



How many species of tulips of the subgenus *Orithyia* (*Tulipa*, Liliaceae) are in Southern Siberia?

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Manuscript received: 02.12.2022

Review completed: 26.12.2022

Accepted for publication: 28.12.2022

Published online: 30.12.2022

ABSTRACT

The subgenus *Orithyia* in the genus *Tulipa* is considered one of the least studied. It includes the most eastern species of tulips. Previously, three species of tulips from this subgenus, *T. heteropetalata*, *T. uniflora*, and *T. mongolica* were reported for the territory of Southern Siberia. The morphological analysis showed that studied plants, from populations previously reported in Southern Siberia under the name *T. heteropetalata*, are large individuals of *T. uniflora*. There was a high level of morphological differences between all populations of *T. uniflora* and *T. mongolica*. Karyotype analysis showed that *T. mongolica*, *T. uniflora*, and *T. siniangensis* have similar karyotype structures. In *T. heterophylla*, in comparison with them, a lower level of intrachromosomal asymmetry was noted. Molecular genetic analysis confirms previous data and separates the subgenus *Orithyia* among other subgenera in the genus *Tulipa*. Molecular data confirmed taxonomic affiliation of specimens from the Zabaykalsky Territory (Russia) previously assigned by morphological characters to *T. mongolica*. Thus, it was confirmed that two species of tulips of the subgenus *Orithyia*, *Tulipa uniflora* and *T. mongolica*, grow in the studied area.

Keywords: *Tulipa*, biology, ecology, morphology, karyotype structure, phylogenetic analysis

РЕЗЮМЕ

Чернышева О.А., Букин Ю.С., Кулакова Н.В., Митренина Е.Ю., Мурашко В.В., Хадеева Е.Р., Эрст А.С., Кривенко Д.А. Сколько видов тюльпанов подрода *Orithyia* (*Tulipa*, Liliaceae) в Южной Сибири? В роде *Tulipa* подрод *Orithyia* по праву является одним из малоизученных. К нему относятся самые восточные виды тюльпанов. Ранее для территории Южной Сибири из этого подрода приводилось три вида тюльпанов – *Tulipa heteropetalata*, *T. uniflora* и *T. mongolica*. Морфологический анализ видов показал, что исследованные растения, в популяциях, ранее приводимых в Южной Сибири под названием *T. heteropetalata*, являются крупными особями *T. uniflora*. Между всеми популяциями *T. uniflora* и *T. mongolica* наблюдается высокий уровень морфологических различий. Анализ кариотипов показал, что *T. mongolica*, *T. uniflora* и *T. siniangensis* имеют сходные структуры кариотипов. У *T. heterophylla*, в сравнении с ними, отмечен более низкий уровень внутрихромосомной асимметрии. Молекулярно-генетический анализ подтверждает ранее проведенные исследования об обособленности подрода *Orithyia* от остальных подродов рода *Tulipa*. Таксономическая принадлежность образцов из Забайкальского края (Россия), ранее отнесенных к виду *T. mongolica* по морфологическим признакам, также подтверждена молекулярными данными. Таким образом, установлено, что на исследуемой территории произрастают два вида тюльпанов подрода *Orithyia* – *Tulipa uniflora* и *T. mongolica*.

Ключевые слова: *Tulipa*, биология, экология, морфология, кариотип, филогения

Tulips (*Tulipa* L., Liliaceae Juss.) are well-known ornamental plants. In the wild, species of tulips are ancestors and an inexhaustible source for breeding new varieties. They have an invaluable advantage over cultivated plants: extremely diverse perianth color and immunity to virus variegation (Bochantseva 1962).

The center of species diversity of the genus *Tulipa* is the foothills of the Tian Shan – the territory of modern Kazakhstan, from here tulips spread to steppes of Southern Siberia and deserts of Iran, to Mongolia and mountains of Southern Europe (Ivashchenko 2005).

The genus *Tulipa* includes about 80 species belonging to four subgenera: *Clusianae* (Baker) Zonn., *Eriostemones* (Boiss.) Raamsd., *Orithyia* (D. Don) Baker, and *Tulipa* (Everet et al. 2013). *Orithyia* is one of the least studied subgenera (Chris-

tenhusz et al. 2013) consisting of species with the easternmost distribution: *Tulipa heteropetalata* Ledeb., *T. heterophylla* (Regel) Baker, *T. mongolica* Y.Z. Zhao, *T. siniangensis* Z.M. Mao and *T. uniflora* (L.) Besser ex Baker. (Fig. 1).

