

Iris tibetica, a new combination in *I. ser. Lacteae* (Iridaceae) from China: evidence from morphological and chloroplast DNA analyses

EUGENY V. BOLTENKOV^{1,2*}, ELENA V. ARTYUKOVA³, MARINA M. KOZYRENKO³ & ANNA TRIAS-BLASI⁴

¹Botanical Garden-Institute, Far Eastern Branch, Russian Academy of Sciences, ul. Makovskogo 142, 690024 Vladivostok, Russian Federation; e-mail: boltenkov@rambler.ru

²Komarov Botanical Institute, Russian Academy of Sciences, ul. Prof. Popova 2, 197376 St. Petersburg, Russian Federation

³Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, prospect 100-letiya Vladivostoka 159, 690022 Vladivostok, Russian Federation

⁴Royal Botanic Gardens, Kew, Richmond TW9 3AE, U.K.

*author for correspondence

Abstract

Historically, the species composition of *Iris* ser. *Lacteae* has been controversial. Morphological and molecular analyses have been conducted here including specimens covering most of their distribution range. The results suggest *I. ser. Lacteae* includes three species: the well-known *I. lactea* and *I. oxypetala*, plus a newly defined taxon which is endemic to the Gansu and Qinghai provinces, China. We here propose it as a new combination at the species rank, *I. tibetica*. Morphologically, this species is close to *I. lactea* but differs by its horizontal, creeping rhizome, scapes with no more than two flowers, its bracts reach the middle of the first flower, its broader inner perianth segments, its obovate with obtuse apex outer perianth segments, and its fruit apex always abruptly narrowed to a very short beak. The most useful qualitative and quantitative morphological characters used to distinguish this species are highlighted. Additionally, all three names are here typified, and a neotype for the name *I. lactea* and a lectotype for the name *I. tibetica* are designated. Notes on distribution and habitat are provided for all the accepted species.

Key words: chloroplast DNA, diagnostic key, molecular phylogeny, taxonomy

Introduction

Iris Linnaeus (1753: 38), when considered in a wide sense (Mathew 1989, Goldblatt *et al.* 2008), is one of the most important genera within the family Iridaceae as it contains the largest number of species, is of high ornamental value, and there is much interest in its taxonomy (Goldblatt *et al.* 2008). Some critical groups of *Iris* are in need of a modern taxonomic revision. One of these groups is *I. ser. Lacteae* Doronkin (1990: 412), the taxonomic distinctiveness of which is indisputable, while the species composition has long been controversial. Initially, it was recognized as *I. subg. Eremiris* Spach (1846: 32), then *I. subsect. Ensatae* Diels (1930: 502), *I. ser. Ensatae* (Diels) Lawrence (1953: 362), and then as the genus *Eremiris* (Spach) Rodionenko (2006: 1707), a generic treatment recently supported by Crespo *et al.* (2015). The species in *I. ser. Lacteae* are perennial herbaceous rhizomatous plants widely distributed in Asia, ranging from the Fergana Valley (Central Asia) in the west to the Korean Peninsula in the east, and from southern Siberia to Tibet and northern India. Traditionally and confirmed in the present study, these plants are distinguished from other irises by the short rhizome densely clothed with leaf remains, the numerous long adventitious roots, the unbranched stem, the ribbed leaves, tough, the extremely short perianth tube, measuring up to 3 mm in length, the long, narrow ovary, and the cylindrical fruit with six equidistant ribs and born on a long pedicel.

According to Grubov (1970), species in *I. ser. Lacteae* were repeatedly described and confused with each other by many authors. Originally, *I. lactea* Pallas (1776: 713) was described from southeastern Siberia. This species was for a long time cited under different names, mostly as *I. ensata* Thunberg (1794: 328). However, this binomial belongs to another species within *I. ser. Laevigatae* (Diels 1930: 502) Lawrence (1953: 361). This is largely due to the taxonomic

confusion admitted by Baker (1876). He erroneously cited *I. lactea* as *I. ensata*, and the latter species was included under the name *I. kaempferi* Siebold ex Lemaire (1858: t. 157), as a synonym of *I. laevigata* Fisch. in Fischer & Meyer (1839: 36). Although Maximowicz (1880) questioned that the names *I. ensata* and *I. lactea* may belong to the same species, he kept the name *I. ensata* for both species. Unfortunately, this taxonomic misunderstanding became entrenched in subsequent works (Lynch 1904, Dykes 1912, 1924, Krylov 1929, Fedtschenko 1935, Lawrence 1953, Sergievskaya 1972) and despite Koidzumi (1925) corrected this mistake, many authors have not taken this into account until now (Akhter *et al.* 2012, Andrabi *et al.* 2015). For this reason, the *I. ser. Lacteae* specimens are often erroneously identified as *I. ensata* in some herbaria.

In the present study, some 14 taxa at the species rank were assigned to *I. ser. Lacteae*. Six taxa were described based on material from Siberia: *I. lactea*, *I. biglumis* Vahl (1805: 149), *I. caricifolia* Pall. ex Link (1820: 72), *I. haematophylla* Fisch. ex Link (1821: 60), *I. pallasii* Fisch. ex Treviranus (1821: without pagination), and *I. doniana* Spach (1846: 34). *Iris triflora* Balbis (1804: 6) and *I. longispatha* Fisch. ex Sims (1825: t. 2528), *nom. illeg.* were described based on cultivated plants. Other taxa were described from northern India: *I. longifolia* Royle ex D. Don in Royle (1839: 372), *I. fragrans* Lindley (1840: t. 1), *nom. illeg.*, *I. moorcroftiana* Wall. ex Don (1840: 315), and *I. pabularia* Naudin ex Ermens in Grisard (1888: 698). *Iris oxypetala* Bunge (1833: 63) was described from northeastern China and *I. iliensis* Poljakov (1950: 88), from southeastern Kazakhstan.

Some authors (Dykes 1912, 1924, Grubov 1970, Tscherneva 1971, Rodionenko 2006) have made an important contribution to the understanding of the *I. ser. Lacteae* taxonomy. They found that the difficulties in delimitation and interpretation of species are due to morphological variability related to environmental factors. Many authors recognised only a single polymorphic species, *I. lactea* (Mathew 1989, Waddick & Zhao 1992, Zhao *et al.* 2000, Blanco White 2012), while other authors (Tscherneva 1971, Grubov 1977, Rodionenko 2006) recognised two species, *I. lactea* and *I. oxypetala*. The latter taxon was placed by Ledebour (1833) in synonymy of *I. pallasii* var. *chinensis* Fisch. ex Sims (1822). For this reason, *I. oxypetala* was not accepted in some works (Zhao 1980, Lee 1996), and was cited for China and Korea under the name *I. lactea* var. *chinensis* (Fisch. ex Sims) Koidzumi (1925: 300) or under the name *I. lactea* subsp. *chinensis* (Fisch. ex Sims) Kitagawa (1940: 115).

