of *trnL-trnF* sequences, with all samples collected either in China or Korea. *I. setosa* is heterogeneous and taxonomically quite clearly delimited from other species, because of this new species and intraspecific taxa have been identified from its composition (Ilyushko et al., 2001). N.B. Alexeeva (2013b) came to the conclusion that the polymorphic complex of *I. setosa* consists of five species that differ in morphology and ecology. Of course, to verify this, more factual material needs to be analyzed.

All five *trnL-trnF* sequences of *I. lactea* are identical to each other, regardless of their habitat. *Iris* Linnaeus ser. *Lacteae* Doronkin (Doronkin, 1990) includes species distributed in the temperate Asian regions of the Northern Hemisphere (Rodionenko, 2006; Boltenkov, 2018). Initially, *I. lactea* was classified in the subgenus *Limniris*, section *Limniris* (Rodionenko, 1961; Mathew, 1989), then in the subgenus *Eremiris*, section *Haloiris* (Doronkin, 2006). Subsequently, it began to be considered as part of a separate genus *Eremiris* (Rodionenko, 2006), with which C.A. Wilson (2011), based on molecular genetic studies, does not agree. For a long time, it was believed that the *Lacteae* Doronkin series was represented by only one

polymorphic species, but molecular studies have confirmed phylogenetic branching in at least three lineages corresponding to the taxa *I. lactea*, *I. oxypetala* and *I. tibetica* (Boltenkov et al., 2018b).

Thus, we can state that morphological and molecular phylogenetic analyses show the same results regarding the phylogeny analysis of the studied species of the genus *Iris*, which means that the identified morphological indicators work in a taxonomic direction.

Conclusion

Based on the conducted assessment of the structure of morphological variability of representatives of the genus *Iris*, 10 taxonomic indicators were identified: the length and width of the outer perianth lobes, the length and width of the inner perianth lobes, the length of the filament, anther and pistil, the width of the fruit, and the length and width of the seed.

The establishment of phylogenetic relationships of the studied species based on the identified taxonomic indicators and the molecular phylogenetic analysis based on *trnL-trnF*, *matK* and *trnS-trnG* chloroplast DNA made it possible to combine the studied *Iris* species into several phylogenetic groups. Thus, it can be concluded that both morphological and molecular phylogenetic analyses yield consistent results regarding the phylogeny of the studied species of the genus *Iris*, which indicates that the identified morphological indicators reliably demonstrate taxonomic validity.

References

Alexeeva N.B. *Iris lokiae*, a new species of genus *Iris* (*Iridaceae*). *Botanicheskii Zhurnal*. 2013a;98(11):1415-1420 (in Russian)

Alexeeva N.B. On the taxa of *Iris setosa* Pall. ex Link affinity (Iridaceae). *Turczaninowia*. 2013b;16(2):13-29 (in Russian)

Alexeeva N.B. *Iris* L. (Iridaceae Juss.) in the Russia. Saint Petersburg, 2020 (in Russian)

Boltenkov E.V. Taxonomic notes on *Iris* ser. *Lacteae* (Iridaceae) with typifications of fifteen names and one new combination. *Phytotaxa*. 2018;383(3):283-292. doi 10.11646/phytotaxa.383.3.5

Boltenkov E.V., Artyukova E.V. Updated taxonomy of *Iris scariosa* (Iridaceae) inferred from morphological and chloroplast DNA sequence data with remarks on classification of *Iris* subg. *Iris*. *Plants*. 2024;13(17):2349. doi 10.3390/plants13172349

Boltenkov E.V., Artyukova E.V., Kozyrenko M.M. Species divergence in *Iris* series *Lacteae* (Iridaceae) in Russia and adjacent countries based on chloroplast DNA sequence data. *Russ J Genet*. 2016;52(5): 507-516. doi 10.1134/S1022795416040037

Boltenkov E.V., Artyukova E.V., Kozyrenko M.M. A taxonomic study of the genus *Iris* (Iridaceae): morphological and molecular data. In: Botany in the Contemporary World. Vol. 1. Mahachkala, 2018a;13-16. Available: https://www.binran.ru/files/publications/Proceedings/Proceedings_RBO/XIV_RBO_Proceedings_T1.pdf (in Russian)