An extensive range of *T. uniflora* covers eastern Kazakhstan, the entire southern Siberia, Mongolia and northern China (Gubanov 1996, Xinqi & Mordak 2000, Ivashchenko 2005, Urgamal et al. 2014, Xing et al. 2017, Chernysheva et al. 2018). Other species are locally distributed: *T. heteropetalata* in northeast of Kazakhstan, Russian Altai (Vlasova 1987, Ivashchenko 2005), *T. siniangensis* in mountains of northern China (Xinqi & Mordak 2000), *T. heterophylla* in northeastern China, Trans-Ili Alatau mountain range in Kazakhstan (Ivashchenko 2005, Xinqi & Mordak 2000), *T. mongolica* in the south of the Trans-Baikal Territory of

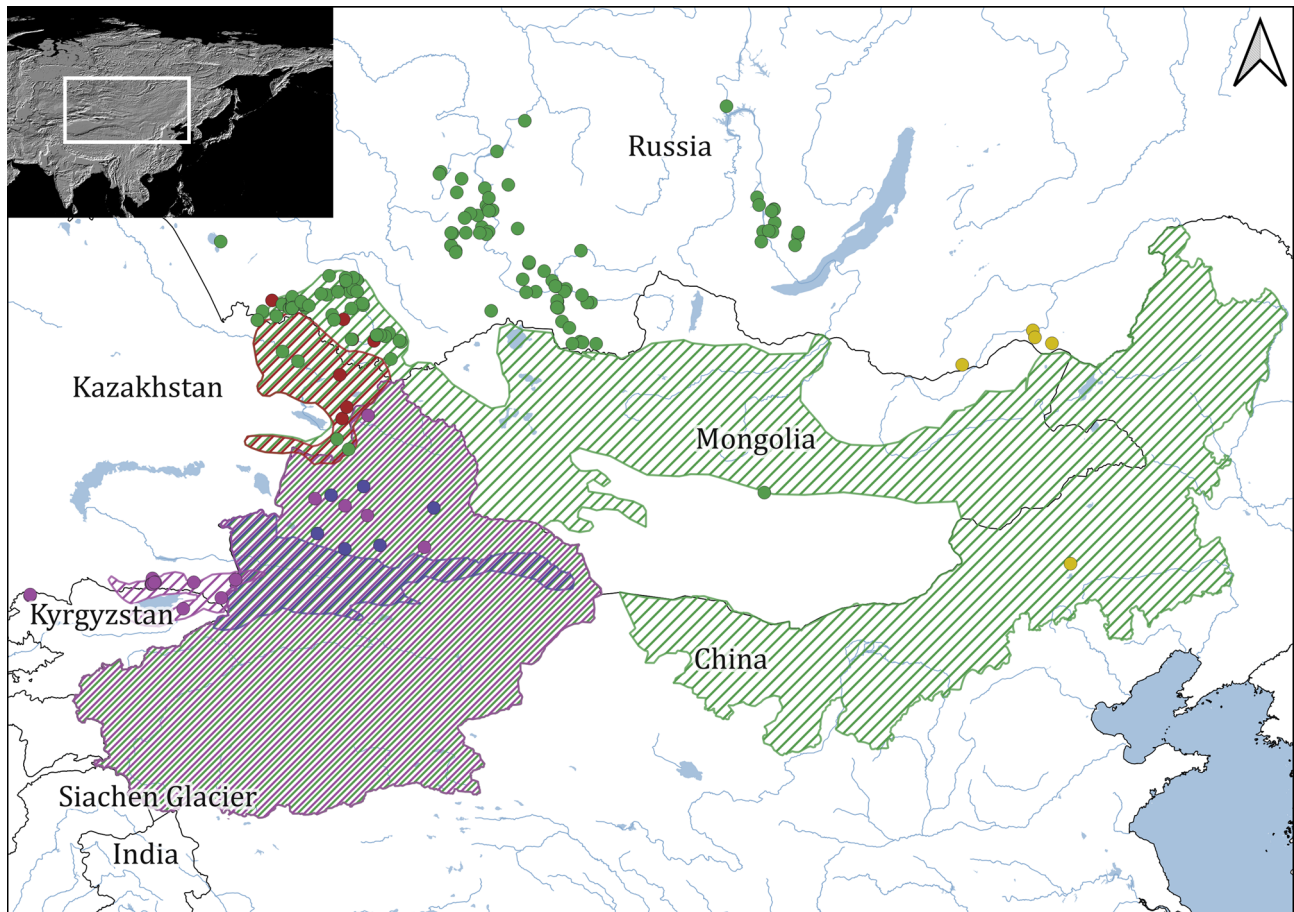


Figure 1 Distribution map of tulips of the subgenus *Orithyia*: *Tulipa heteropetalata* Ledeb. (red), *T. heterophylla* (Regel) Baker (purple), *T. mongolica* Y.Z. Zhao (yellow), *T. sinkiangensis* Z.M. Mao (blue), and *T. uniflora* (L.) Besser ex Baker (green). Shaded parts do not represent continuous range, but reflect published data (Ivashchenko 2005, Urgamal et al. 2014, Xinqi & Mordak 2020). Visualization of cartographic data was carried out in QGIS version 3.16, using standard analysis tools, GDAL and SAGA. Administrative boundaries and hydrological features are based on the OpenStreetMap geodatabase under Open Data Commons Open Database License (ODbL)

Russia, northeast of China (Inner Mongolia) (Zhao 2003, Verkhozina et al. 2020).

There are three species reported as occurring in Southern Siberia: *T. heteropetalata* (Vlasova 1987), *T. mongolica* (Verkhozina et al. 2020), and *T. uniflora*. Tulips' populations in this area are tertiary relics (Peshkova 1972). We added *T. mongolica* to the list in 2020. Previously it was considered that this species is endemic to Inner Mongolia (Zhao 2003). Locations in the Trans-Baikal Territory were previously listed as *T. uniflora* (Sergievskaya 1972, Peshkova 1979, Vlasova 1987, Galanin & Belikovich 2011).