For evolutionarily young and closely related groups of plants, species delimitation and establishment of their relationships are often very complicated (Schanzer 2013). In the past decades, molecular techniques have been widely applied to analyse genetic diversity in species, including some *Iris* taxa (e.g., Artyukova *et al.* 2001, Lamote *et al.* 2002, Al-Gabbiesh *et al.* 2006, Wróblewska & Brzosko 2006, Meerow *et al.* 2007, Kostrakiewicz & Wróblewska 2008, Wang *et al.* 2009) and to distinguish between taxa at different taxonomic levels, including taxa with no morphological differences in the characters usually used for species delimitation (e.g., Cornman & Arnold 2007, Kozyrenko *et al.* 2009).

A recent phylogenetic study of *I. ser. Lacteae* using three chloroplast DNA (cpDNA) regions has shown (Boltenkov *et al.* 2016) that at least two genetically distinct species occur in Russian Federation and adjacent countries: *I. lactea* in Kazakhstan, Mongolia, and Siberia and *I. oxypetala* in the Russian Far East. In this study, an extended sampling of irises was used from habitats corresponding to the distribution ranges of almost all the previously described taxa of *I. ser. Lacteae*, to further clarify their taxonomy and distribution. Our specific objectives were (1) to study the morphological characters of living plants and herbarium specimens from the geographical range of *I. ser. Lacteae*; (2) to resolve the phylogenetic relationships within *I. ser. Lacteae* using four cpDNA regions; (3) to assess the levels of cpDNA sequence divergence within *I. ser. Lacteae* as compared to species of other series in *I. sect. Limniris* Tausch (1823: without pagination); and (4) to collate the results of morphological and molecular studies to determine the number of taxa in *I. ser. Lacteae*.

Materials and Methods

Morphological analysis:—The study is based on an analysis of relevant species descriptions available in the taxonomic literature (Krylov 1929, Sergievskaya 1972, Doronkin 1987, Pavlova 1987) and a revision of all the herbarium specimens (including the original material for the names studied) belonging to *I. ser. Lacteae*, kept at E, IRK, K, LE, MHA, MW, NS, NSK, TASH, TK, and VBG (acronyms according to Thiers 2017). Some specimens were examined as digital images (available at <http://www.cvh.org.cn/> and <http://pe.ibcas.ac.cn/>). When possible, data were obtained from living specimens, either cultivated or collected from wild populations. The following 15 characters were examined in all the species: plant height (cm), rhizome morphology, flowering stem height (cm), number of cauline leaves, number and

colour of flowers, bract length (cm), pedicel length (cm), length of outer (falls) and inner (standards) perianth segments (cm), width of falls and standards (cm), shape of fall blade, length of fruit, and fruit apex morphology.

DNA extraction, amplification and sequencing:—One hundred thirty-six specimens of *I. ser. Lacteae* from 59 different locations in Asia (Fig. 1, Table 1) were available for our study, including 92 samples (both DNA and sequence data) previously presented (Boltenkov *et al.* 2016). Our sampling covering almost all the geographical range (China, India, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, and Russian Federation) of *I. ser. Lacteae* allows testing differentiation within this group. Our sampling also comprised 13 outgroup specimens, including: four specimens of *I. uniflora* Pall. ex Link (1920: 71) from *I. ser. Ruthenicae* (Diels 1930: 502) Lawrence (1953: 360), three specimens of *I. sibirica* Linnaeus (1753: 39), and a single specimen each of *I. bulleyana* Dykes (1910: 418) and *I. wilsonii* Wright (1907: 321) from *I. ser. Sibiricae* (Diels 1930: 501) Lawrence (1953: 359), as well as two specimens of *I. ensata*, and a single specimen each of *I. laevigata* and *I. pseudacorus* Linnaeus (1753: 38) from *I. ser. Laevigatae*. The complete specimen list, including the sampling locations and the voucher information is given in Table 1. Methods for DNA extraction, amplification, and direct sequencing of non-coding cpDNA regions (the *trnS–trnG*, *trnL–trnF*, *rps4–trnS^{GGA}*, and *psbA–trnH*) have been presented elsewhere (Kozyrenko *et al.* 2004, 2009, Boltenkov *et al.* 2016). The research was done using equipment of the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Center of the East Asia Terrestrial Biodiversity (Vladivostok, Russian Federation). Forward and reverse sequences were determined with an ABI PRISM 310 sequencer (Applied Biosystems, USA) and assembled using the Staden Package v. 1.4 (Bonfield *et al.* 1995) for each locus and individual. All DNA fragments that contained substitutions and/or microsatellite variants were retested (reamplified and resequenced) to verify that our results were repeatable. The sequences were deposited in the EMBL/GenBank database (accession numbers are available in Table 1).

TABLE 1. List of sampled *Iris* specimens with data including locality codes (number of analyzed individuals and haplotypes defined using the combined sequences of four cpDNA regions), locality and voucher information (herbarium acronyms according to Thiers 2017), and GenBank accession numbers for each particular cpDNA region. The GenBank numbers in bold are new submissions. Abbreviation: cult., cultivated.

Code	Locality, voucher	GenBank accession numbers			
		<i>trnL–trnF</i>	<i>trnS–trnG</i>	<i>rps4–trnS</i>	<i>psbA–trnH</i>
<i>I. ser. Lacteae</i>					
AEM (1; AEM)	Kazakhstan, Mynbulak, <i>Rae et al.</i> 66 (E)	LN871639	LN871674	LN871718	LT627882
AKB (1; AKB)	Kazakhstan, Akmola, <i>Isachenko</i> 3548 (LE)	LN871636	LN871672	LN871716	LT627883
ALB (1; H12)	Russian Federation, Altai Krai, Barnaul, <i>Ignatov s.n.</i> (MHA)	LN871635	LN871671	LN871715	LT627859
ALK (1; ALK)	Russian Federation, Altai Republic, Kokorya, <i>Shaulo s.n.</i> (NS)	LT627921	LT627985	LT627960	LT627860
ALM (1; H1)	Kazakhstan, Chilik, <i>Vickery</i> KZ5 (cult. K)	LT627905	LT627969	LT627944	LT627838
ALT (10; H7–H10)	Russian Federation, Altai Republic, Aktal, <i>Doronkin s.n.</i> (NSK)	LN871630– LN871634	LN871666– LN871670	LN871710– LN871714	LT627863
ARK (1; H4)	Mongolia, Arkhangai, <i>Tevshruleh, Gubanov</i> 349 (MW)	LN871621	LN871659	LN871703	LT627872
BAY (1; BAY)	Mongolia, Bayan-Ulgii, Ulgii, <i>Krasnoborov et al.</i> 1018 (NS)	LT627924	LT627988	LT627963	LT627878
BEI (1; BEI)	China, Beijing, <i>Guofeng s.n.</i> (cult. PE)	LT627913	LT627977	LT627952	LT627846
BSH (6; H1)	Kyrgyzstan, Beshkent, <i>Popova s.n.</i> (IRK)	LT627906	LT627970	LT627945	LT627839
BUG (1; H3)	Mongolia, Bulgan, Uubulan, <i>Holubec</i> 696 (cult. K)	LT627925	LT627989	LT627964	LT627879
BUL (1; BUL)	Mongolia, Bulgan, <i>Neronov</i> 108 (MW)	LN871619	LN871657	LN871701	LT627876
BUR (11; H3–H6)	Russian Federation, Buryatia, Saratovka, <i>Verkhovina & Krivenko</i> 282 (IRK)	LN871611	LN871646– LN871649	LN871690– LN871693	LT627866
EK1 (2; H7, EK1B)	Kazakhstan, Sarybulak, <i>Karamysheva et al.</i> 761 (LE)	LN871628	LN871673, LN871679	LN871717	LT627884