Boltenkov E.V., Artyukova E.V., Kozyrenko M.M., Trias-Blasi A. *Iris tibetica*, a new combination in *I.* ser. *Lacteae* (Iridaceae) from China: evidence from morphological and chloroplast DNA analyses. *Phytotaxa*. 2018b;338(3):223-240. doi 10.11646/phytotaxa.338.3.1

Boltenkov E., Artyukova E., Kozyrenko M., Erst A., Trias-Blasi A. *Iris sanguinea* is conspecific with *I. sibirica* (Iridaceae) according to morphology and plastid DNA sequence data. *Peer J*. 2020;8:e10088. doi 10.7717/peerj.10088

Choi T.-Y., Lee S.-R. Complete plastid genome of *Iris orchioides* and comparative analysis with 19 *Iris* plastomes. *PLoS One*. 2024;19(4): e0301346. doi 10.1371/journal.pone.0301346

Crespo M.B., Martínez-Azorín M., Mavrodiev E.V. Can a rainbow consist of a single colour? A new comprehensive generic arrangement of the '*Iris sensu latissimo*' clade (Iridaceae), congruent with morphology and molecular data. *Phytotaxa*. 2015;232(1):1-78. doi 10.11646/phytotaxa.232.1.1

Dorofeeva M.M., Zhurbenko P.M. Comparative study of ovule structure and development in some species of *Iris* subgenus *Limniris* (Iridaceae). *Botanicheskii Zhurnal*. 2020;105(1):15-31. doi 10.31857/S0006813620010068

Dorogina O.V., Doronkin V.M., Selyutina I.Yu., Konichenko E.S. Structure of the genus *Iris* L. (Iridaceae Juss.) in Asiatic Russia, revealed by SDS-electrophoresis of seed storage proteins. *Turczaninowia*. 2012;15(4):76-81 (in Russian)

Doronkin V.M. The synopsis of Siberian species of the genus *Iris* (Iridaceae). *Botanicheskii Zhurnal*. 1990;75(3):409-416 (in Russian)

Doronkin V.M. Taxonomy of the genus *Iris* L. (Iridaceae Juss.) in Asian Russia. In: Role of Botanical Gardens in the Preservation of Vegetation Diversity in Asian Russia: the Present and the Future. Novosibirsk, 2006;101-103 (in Russian)

Doronkin V.M., Krasnikov A.A. Cytotaxonomic studies in some Siberian species of the genus *Iris* (Iridaceae). *Botanicheskii Zhurnal*. 1984;69(5):683-685 (in Russian)

Doronkin V.M., Baikov K.S. Modeling phylogenetic relationships of Siberian species of the genus *Iris* (Iridaceae) using SYNAP method. *Turczaninowia*. 2004;7(2):45-57 (in Russian)

Dospekhov B.A. Methodology of Field Experiments (with the Basics of Statistical Processing of Research Results). Moscow, 1985 (in Russian)

Golubev V.N. Fundamentals of Biomorphology of Herbaceous Plants in the Central Forest-steppe. Voronezh, 1962 (in Russian)

Ilyushko M.V., Karatavcev Yu.F., Zhuravlev Yu.N. Variability of morphological characters of *I. setosa* (Iridaceae) in the Russian Far East. *Botanicheskii Zhurnal*. 2001;86(3):60-72 (in Russian)

Ishbirdin A.R., Ishmuratova M.M., Zhirnova T.V. Life strategies of the cenopopulation *Cephalanthera rubra* L. (Rich) on the territory of the Bashkir State Nature Reserve. *Vestnik of Lobachevsky University of Nizhni Novgorod*. 2005;1:85-98 (in Russian)