Tulipa heteropetalata is taxonomically controversial species. Some researchers synonymise it with *T. uniflora* (Mordak 1990, 1992, Cherepanov 1995, Xinqi & Mordak 2000) while others consider it an independent endemic species of the Kazakh and Russian Altai and Tarbagatai (Vvedensky 1935, Kamelin 1998, Grebenjuk 2008). Analysis of herbarium collections ALTB!, IRK!, IRKU!, KRAS!, MOSP!, MW!, NS! and NSK! (herbarium acronyms are given in accordance with Thiers (2022)) showed that *T. heteropetalata* is distributed from eastern Kazakhstan to Eastern Siberia, within the range of *T. uniflora*. However, the identification of these species is hampered by high plasticity in the morphological traits of a flower, which are poorly preserved in herbarium collections, and the specimens with fruits do not differ significantly (Grebenjuk 2008).

Recent molecular genetic studies showed the monophyly of the genus *Tulipa* (Fay et al. 2001, Clennett et al. 2012) and its division into four subgenera (Zonneveld 2009, Christenhusz et al. 2013, Everett 2013, Turktas et al. 2013, Dekhkonov et al. 2022). However, species of the subgenus *Orithyia* were not sufficiently involved in these studies.

This study aimed to clarify the number of species in the subgenus *Orithyia* of the genus *Tulipa* in Southern Siberia applying a complex of morphological, ecological, biological, karyological and molecular genetic methods.

MATERIAL AND METHODS

Field studies were carried out in 22 natural populations in the steppes of Southern Siberia in 2017–2019 (Table 1). The collected material was deposited in the IRK Herbarium.

Morphological methods

For each population, up to 12 morphometric traits were measured in 15–30 random individuals in the flowering phase. There were 422 studied tulips in total. Due to a short flowering period in tulips, it was not possible to measure some morphometric characteristics of the flower in populations **B**, **H**, **I**, **L**, **K1**, **K2**, **VK**, **ZT** (Table 1). These parameters were reconstructed using multivariate linear regression according to recommendations of Myers (2000) and Lee et al. (2010).

Table 1. Investigated localities of tulips of the subgenus *Orithyia* in Southern Siberia

Location, population	Coordinates, a.s.l. (m)	Species (comment)	Habitat	Stage of pasture digression (Ramensky et al. 1956)
Irkutsk Region, Bokhan District, Bokhan settlement – B	53.161333°N 103.800806°E, 451	<i>T. uniflora</i>	Cinquefoil-grass-forb steppe, southern slope, soddy calcareous soil	9
Irkutsk Region, Bokhan District, Khandagay settlement – H	53.186806°N 103.617139°E, 445	<i>T. uniflora</i>	Grass-forb steppe, southwestern slope, soddy calcareous soil	3–4
Irkutsk Region, Ekhirit-Bulagat District, Gakhany village – G	53.048988°N 104.898716°E, 600	<i>T. uniflora</i>	Thyme-forb steppe, northwestern slope, soddy calcareous soil	3–4
Irkutsk Region, Nukut District, Tomar cape – M.T	53.840449°N 103.219994°E, 520	<i>T. uniflora</i>	Grass-cinquefoil-sagebrush-forb steppe, southern and southwestern slope, soddy calcareous soil	3–4
Irkutsk Region, Osinsky District, Novo-Lenino village – L	53.688754°N 103.740719°E, 539	<i>T. uniflora</i>	Cinquefoil-forb steppe, southeastern slope, soddy calcareous soil	6–7
Irkutsk Region, Osinsky District, Obusa village – O	53.730567°N 103.847628°E, 528	<i>T. uniflora</i>	Spirea grass-forb steppe, southeastern slope, soddy calcareous soil	5
Irkutsk Region, Ust'-Udinsky District, Igzhay village – I	54.041797°N 103.112555°E, 535	<i>T. uniflora</i>	Grass-forb steppe, southwestern slope, soddy calcareous soil	6–7
Krasnoyarsk Territory, Balakhtinsky District, Primorsk settlement – P	55.204278°N 91.863611°E, 363	<i>T. uniflora</i>	Spirea cinquefoil-cold-sagebrush-grass steppe, southwestern slope, carbonized chernozem	1–2
Krasnoyarsk Territory, Ermakovo District, Verkhneusinskoe village – V.U	52.368806°N 93.269417°E, 797	<i>T. uniflora</i> (Stepanov 2012: 204 as “ <i>T. heteropetala</i> ”)	Spirea sedge steppe, southern slope, leached chernozem	–
Krasnoyarsk Territory, Ermakovo District, Verkhny Kebezh village – V.K	53.241222°N 92.771028°E, 372	<i>T. uniflora</i> (Stepanov 2012: 204 as “ <i>T. heteropetala</i> ”)	Pea shrub sedge steppe, southeastern slope, leached chernozem	1–2
Republic of Khakassia, Abakan city, Samokhval mountain – A.S	53.69575°N 91.531861°E, 394	<i>T. uniflora</i> (Vlasova 1987: 102 as “ <i>T. heteropetala</i> ”)	Grass-forb steppe, southeastern slope, carbonized chernozem	1–2
Republic of Khakassia, Askiz District, Ust'-Khoiza – U.H	53.129528°N 89.902667°E, 633	<i>T. uniflora</i>	Grass-forb steppe, southern slope, carbonized chernozem	8
Republic of Khakassia, Beysky District, Novonikolaevka village – N.N	53.151333°N 91.433944°E, 311	<i>T. uniflora</i>	Grass-tulip-forb steppe, leached chernozem	5
Republic of Khakassia, Bograd District, Abakano-Perevoz village – A.P	54.264139°N 91.374083°E, 308	<i>T. uniflora</i>	Sagebrush-forb steppe, southeastern slope, carbonized chernozem	5
Republic of Khakassia, Shirino District, Chernoe Ozero village – Ch.O	54.679806°N 89.403111°E, 531	<i>T. uniflora</i>	Grass-forb steppe, southeastern slope, carbonized chernozem	5
Republic of Khakassia, Tashtyp District, vicinity of Abaza town – A	52.623972°N 90.097972°E, 616	<i>T. uniflora</i>	Spirea forb steppe, southwestern slope, leached chernozem	–
Republic of Tuva, Kyzyl District, Kaa-Khem settlement – K.I	51.717389°N 94.780306°E, 764	<i>T. uniflora</i> (In NSK as “ <i>T. heteropetala</i> ”)	Grass-forb steppe, southeastern slope, calcareous chestnut soil	3–4
Republic of Tuva, Kyzyl District, Kaa-Khem settlement – K.2	51.671917°N 94.610944°E, 688	<i>T. uniflora</i> (In NSK as “ <i>T. heteropetala</i> ”)	Sagebrush-forb steppe, southeastern slope, soddy calcareous soil	8–9
Republic of Tuva, Piy-Khema District, Seserlig village – S	51.8665°N 94.253694°E, 1014	<i>T. uniflora</i>	Grass-forb steppe, southeastern slope, non-calcareous light chestnut soil	8
Republic of Tuva, Piy-Khema District, Turan town – T	52.126222°N 93.924306°E, 856	<i>T. uniflora</i>	Spirea forb steppe, southeastern slope, leached chernozem	6–7
Trans-Baikal Territory, Onon District, Maly Bator tract – M.B	50.530191°N 115.026008°E, 667	<i>T. mongolica</i> (Peshkova 1979: 225 as “ <i>T. uniflora</i> ”)	Forb-feather grass steppe, southern and southeastern slope, non-calcareous chestnut soil	5
Trans-Baikal Territory, Onon District, near Zun-Torey Lake – Z.T	50.17488°N 115.85037°E	<i>T. mongolica</i> (Peshkova 1979: 225 as “ <i>T. uniflora</i> ”)	Forb-feather grass steppe, non-calcareous chestnut soil	6–7