...continued on the next page

TABLE 1. (Continued)

Code	Locality, voucher	GenBank accession numbers			
		<i>trnL–trnF</i>	<i>trnS–trnG</i>	<i>rps4–trnS</i>	<i>psbA–trnH</i>
GAG (1; GAG)	Mongolia, Govi-Altai, Taishir, <i>Gubanov 7258</i> (MW)	LN871620	LN871658	LN871702	LT627877
GAN (1; H1)	China, Gansu, Tianshui, <i>Petrov s.n.</i> (LE)	LT627908	LT627972	LT627947	LT627841
GAS (1; GAS)	China, Gansu, Jonê, <i>s.coll. 100</i> (E)	LT627930	LT627995	LT627941	LT627890
GUS (1; H1)	China, Gansu, Wenxian, <i>s.coll. 444</i> (cult. E)	LT627909	LT627973	LT627948	LT627842
HEI (1; HEI)	China, Heilongjiang, Harbin, <i>Skvortsov 1112</i> (LE)	LT627927	LT627991	LT627966	LT627881
ILI (1; ILI)	Kazakhstan, Iliisk, <i>Pavlov s.n.</i> (MW)	LN871627	LN871675	LN871719	LT627886
IMD (1; IMD)	China, Inner Mongolia, Hailar, <i>Livanov s.n.</i> (LE)	LT627919	LT627983	LT627958	LT627852
IMH (1; IMH)	China, Inner Mongolia, Wuchuan, <i>Petrov s.n.</i> (LE)	LT627918	LT627982	LT627957	LT627851
IND (1; H1)	India, Sajwar, Jalori Pass, <i>Klyuykov s.n.</i> (cult. MW)	LT627915	LT627979	LT627954	LT627848
INK (1; H1)	India, Jammu-Kashmir, <i>Bohme 155</i> (cult. K)	LT627916	LT627980	LT627955	LT627849
KH1 (1; H1)	Russian Federation, Primorsky Krai, Gvozdevo, <i>Kozhevnikov 669-9</i> (LE)	LN871609	LN871644	LN871688	LT627836
KH2 (1; H1)	Russian Federation, Primorsky Krai, Cape Marble, <i>Kozhevnikov 663-1</i> (LE)	LN871610	LN871645	LN871689	LT627837
KHA (1; KHA)	Russian Federation, Khakassia, Abakan, <i>s.coll. s.n.</i> (cult. MHA)	LN871618	LN871656	LN871700	LT627861
KHK (9; KHK)	Russian Federation, Khakassia, Chests Mountains ridge, <i>Erst s.n.</i> (NS)	LT627922	LT627986	LT627961	LT627862
KIR (2; H1)	Kyrgyzstan, Uzbekli, <i>Tkachenko s.n.</i> (LE)	LT627907	LT627971	LT627946	LT627840
KRA (1; KRA)	Russian Federation, Zlatorunovsk, <i>Poluboyarova s.n.</i> (VBGI)	LN871613	LN871651	LN871695	LT627858
ORD (1; H1)	China, Inner Mongolia, Ordos, <i>s.coll. s.n.</i> (LE)	LT627910	LT627974	LT627949	LT627843
ORK (1; H17)	Mongolia, Ovorkhangai, Kharkhorin, <i>Galanin & Belikovich s.n.</i> (VBGI)	LN871624	LN871663	LN871707	LT627873
PAK (1; H1)	Pakistan, Gilgite-Hunza, Pisan, <i>s.coll. 6</i> (cult. E)	LT627917	LT627981	LT627956	LT627850
PAS (1; PAS)	Pakistan, Gilgit-Baltistan, Yasin, <i>Stober GS8</i> (K)	LT627928	LT627992	LT627967	LT627887
POS (13; H1)	Russian Federation, Primorsky Krai, Posiet, <i>Boltenkov s.n.</i> (VBGI)	LN871606	LN871642	LN871686	LT627834
PPA (2; H4)	Russian Federation, Buryatia, Petropavlovka, <i>Kalinovich s.n.</i> (IRK)	LN871612	LN871650	LN871694	LT627867
QAN (1; QAN)	China, Qinghai, Daewei, <i>s.coll. 47</i> (E)	LT627914	LT627978	LT627953	LT627847
QHA (1; QHA)	China, Qinghai, Qingsha Shan, <i>s.coll. 34</i> (E)	LT627931	LT627996	LT627942	LT627891
QHU (1; H18)	China, Qinghai, Riyue Xiang, <i>Long et al. 60</i> (E)	LT627932	LT627997	LT627943	LT627892
QIH (1; QIH)	China, Qinghai, Xining to Ta Er, <i>Long et al. 3</i> (E)	LT627933	LT627998	LT627939	LT627893
QIN (1; H18)	China, Qinghai, Gonghe, <i>s.coll. s.n.</i> (cult. E)	LT627934	LT627998	LT627939	LT627894
SGB (1; H4)	Mongolia, South Gobi, Bayan-Dalai, <i>Guricheva & Rachkovskaya 2452</i> (LE)	LN871629	LN871677	LN871721	LT627868
SGN (1; SGN)	Mongolia, South Gobi, Nomgon, <i>Gubanov 5458</i> (MW)	LN871622	LN871660	LN871704	LT627870