Kashin A.S., Parkhomenko A.S., Abramova L.M., Kondratyeva A.O., Bogoslov A.V., Shilova I.V., Kryukova A.V. Morphological variability of *Iris pumila* (Iridaceae) in the Lower Volga region and the South Urals. *Botanicheskii Zhurnal*. 2022;107(2):180-197. doi 10.31857/S0006813622020065 (in Russian)

Katoh K., Standley D.M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013;30(4):772-780. doi 10.1093/molbev/mst010

Kozyrenko M.M., Artyukova E.V., Zhuravlev Y.N. Independent species status of *Iris vorobievii* N.S. Pavlova, *Iris mandshurica* Maxim., and *Iris humilis* Georgi (Iridaceae): evidence from the nuclear and chloroplast genomes. *Russ J Genet*. 2009;45(11):1394-1402. doi 10.1134/S1022795409110143

Kulikov P.V. Synopsis of the Flora of the Chelyabinsk Region (Vascular Plants). Ekaterinburg–Miass, 2005 (in Russian)

Makarevich I.F., Doronkin V.M., Shcherbik S.V., Blinov A.G. Application of RAPD-PCR method in the study of phylogenetic relationships between Siberian species of the genus *Iris* L. (Iridaceae). *Turczaninowia*. 2001;4(4):80-92 (in Russian)

Makarevich I., Golovnina K., Scherbik S., Blinov A. Phylogenetic relationships of the Siberian Iris species inferred from noncoding chloroplast DNA sequences. *Int J Plant Sci*. 2003;164(2):229-237. doi 10.1086/346160

Mathew B. The Iris. Portland, 1989

Mitic B. *Iris adriatica* (Iridaceae), a new species from Dalmatia (Croatia). *Phyton*. 2002;42(2):305-314

Pleines T., Jakob S.S., Blattner F.R. Application of non-coding DNA regions in intraspecific analyses. *Plant Syst Evol*. 2009;282:281-294. doi 10.1007/s00606-008-0036-9

Red Book of the Republic of Bashkortostan. Vols. 1 and 2. Ufa, 2021-2024 (in Russian)

Red Book of the Russian Federation. Plants and Mushrooms. Moscow, 2024 (in Russian)

Rodionenko G.I. Genus Iris. Moscow–Leningrad, 1961 (in Russian)

Rodionenko G.I. *Eremiris*, a new genus of the family Iridaceae. *Botanicheskii Zhurnal*. 2006;91(11):1707-1712 (in Russian)

Rodionenko G.I. Understanding the Secrets of Nature (My Destiny is Irises). St. Petersburg, 2013 (in Russian)

Rostova N.S. Correlations: Structure and Variability. St. Petersburg, 2002 (in Russian)

Trifinopoulos J., Nguyen L.-T., Haeseler A., Minh B.Q. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* 2016;44(W1):W232-W235. doi 10.1093/nar/gkw256

Tsvelev N.N. Genus Iris L. In: Flora of the European Part of the USSR. Vol. 4. 1979;299-307 (in Russian)

Volkova G.A. The results of introduction of natural species of the *Iris* L. genus in northern Europe. In: Botanical Gardens: Issues of Introduction. Tomsk, 2010;274:127-129 (in Russian)

Weber T., Jakše J., Sladonja B., Hruševar D., Landeka N., Brana S., Bohanec B., Milović M., Vladović D., Mitić B., Poljuha D. Molecular study of selected taxonomically critical taxa of the genus *Iris* L. from the broader Alpine-Dinaric area. *Plants*. 2020;9(9):1229. doi 10.3390/plants9091229

Wilson C.A. Subgeneric classification in *Iris* re-examined using chloroplast sequence data. *Taxon*. 2011;60(1):27-35. doi 10.1002/tax.601004

Zaitsev G.N. Mathematical Statistics in Experimental Botany. Moscow, 1984 (in Russian)].

Zaitsev G.N. Mathematics in Experimental Botany. Moscow, 1990 (in Russian)

Zhao Y.T. A new species of Iris from China. *J Syst Evol*. 1992;30(2):181-182

Conflict of interest. The authors declare no conflict of interest.

Received April 30, 2025. Revised August 10, 2025. Accepted September 9, 2025.