In addition, for reliable data processing, the population **M.T** was divided into **M.T1** which consists of small individuals from the arid slope of southern exposition, and **M.T2** with large individuals from the moisture-provided slope of southwestern exposition. The split of the population **Z.T** is based on data from 2016 (**Z.T16**) and 2019 (**Z.T**).

Methods of multidimensional statistics, the principal component analysis (PCA) and K-means cluster analysis, were used to analyze and visualize the distribution of studied individuals by combination of morphometric traits. The groups identified by cluster analysis were visualized

on a PCA scatterplot. The factoextra package (Kassambara 2015, 2017) was used for multivariate data analysis.

The Fisher's one-way analysis of variance (ANOVA) ($\alpha = 0.05$) was used to assess the degree of differences in morphometric parameters in studied populations (Chambers et al. 1992). Differences between populations were considered significant at p-value < 0.05. The degree of differences (H) between populations by studied traits was calculated with the formula: $H = 1 - 1/F$, where F is the value of the Fisher criterion. All of the statistical analysis and diagrams were performed using R Statistical Software.

Karyotype analysis

The comparative karyotype analysis was conducted for *T. mongolica* (**M.B**) and *T. uniflora* (**G**). Somatic chromosomes of *Tulipa* were studied from root tip cells. Bulbs were germinated in wet moss at ~15°C for 2–3 weeks. Newly formed 1–2 cm long roots were excised and pretreated in 0.2 % colchicine solution for 3–4 h. Roots were fixed in a mixture of 96 % ethanol and glacial acetic acid (3:1). Root tips were stained with 1% aceto-haematoxylin, and the karyotypes were investigated by the squash method (Smirnov 1968). Mitotic metaphase chromosome plates were studied using an AxioStar microscope and photographed using an Axio Imager A1 microscope with AxioVision 4.7 software and AxioCam MRc5 CCD-camera (Carl Zeiss, Germany) at 1000× magnification in the Laboratory for Ecology, Genetics and Environmental Protection (Ecogene), National Research Tomsk State University (Tomsk, Russia). KaryoType software (Altinordu et al. 2016) was used for karyotyping, and Adobe Photoshop CS5 (Adobe Systems, USA) were used for image editing.

Karyotype formulas were derived based on measurements of the photographed mitotic metaphase chromosomes with a similar degree of condensation that was estimated from the total haploid length of the chromosome set. The symbols used to describe the karyotypes corresponded to those coined by Levan et al. (1964). Mean values of arm ratio (r), centromeric indices (CI), mean chromosome length (CL), and relative chromosome length (RL) for each chromosome pair, and mean total haploid length (THL) were determined. In addition, we calculated Coefficient of Variation of Chromosome Length (CVCL; Paszko 2006), Coefficient of Variation of Centromeric Index (CVCI; Paszko 2006), and Mean Centromeric Asymmetry (MCA; Peruzzi & Eroğlu 2013).