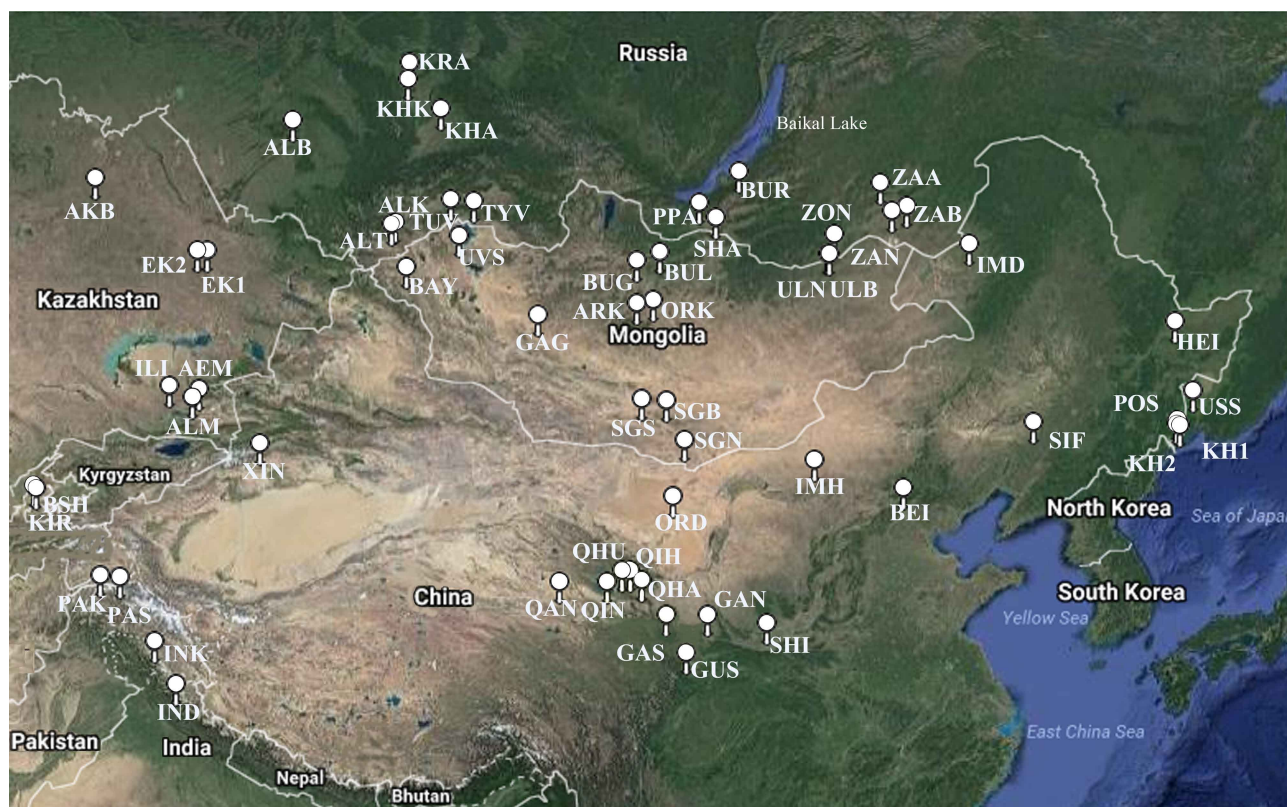
...continued on the next page

TABLE 1. (Continued)

Code	Locality, voucher	GenBank accession numbers			
		<i>trnL–trnF</i>	<i>trnS–trnG</i>	<i>rps4–trnS</i>	<i>psbA–trnH</i>
SHA (1; H17)	Mongolia, Selenge, Shamar, <i>Zaslavskaya s.n.</i> (LE)	LN871638	LN871678	LN871722	LT627869
SHI (1; H1)	China, Shaanxi, Suyde, <i>Kabanov s.n.</i> (LE)	LT627911	LT627975	LT627950	LT627844
SYF (1; H1)	China, Liaoning, Shenyang forest, <i>Li s.n.</i> (cult. K)	LT627912	LT627976	LT627951	LT627845
TUV (1; TUV)	Russian Federation, Tuva, Solchur, <i>Korotkova & Kovaleva 446</i> (NS)	LT627923	LT627987	LT627962	LT627865
TYV (2; TYVA, TYVB)	Russian Federation, Tuva, Ak-Chyr, <i>Timokhina & Amelchenko 1770</i> , (MW)	LN871623	LN871661	LN871705, LN871706	LT627864
ULB (1; ULB)	Mongolia, Dornod, Bayan-Uul, <i>Galanin s.n.</i> (VBGI)	LN871615	LN871653	LN871697	LT627874
ULN (1; ULB)	Mongolia, Khentii, Norovlin, <i>Belikovitch s.n.</i> (VBGI)	LN871615	LN871654	LN871698	LT627875
USS (15; H1, USS2)	Russian Federation, Primorsky Krai, Putsilovka, <i>Boltenkov s.n.</i> (VBGI)	LN871607, LN871608	LN871643	LN871687	LT627835
UVS (1; UVS)	Mongolia, Uvs, Zel, <i>Holubec 862</i> (cult. K)	LT627926	LT627990	LT627965	LT627880
XIN (1; XIN)	China, Xinjiang, Baicheng, <i>Junatov 781</i> (MHA)	LT627929	LT627993	LT627968	LT627888
ZAA (1; ZAA)	Russian Federation, Zabaykalsky Krai, Dogoy, <i>Kuvaev 185-1</i> (MW)	LN871626	LN871665	LN871709	LT627856
ZAB (16; H7, H12–H14, H16)	Russian Federation, Zabaykalsky Krai, Kharanor, <i>Chernova s.n.</i> (IRK)	LN871625	LN871662, LN871664, LN871680– LN871683	LN871708	LT627854, LT627855
ZAN (1; ZAN)	Russian Federation, Zabaykalsky Krai, Novaya Zarya, <i>Roenko s.n.</i> (VBGI)	LT627920	LT627984	LT627959	LT627857
ZON (1; ZON)	Russian Federation, Zabaykalsky Krai, Verkhny Ulhun, <i>Galanin s.n.</i> (VBGI)	LN871614	LN871652	LN871696	LT627853
Outgroup specimens					
<i>I. ser. Laevigatae</i>					
<i>I. ensata</i>					
ZAR (2; ZARA, ZARB)	Russian Federation, Primorsky Krai, Zarubino, <i>Boltenkov s.n.</i> (VBGI)	LT628002	LT628022, LT628023	LT628012	LT627896
<i>I. laevigata</i>					
ROS (1; ROS)	Russian Federation, Primorsky Krai, Roshchino, <i>Pshennikova s.n.</i> (cult. VBGI)	LT628003	LT628024	LT628013	LT627897
<i>I. pseudacorus</i>					
VLA (1; VLA)	Russian Federation, Vladivostok, <i>Boltenkov s.n.</i> (cult. VBGI)	LT628004	LT628025	LT628014	LT627898
<i>I. ser. Ruthenicae</i>					
<i>I. uniflora</i>					
ANIS (1; ANIS)	Russian Federation, Primorsky Krai, Anisimovka, <i>Orlovskaya s.n.</i> (VBGI)	LN871604	LN871640	LN871684	LT627832
NAR (1; NAR)	Russian Federation, Primorsky Krai, Narvinsky Pass, <i>Mironova s.n.</i> (cult. VBGI)	LT628009	LT628030	LT628019	LT627903
SIN (1; SIN)	Russian Federation, Primorsky Krai, Sinelnikovo, <i>Vrzhosek s.n.</i> (cult. VBGI)	LN871605	LN871641	LN871685	LT627833
ZKY (1; ZKY)	Russian Federation, Kyrinsky District, <i>Vologdina s.n.</i> (VBGI)	LT628008	LT628029	LT628018	LT627902
<i>I. ser. Sibiricae</i>					
<i>I. bulleyana</i>					
ZHO (1; ZHO)	China, Yunnan, Zhongdian, <i>Pimenov et al. 432</i> (MW)	LT628001	LT628021	LT628011	LT627895
<i>I. sibirica</i>					

...continued on the next page

Code	Locality, voucher	GenBank accession numbers			
		<i>trnL-trnF</i>	<i>trnS-trnG</i>	<i>rps4-trnS</i>	<i>psbA-trnH</i>
ORL (1; ORL)	Russian Federation, Primorsky Krai, Orlovka, <i>Boltenkov s.n.</i> (VBGI)	LT628006	LT628027	LT628016	LT627900
ZOR (1; ZOR)	Armenia, Zorakert, <i>Fayvush et al. 09-1696</i> (ERE)	LT628007	LT628028	LT628017	LT627901
<i>I. wilsonii</i>					
DAL (1; DAL)	China, Sichuan, Daliang Shan, <i>s.coll. 1229</i> (E)	LT628010	LT628031	LT628020	LT627904