DNA extraction, PCR amplification and sequencing

Five specimens of *Tulipa* were studied using molecular genetic techniques. DNA was extracted from dried tulip leaves (0.5 cm²) by the method of Doyle & Doyle (1990).

The PCR amplification was performed in a 20 µl reaction mixture with 80–100 ng of DNA using BioMaster HS-Taq PCR Kit (Biolabmix, Russia) according to manufacturer's instruction.

The ITS1–5.8S–ITS2 region was amplified by PCR and sequenced with forward and reverse with primers ITS5, ITS4 (White et al. 1990). The PCR amplification settings include initial denaturation for 3 minutes at 95°C followed by 30 cycles: 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min. Nucleotide sequences were analyzed and assembled using BioEdit 7.0.5.3 program (Hall 1999). Comparison search for nearest homologues was performed using the BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The nucleotide sequences of ITS1–ITS2 obtained in this study were submitted to the GenBank database and available under the accession numbers *T. uniflora*: OP630729 – **I**, OP630730 – **N.N**; *T. mongolica*: OP532978, OP532980 – **M.B**, OP532982 – **Z.T**.

Phylogenetic analysis

Phylogenetic reconstruction of the genus *Tulipa* was based on the ITS1–5.8S–ITS2 region. The alignment consists of 71 sequences of 585 nucleotides in length. The best substitution model TrN+I+G for the dataset was calculated by JModelTest 2.1.10 (Darriba et al. 2012).

The evolutionary history was inferred using the Bayesian reconstruction and Maximum Likelihood (ML) methods. The Markov chain Monte Carlo (MCMC) calculations were carried out in triplicates using MrBayes 3.2.6 (Ronquist et al. 2012). Analysis was run until a stable value of ESS statistics (300 units) was achieved. ML tree was calculated using Mega X (Kumar et al. 2018). Bootstrap test was run for 500 replicates.

RESULTS AND DISCUSSION

Morphological analysis, ecological and biological features of studied species

Morphological analysis showed that studied plants in populations in Southern Siberia, previously reported under the name *T. heteropetala* (Vlasova 1987, Stepanov 2012), are large individuals of *T. uniflora*. In our opinion, their morphological traits are explained by edaphic conditions of the habitat. These populations are confined to leached chernozems with a high content of organic matter (Table 1). Typical specimens of *T. uniflora* are smaller and occur on open dry slopes, in xerophilous herb communities confined to soddy calcareous soils or carbonized chernozems. We noted that butterflies *Aglais urticae* (Linnaeus, 1758) (**A.S**) and *Papilio machaon* Linnaeus, 1758 (**L** – Fig. 2) participate in cross-pollination of *T. uniflora*. Edificators of communities with *T. uniflora* in the eastern part of the range (**B, H, I, G, L, and O**) are *Potentilla acaulis* L. and in the western part (**A, A.P, A.S, K.1, K.2, N.N, P, S, T, V.K, and V.U**) – *Coluria goeides* (Pall.) Ledeb. *T. mongolica* grows on non-calcareous chestnut soils in shrubby forb-feather grass steppes along slopes (Table 1).

Results of one-way ANOVA for morphometric parameters show significant differences between populations (p -value < 0.05). The total data set was divided into four clusters with a high degree of differences. The selected clusters group with a small overlap (Fig. 3).



Figure 2 Participation of *Papilio machaon* Linnaeus, 1758 in cross-pollination of *Tulipa uniflora*

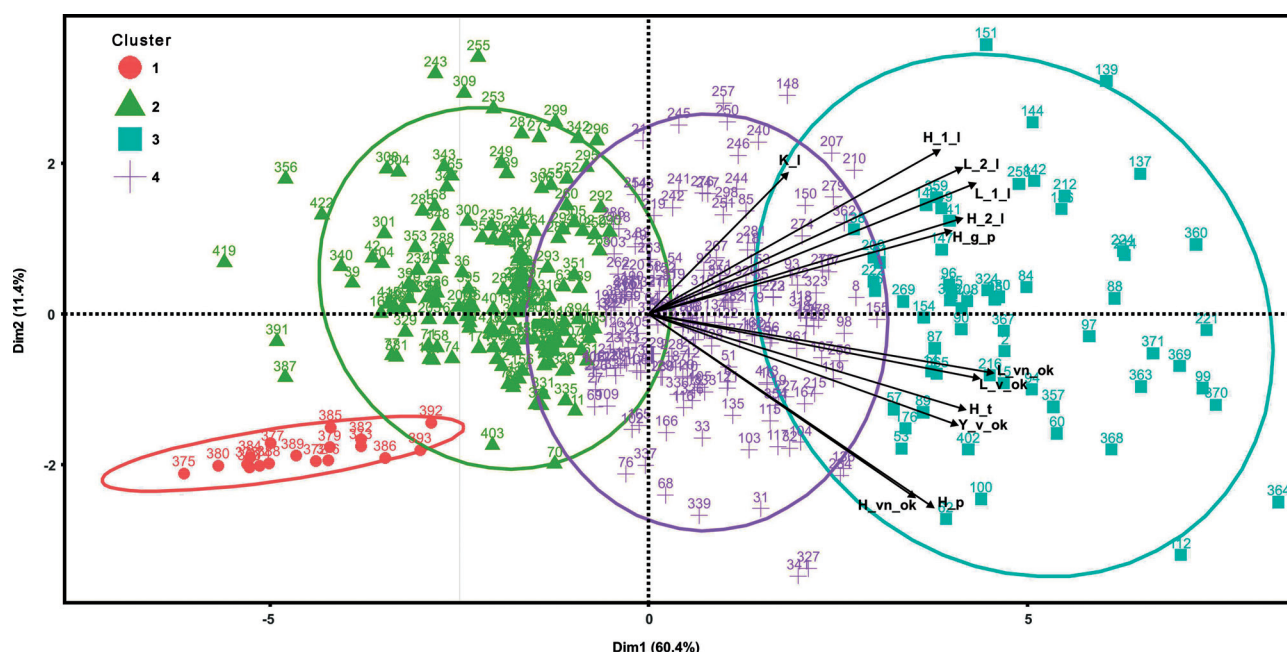


Figure 3 Scatterplot of the first two principal components. Ellipses show point clouds of clusters at 95 % confidence interval combining K-means method derived data of plants with similar morphometric parameters. Vectors show the directions of gradient changes of the studied morphometric parameters.

Clusters are distinguished by a combination of dimensional parameters of individuals. An increase in dimensional characteristics for all studied traits occurs from 1, 2, and 4 to the 3 cluster. Cluster 3 mainly contains the largest plants, it includes individuals previously identified as *T. heteropetala*. Cluster 1 includes individuals of *T. mongolica*, which differ by the smallest values of the studied traits. These two clusters are not numerous in terms of the number of populations they combine (Fig. 4).

On the scatterplot, the vectors of gradient changes of morphometric parameters can be divided into two groups (Fig. 3). The first group of co-directed vectors combines parameters of the number of leaves (K_l), the width (H_{1_l} , H_{2_l}) and the length (L_{1_l} , L_{2_l}) of the first and second leaves and the height of the generative shoot (H_{g_p}). All these traits show a direct correlation and an increase in the value of one of them is mostly accompanied by an increase in the rest. The second group of vectors combines parameters of the length (L_{vn_ok}) and the width (H_{vn_ok}) of the inner and outer (L_{v_ok} , Y_{v_ok} , respectively) perianths, pistil height (H_p) and stamen height (H_t). There is also direct correlation between them. The vectors between these two groups are connected by a positive but less pronounced correlation. Our data on the correlation of morphological parameters are consistent with the results of Mukhametshina et al. (2014) obtained for the South Ural populations of *T. sylvestris* subsp. *australis* (Link) Pamp. (incl.: *T. biebersteiniana* Schult. & Schult.f., *T. patens* C.

Agardh, and *T. riparia* Knjaz., Kulikov & E.G. Philippov).

The noted variability of morphometric traits in tulips, on the one hand, is explained by their relict nature, and on the other hand, it is the result of anthropogenic impact on their populations. In habitats with a high degree of pasture digression (Table 1), plants show various adaptations to unfavorable conditions, such as an increase in the depth of the bulb, a decrease in the height of the generative shoot and the entire vegetative sphere. At the same time, the morphometric traits of the generative sphere remain stable, since the flower plays an exceptional role as a special morphological structure, due to the fact that it completely combines all the processes of asexual and sexual reproduction.

Karyotypes structure

Most of *Tulipa* species have the same somatic chromosome number $2n = 2x = 24$ although there are polyploid races. Karyotype structure was studied in detail for many of them (Woods & Bamford 1937, Masoud et al. 2002, Zonneveld 2009, Qu et al. 2018). However, tulips native to North Asia have not been investigated in this regard. We have studied karyotypes (chromosome sets) of two specimens of *T. mongolica* and *T. uniflora* from Trans-Baikal Territory and Irkutsk Region, respectively. The karyotypes were similar in basic karyomorphological parameters (Table 2). The somatic chromosome numbers were $2n = 2x = 24$ in the both specimens. The chromosome sets included submeta-

Table 2. The karyotype parameters in *Tulipa mongolica* and *T. uniflora*

Species	PL	$2n$	Karyotype formula	THL (μm)	r	RL (%)	CV _{CL}	CV _{CI}	M_{CA}
<i>T. mongolica</i>	$2x$	24	8sm + 6sm/st + 10st	71.98(5.36)	2.06–4.27	3.09–5.27	16.09(1.55)	17.31(1.70)	48.68(1.44)
<i>T. uniflora</i>	$2x$	24	14sm + 10st	73.81(0.65)	2.02–4.14	3.30–5.13	15.22(0.73)	19.22(0.28)	45.78(1.39)

Notes: PL – ploidy level, $2n$ – somatic chromosome number.