Data analysis:—The sequences of each cpDNA region were aligned manually with the program Sea View (Gouy *et al.* 2010) and concatenated for each specimen. Our dataset included length variation in mono- and dinucleotide repeats and indels because repeatability tests allowed us to exclude PCR errors. The haplotypes were identified based on combined DNA sequences using the DnaSP v.5 (Librado & Rozas 2009). Relationships among haplotypes were examined with the program Network 4.6 (Bandelt *et al.* 1999), using the median joining (MJ) algorithm with default settings. An 8-bp inversion within the *psbA–trnH* spacer and each deletion/insertion, regardless of their size were treated as a single mutational event. We used a Bayesian clustering approach implemented in the software package BAPS v. 6.0 (Corander *et al.* 2008) to identify subdivision of the cpDNA sequences of plants from all collection locations into genetically distinct groups/clusters (K). Using the codon linkage model implemented in the ‘Clustering with linked loci’ option, we carried out 10 repetitions of the algorithm for each K ranging between 2 to 59. Based on mixture clustering, an admixture analysis was performed in order to calculate proportions of admixture of each individual to each cluster. Evidence for admixture was considered significant for individuals with *P*-values < 0.05. The distribution of genetic variation within and among groups identified in MJ and BAPS analyses and pairwise distances (F_{ST}) between them were determined with AMOVA as implemented in the program Arlequin v. 3.5 (Excoffier & Lischer 2010). The significance of the variance components and genetic distances was tested using 10000 random permutations. The degrees of divergence between the clusters and haplogroups as well as between species in series *Sibiricae* and *Laevigatae* were calculated on the basis of nucleotide substitutions using the program DnaSP v. 5.0 (Librado & Rozas 2009). Phylogenetic analyses and tree construction were performed using maximum likelihood

(ML), neighbour joining (NJ), and maximum parsimony (MP) methods as implemented in the PAUP v. 4.0b10 software package (Swofford 2003) and using Bayesian inference (BI) with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The sequences of species from *Laevigatae*, *Ruthenicae*, and *Sibiricae* series were used as outgroup taxa. The ML and BI analyses were based on the sequence evolution model GTR + I + G, which was selected with Modeltest 3.6 (Posada & Crandall 1998). In BI, two parallel MCMC runs were carried out for ten million generations, sampling every 1000 generations for a total of 10000 samples. Convergence of the two chains was assessed, and the posterior probabilities (PP) were calculated from the trees sampled during the stationary phase. The robustness of nodes in ML, MP, and NJ trees was tested using the bootstrapping method with 1000 replicates (bootstrap percentage, BP). BP < 50% and PP < 0.95 were not taken into account.

Results

Genetic divergence within *I. ser. Lacteae*:—Four regions of cpDNA were amplified for *I. ser. Lacteae* specimens from 59 localities as well as for 13 specimens of seven species from other series in *I. sect. Limniris*. The total aligned length of the combined dataset for 4 cpDNA regions of the 136 *I. ser. Lacteae* specimens was 3834 bp, in which variations in length of mono- and dinucleotide repeats, short (1–2 bp) and multi-base indels, an inversion (8 bp) and 62 polymorphic sites were identified. Altogether, these variable positions defined 49 haplotypes, most of them (> 80%) were unique, i.e. found in a single individual. Only nine haplotypes were found in more than one individual, seven of which were shared between specimens from different localities which are far apart (ca. 900 km or more). Haplotype H1 is the most widespread and shared by the plants from the Russian Far East (KH1, KH2, POS, and USS), China (ORD, GAN, GUS, SHI, and SYF), India (IND and INK), Kyrgyzstan (BSH and KIR), Kazakhstan (ALM) and Pakistan (PAK). Haplotype H4 is found in both populations from Buryatia (PPA and BUR; Boltenkov *et al.* 2016) and in plants from two localities in Mongolia (ARK and SGB), while another haplotype from population BUR (H3) is shared with BUG from Mongolia. Haplotype H12 is found only in specimens from Siberia and is shared among samples from three localities: it is found in 12.5% plants from Zabaykalsky Krai (ZAB), in all plants from Khakassia Republic (KHK) and in specimen ALB from Altai Krai. Another common haplotype H7 is shared between populations from Siberian regions (Altai Republic, ALT; Zabaykalsky Krai, ZAB) that are distant from each other and is also found in EK1 from East Kazakhstan Region, Kazakhstan. In addition, two specimens from Qinghai, China (QHU and QIN) share a common haplotype (H18) as well as two specimens from Mongolia (ORK and SHA) have a single haplotype (H17).

The median joining network revealed three main haplogroups separated from each other by more than 20 mutational steps (Fig. 2). Two haplogroups (A and C) are located at the terminal tips of the network. Haplogroup A contained four haplotypes and was dominated by H1, the most common haplotype in our dataset, which is distributed along the southern part of the *I. ser. Lacteae* range, from the Russian Far East (KH1, KH2, POS, and USS) to the Fergana Valley (BSH and KIR). The other three haplotypes in this group (BEI, QAN, and USS2) differed by only one or two mutational steps from H1 and occurred in plants from the Russian Far East and China. Haplogroup C was found at another tip of the network and comprised four haplotypes from China. The haplotype GAS from Gansu differs in length of poly-TA repeat from H18, which is common for QIN and QHU specimens from Qinghai while the two other specimens from Qinghai (QIH and QHA) have divergent haplotypes separated from H18 by three and nine mutational steps, respectively. Haplogroup B occupies the inner part of the network and contains 38 haplotypes including all haplotypes from Mongolia, Siberia (except ZON), and Kazakhstan (except ALM), as well as HEI and IMD from China and PAS from Pakistan. Within haplogroup B, most haplotypes are separated from the neighbouring ones by a single mutational step and form a number of star-like structures, the centers of which occupy the haplotypes of population ZAB. Alternative connections between some of these haplotypes (loops in the network, not shown) point to homoplasy that hampers the identification of genetic relationships between them uniquely. Haplotypes IMH, XIN, and ZON are not part of any haplogroup, they are located at isolated positions and connected with the rest haplotypes in a single network by many mutational steps (from 9 to 14).

From the BAPS analysis, three genetic clusters were identified among the samples from 59 localities with posterior probability (PP) of 0.94 (Fig. 3A). Cluster 1 contains haplotypes of terminal haplogroup A and haplotypes ZON and IMH, which occupy isolated positions in the median-joining network (Fig. 2A). Cluster 3 includes haplotypes of terminal haplogroup C together with isolated haplotype XIN, while all haplotypes forming the haplogroup B in the median-joining network fell into the cluster 2. Admixture analysis showed that nearly every individual was unambiguously assigned to a respective cluster, and only four haplotypes (Fig. 3B) showed significant admixture ($P < 0.05$) among clusters 1 and 2 (IMH, PASS, and ZON) and among clusters 2 and 3 (XIN).

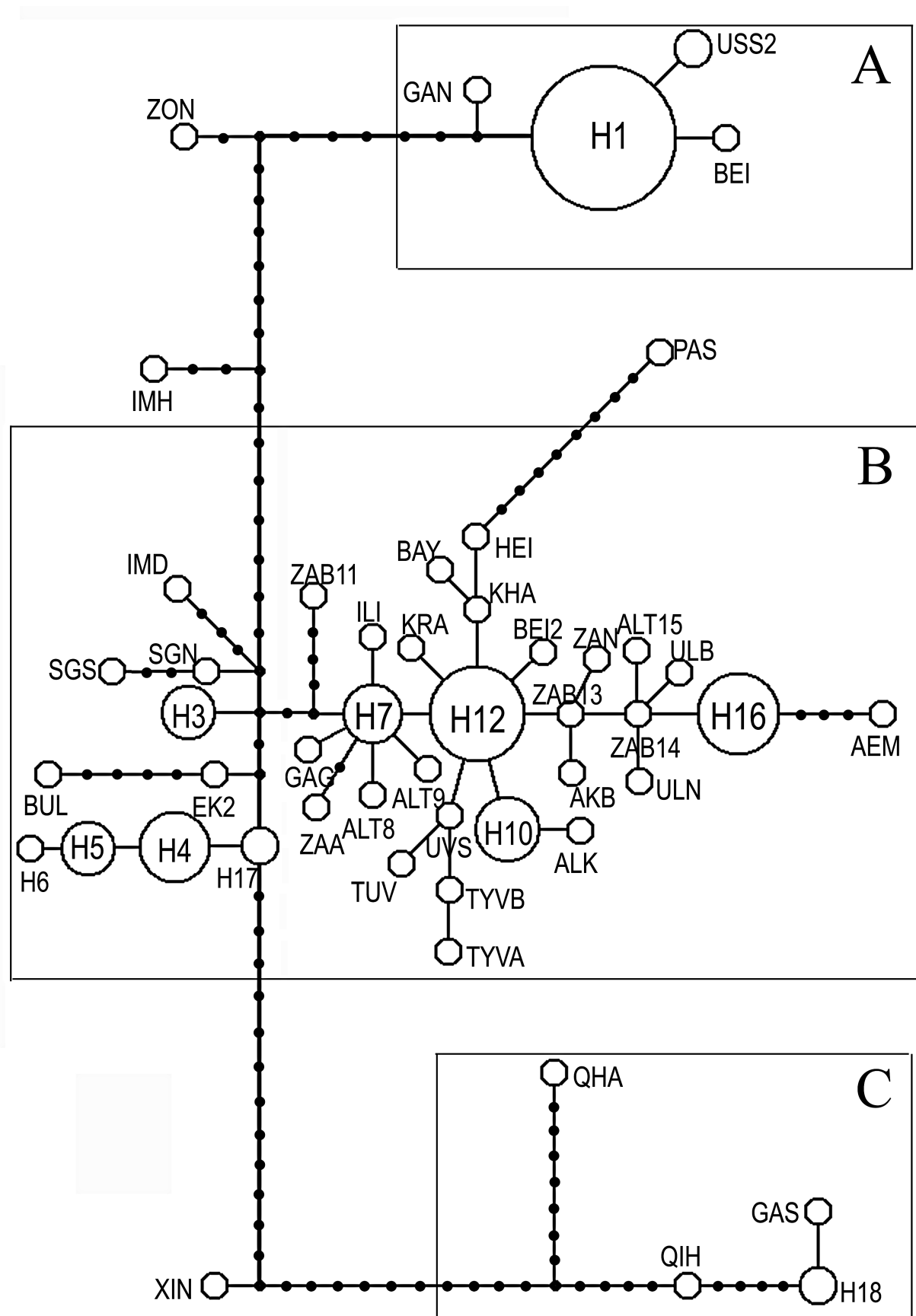


FIGURE 2. Median-joining network based on combined sequences of four cpDNA regions, depicting the relationships among haplotypes of 136 individuals from 59 localities across the geographic range of *Iris* ser. *Lacteae*. Each white circle represents a haplotype and the size of the circle is proportional to the number of individuals with that haplotype. Haplotype codes correspond to those in Table 1. Black dots indicate missing haplotypes (extinct or not sampled). Haplotypes of A, B, and C haplogroups are encircled with thin lines.

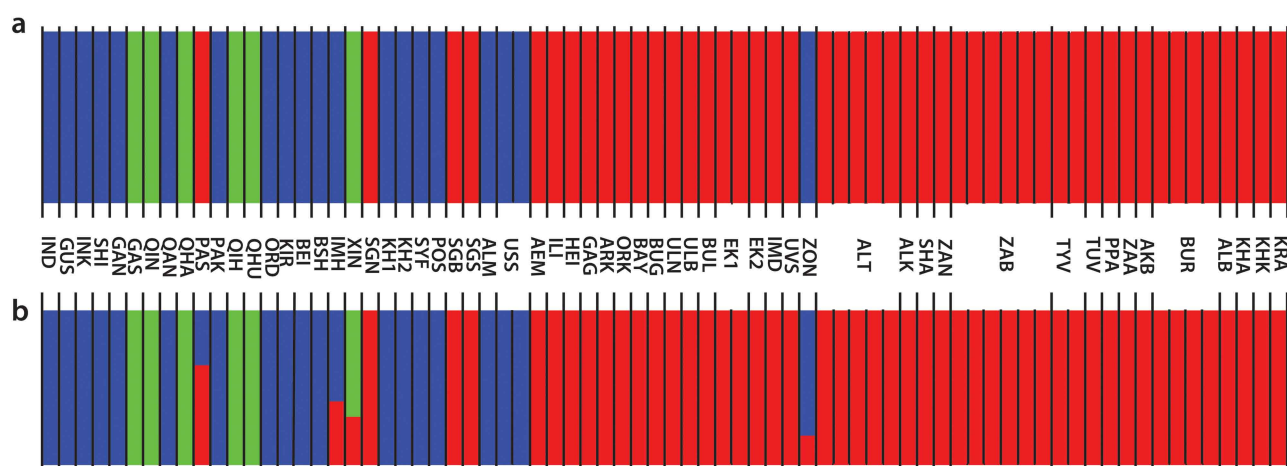


FIGURE 3. Genetic clustering of *Iris* ser. *Lacteae* haplotypes from 136 individuals inferred with the program BAPS. Each vertical column corresponds to one haplotype, and black vertical lines separate haplotypes from the different localities. Locality codes are given in Table 1. Different colours represent the different genetic cluster (blue-cluster 1, red-cluster 2, green-cluster 3). (a) Mixture clustering results revealing three clusters with a posterior marginal probability of 0.80; (b) Bar plot from the admixture results. The vertical bars are split into several colours when there is evidence for the admixture ($P < 0.05$). Localities are ordered according to latitude, from south to north.