Figure 4 Bar graph shows the occurrence of plants with similar morphometric traits from different populations in clusters identified using the K-means method

centric and subtelocentric types of chromosomes (Fig. 5). The karyotype formula of *T. mongolica* was $2n = 2x = 8sm + 6sm/st + 10st$, while of *T. uniflora* $2n = 2x = 14sm + 10st$. We referred six chromosomes of *T. mongolica* to sm/st type because the mean values of their centromeric indices varied around 3. The lowest and highest values of arm ratio (r) and relative chromosome length (RL) were similar for both specimens. In addition, we calculated the levels of interchromosomal asymmetry via CVCL, intrachromosomal asymmetry via MCA, and heterogeneity in centromere position via CVCI. These karyotype parameters also had close values in *T. mongolica* and *T. uniflora*.

Chromosome sets of two other native to China species of the subgenus *Orithyia* (*T. heterophylla* and *T. sinkiangensis*) were previously described (Qu et al. 2018). The karyotype formula of *T. sinkiangensis* was $2n = 2x = 24 = 8sm + 16st$. Thus its chromosomes were submetacentric and subtelocentric types just as in the species we've studied. The level of interchromosomal asymmetry,

estimated via CVCL, was 17.30. It was close to the value of this parameter in *T. mongolica* and *T. uniflora*. The second species, *T. heterophylla*, had karyotype formula $2n = 2x = 24 = 4m + 10sm + 10st$. This chromosome set included

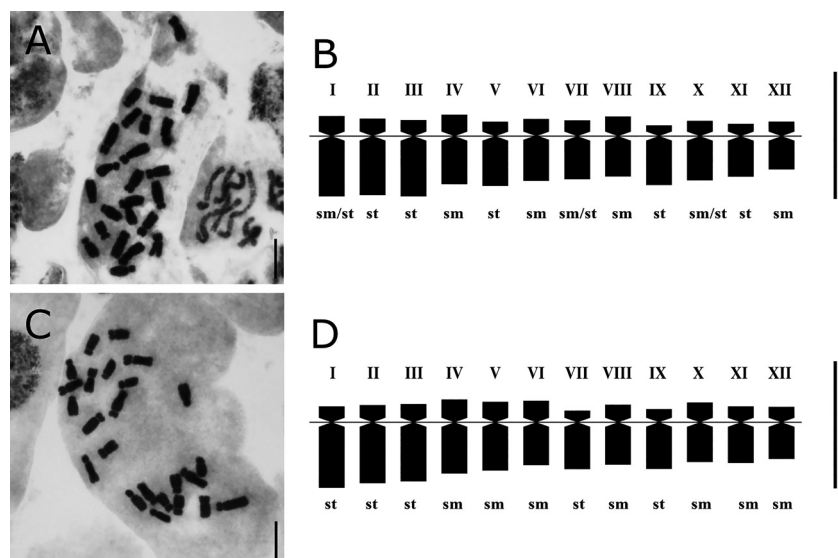


Figure 5 Mitotic metaphase plates and haploid idiograms of *Tulipa mongolica*, $2n = 24$ (A, B) and *T. uniflora*, $2n = 24$ (C, D). I–XII – chromosome pairs; sm – submetacentric chromosome; st – subtelocentric chromosome. – Scale bars = 10 μ m

four metacentric chromosomes, i.e., had a lower level of intrachromosome asymmetry than the other three. On the other hand, the CVCL value, which estimates the level of interchromosome asymmetry, was higher (23.45). Consequently, three species of the subgenus *Orithyia*, *T. mongolica*, *T. uniflora*, and *T. sinkiangensis*, had similar karyotype structures.

Molecular phylogenetic analysis

The molecular genetic analysis showed the distinctive clade of the subgenus *Orithyia* (bootstrap support BPs 98) among other subgenera of the genus *Tulipa* (Fig. 6). On the phylogenetic tree, studied specimens of *T. uniflora* clustered together within the *Orithyia* phylogenetic lineage. *T. hetero-*

phylla and two close related studied specimens of *T. uniflora* (accessions OP630729, OP630730) present a clearly distinct phylogenetic lineages. The studied *T. uniflora* specimens form a sister lineage (PP and BP support 1/100) to the only one available in GenBank ITS sequence of *T. uniflora* (HF952979). It is not possible to state that accession HF952979 belongs to *T. uniflora*, as there is no voucher accession and information about its geographic origin (Christenhusz et al. 2013, GenBank). Also, the nucleotide sequences of a typical *T. heteropetala* are not presented in GenBank, which complicates a phylogenetic analysis.

Analysed in this study sequences of *T. mongolica* formed another well supported (1/95) lineage which is sister to all of *T. uniflora*.