Phylogenetic reconstruction methods (MP, NJ, ML, and BI) all resulted in basically similar topologies with few differences in statistical support (Fig. 4) that broadly consistent with a network topology (Fig. 2). As expected, specimens of all species formed independent and well supported clades according to their affiliation to series of *I. sect. Limniris*. In agreement with earlier studies (Tillie *et al.* 2000, Wilson 2009, Mavrodiev *et al.* 2014) samples of *I. uniflora* (*I. ser. Ruthenicae*) were positioned as a sister group to the highly supported (BP 99–100%) monophyletic clade comprising all *I. ser. Lacteae* samples. *Iris* ser. *Lacteae* haplotypes segregate into two highly supported clusters, one of which includes all haplotypes constituting haplogroup C (BP 94–95%, PP 1.00), haplotype XIN is placed in this cluster with moderate support only in NJ and MP analyses (BP 74 and 84%). The second cluster (BP 89–98%, PP 1.00) includes all other members of *I. ser. Lacteae* specimens; the relationships among most of them are, however, poorly resolved. Only specimens of haplogroup A together with haplotype ZON formed a well-supported group within this cluster (BS 96–99%, PP 1.00).

When all specimens were grouped according to clusters identified in the BAPS analysis or to haplogroups revealed with the MJ method, the high levels of pairwise genetic distances and nucleotide divergence were observed between them (Table 2). The level of nucleotide sequence divergence between species (K_s) varied in different series of *I. sect. Limniris* (Table 3). The K_s values obtained for nucleotide divergence between clusters and haplogroups identified in *I. ser. Lacteae* species are comparable with divergence between some species in the *Sibiricae* and *Laevigatae* series (Tables 2, 3). The results obtained from AMOVA also revealed significant levels of genetic differentiation ($P < 0.0001$) between clusters as well as between haplogroups ($\Phi_{ST} = 0.7800$, $\Phi_{ST} = 0.7995$, respectively).

TABLE 2. Nucleotide divergence (K_s , below diagonal) and pairwise genetic distances (F_{ST} , above diagonal) between haplogroups and clusters identified in MJ and BAPS analyses of *Iris* ser. *Lacteae* from 59 locations as inferred from the cpDNA data. An asterisk (*) denotes significance level, $P < 0.0001$.

Haplogroup/Cluster	A/1	B/2	C/3
A/1	–	0.80024/0.78786*	0.97384/0.92954*
B/2	0.00454/0.00440	–	0.68850/0.64791*
C/3	0.00938/0.00879	0.00711/0.00675	–

TABLE 3. Nucleotide divergence (K_s) between species within *Laevigatae* and *Sibiricae* series.

<i>Laevigatae</i>			<i>Sibiricae</i>		
	<i>I. ensata</i>	<i>I. laevigata</i>		<i>I. sibirica</i>	<i>I. bulleyana</i>
<i>I. ensata</i>	–		<i>I. sibirica</i>	–	
<i>I. laevigata</i>	0.01022	–	<i>I. bulleyana</i>	0.00893	–
<i>I. pseudacorus</i>	0.00765	0.01223	<i>I. wilsonii</i>	0.00837	0.00451

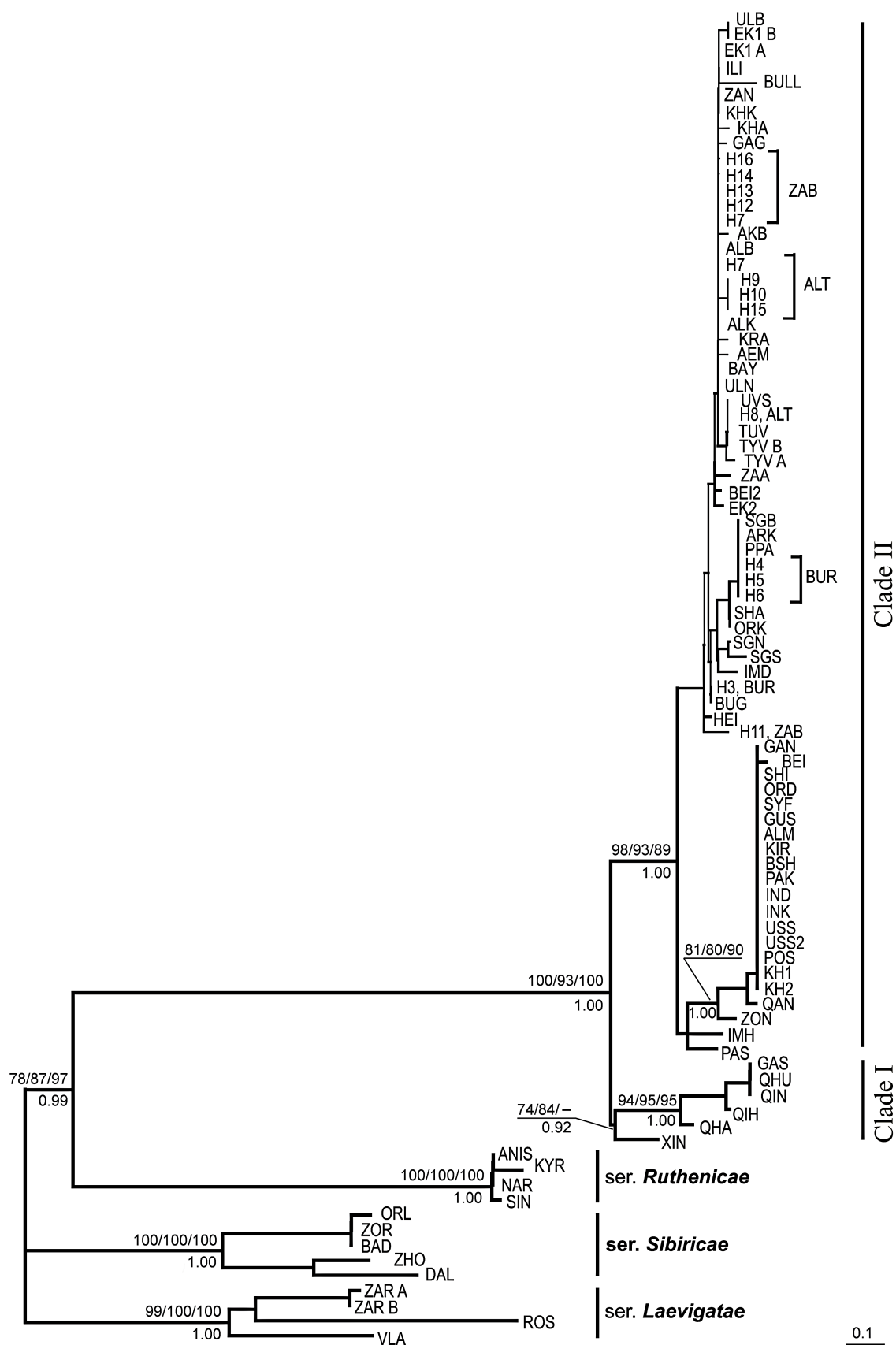


FIGURE 4. Phylogenetic analysis of *Iris* ser. *Lacteae* based on *psbA-trnH/rps4-trnS/trnS-trnG/trnL-trnF* sequences. Neighbour-joining tree is shown with bootstrap values in 1000 replicates for NJ/MP/ML analyses (shown only when > 50%) above the branches and Bayesian posterior probabilities more than PP = 0.90 below the branches. Clades I and II within *I. ser. Lacteae* are indicated. Haplotypes and locality codes are given in Table 1.