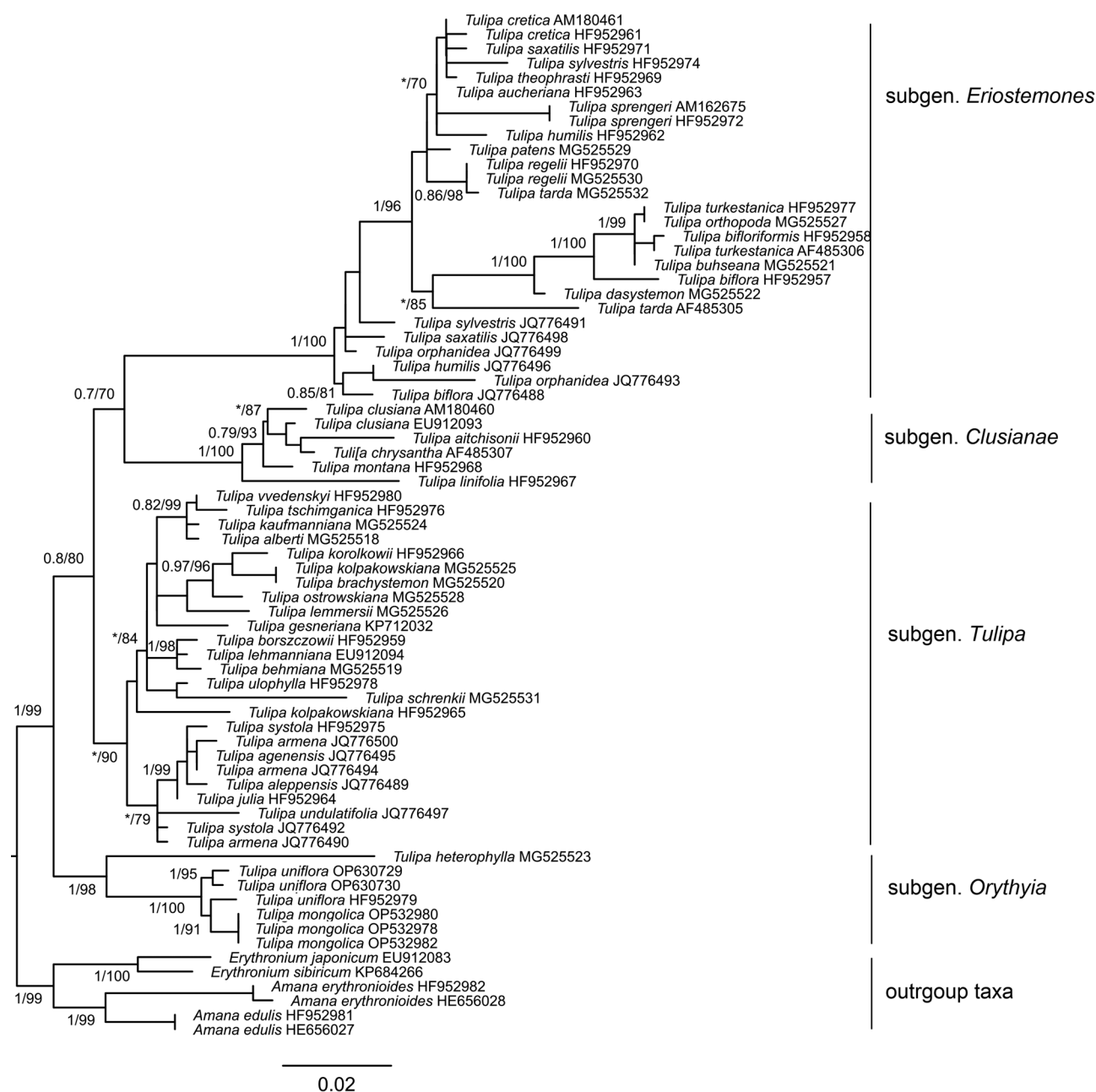


Figure 6 The ML phylogenetic tree of the genus *Tulipa* based on ITS1-ITS2 region, reconstructed using Maximum Likelihood method. Studied specimens (OP630729, OP630730) belong to the sister phylogenetic lineage in relation to the *Tulipa uniflora* lineage. Bootstrap values and posterior probabilities are given next to the nodes (BP/ML) (BP – Bayesian probabilities, ML – Maximum Likelihood). The scale bar indicates the number of nucleotide substitutions per site

The results presented are preliminary. It is necessary to use samples collected at the locus classicus to obtain reliable information on the phylogenetic relationships of tulips in the subgenus *Orithyia*.

CONCLUSION

Morphological analysis of *Tulipa* species of the subgenus *Orithyia* showed that studied plants from populations previously described in Southern Siberia under the name *Tulipa heteropetala* are large individuals of *T. uniflora*. There is a high level of morphological differences between all populations of *T. uniflora* and *T. mongolica*.

The analysis of karyotypes of tulips of the subgenus *Orithyia* showed that *Tulipa mongolica*, *T. uniflora*, and *T. sinianensis* have similar karyotype structures. In *T. heterophylla*, in comparison with other, a lower level of intrachromosomal asymmetry was noted.

Molecular genetic analysis confirms previous studies on the isolation of the subgenus *Orithyia* from other subgenera of the genus *Tulipa*. The taxonomic affiliation of specimens from the Trans-Baikal Territory (Russia) assigned to the species *T. mongolica* using morphological traits, is also confirmed by molecular data.

Thus, there are two species of tulips, *T. uniflora* and *T. mongolica* of the subgenus *Orithyia* in Southern Siberia of Russia.

ACKNOWLEDGEMENTS

We are grateful to N.V. Vlasova for valuable consultations, N.V. Stepanov for help with finding tulip locations in the Krasnoyarsk Territory, and T.A. Agafonova for identification of butterfly species.

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, the grant 075-15-2020-787 for implementation of Major scientific projects on priority areas of scientific and technological development (the project "Fundamentals, methods and technologies for digital monitoring and forecasting of the environmental situation on the Baikal natural territory").

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