Morphological analysis:—We performed a detailed comparison between the three groups of *I. ser. Lacteae* revealed genetically, which included a study of the relevant literature, type material, and herbarium specimens. These groups corresponded to *I. lactea*, *I. oxypetala*, and a new taxon, which is named here as *I. tibetica* (see below). A total of 57 herbarium specimens from Gansu and Qinghai were selected for the morphological study of *I. tibetica* (Appendix 1). The 15 characters, listed in Table 4, were examined. After the morphological examination of the herbarium material, we concluded that *I. ser. Lacteae* species are very variable, especially in the stature and in the length of stem, bracts, pedicels, and fruit; the flower colour varies in *I. lactea* and *I. tibetica*. The analysis suggests that a combination of several characters should be considered before identification. Morphologically, the specimens of *I. tibetica* under study are more similar to *I. lactea* in the following characters: stem, leaves, flowers and fruit, but differ in the morphology of rhizome, the number of flowers, the shape of falls, the standard width, the bracts-ovary/perianth length ratio, and the fruit apex length. Fall shape is a character regarded to be of high diagnostic value in *I. ser. Lacteae* species.

TABLE 4. Comparison of morphological characters between the *Iris ser. Lacteae* species.

Characters	<i>I. lactea</i>	<i>I. oxypetala</i>	<i>I. tibetica</i>
Plant height (cm)	10–50	22–70	15–60
Rhizome	Thickened, vertical or oblique, forming closely compacted tufts	Thickened, vertical or oblique, forming closely compacted tufts	Slender, creeping, horizontal
Flowering stem height (cm)	(3–)10–27(–35)	(7–)25–35	(8–)11–22
Number of cauline leaves	3–4	3–5	3–4
Number of flowers	1–3(–4)	1–4(–5)	(1–)2
Flower colour	Light blue; pale purple; milky white rarely	Pale lilac, almost creamy white	Blue; falls white with creamy centre veined mauve, standards mauve; yellowish green
Bract length (cm)	(4–)7.5–8(–9.5); often reach the apex of or shorter than ovary	5–9(–10); not longer than the middle of ovary	6.5–10.3; reach the middle of or slightly shorter than perianth
Pedicel length (cm)	2.5–7.5	3–10.3	2.5–7(–10)
Fall length (cm)	5.5–7	5–6.1	5.5–6
Fall width (cm)	1.5–2	1.2–1.5	1.7–2.1
Standard length (cm)	4.5–6	4.6–5.1	5–5.5
Standard width (cm)	0.7–1.1	0.8–1	1.5
Fall blade shape	Oblong-elliptic or obovate; apex rounded or obtuse	Ob lanceolate; gradually narrowed to an acute apex	Obovate; apex obtuse
Fruit length (cm)	2–6	4–9	3–8.5
Fruit apex	Narrowed to distinct beak 0.2–0.5(–0.8) cm	Gradually narrowed to elongate beak (0.5–)0.7–1.1(–1.4) cm	Abruptly narrowed to short beak 0.1–0.4 cm

Discussion

Genetic relationships in *I. ser. Lacteae*:—In the present study, analyses of the chloroplast genome based on extended *I. ser. Lacteae* sampling and polymorphism of four cpDNA regions showed that the *I. ser. Lacteae* members are subdivided into three distinct groups/clusters. Two of them (haplogroups A and B) corresponded to genetically and geographically distinct species, *I. oxypetala* and *I. lactea*, the independence of which had been confirmed previously (Boltenkov *et al.* 2016). *Iris lactea* (haplogroup B) is characterised by high chloroplast genome variability. The incorporation of some additional *Iris* specimens from northern-east China (IMD and HEI) and one specimen from Pakistan (PAS) into the *I. lactea* cluster indicates that this species occurs not only in Siberia, Mongolia, and Kazakhstan but also in China, and in Pakistan. The star-like structures identified in the median-joining network can be indicative of a rapid range expansion in the past and a recent common history of *I. lactea* in the territories distant from each other. Previously it was shown (Boltenkov *et al.* 2016) that *I. oxypetala* is distributed in the south of the Russian Far East and is characterized by a low level of genetic variation, while *I. lactea* is highly genetically variable species. In this study, we established that geographical range of *I. oxypetala* (haplogroup A) includes also China (BEI, GAN, GUS, ORD, SHI, and SYF), India (IND and INK), Kyrgyzstan (BSH and KIR), Kazakhstan (ALM), and Pakistan (PAK). One more genetically divergent group (haplogroup C) consisting of five individuals from a limited area in the Gansu and Qinghai provinces (on the northeastern part of the Qinghai-Tibet Plateau) was revealed in genealogical, phylogenetic and Bayesian clustering analyses and the specimen XIN from the west of China was closer to this group than to any of the others.

Lack of common haplotypes, as well as high values of pairwise F_{ST} and high levels of differentiation indicated significant isolation and a high degree of genetic disunity of *I. ser. Lacteae* species belonging to the three revealed groups. The K_s values obtained for nucleotide divergence between these groups are comparable with divergence between some species of *I. ser. Sibiricae*, *I. ser. Laevigatae* (Table 2, 3), and *I. ser. Psammiris* (Kozyrenko *et al.* 2009). Based on these results, it might be inferred that all three groups represent separate but closely related species, *I. lactea*, *I. oxypetala*, and a distinct one occurring in Gansu and Qinghai provinces of China. In this study, it is named *I. tibetica*.

The taxonomic state of *I. ser. Lacteae* species and their distribution:—Historically, the views on the taxonomic composition of *I. ser. Lacteae* and the geographical distribution of its species has varied. The first taxonomic revision of this group was performed by Poljakov (1950). He came to the conclusion that in the region of the former Soviet Union this group is represented by three species: *I. biglumis*, *I. iliensis*, and *I. pallasii*. Grubov (1970) criticized this work and concluded that the group comprises two species, whose distribution ranges overlap in China. According to Grubov, the “northern species”, *I. lactea*, occurs in southern Siberia, Mongolia, China, and south eastern Kazakhstan. The “southern species”, *I. oxypetala*, is distributed in the south of the Russian Far East, as well as in Korea, China, Kashmir, and the Pamir-Alay. Subsequently, Grubov’s opinion was supported by Rodionenko (2006). Peschkova (1975) accepted three species: *I. lactea* (for northeastern China, eastern Mongolia, and southern Transbaikalia), to which she attributed *I. oxypetala*; *I. biglumis* (for the south of West Siberia and Buryatia, and northern Mongolia); and *I. pallasii* (for Altai, Kazakhstan, and northwestern China), to which she attributed *I. iliensis*. More recent publications (Alekseeva 2008, Doronkin 2012) have accepted that *I. biglumis*, *I. lactea*, *I. oxypetala*, and *I. pallasii* are independent species. However, following Rodionenko (2006) we have established that *I. biglumis*, *I. iliensis*, and *I. pallasii* should be considered as a single variable species, *I. lactea* (Boltenkov *et al.* 2016).

Iris ser. Lacteae species were for a long time erroneously referred to as *I. ensata* (see Introduction). Dykes (1912) attributed two varieties to this taxon: *I. ensata* var. *grandiflora* Dykes (1912: 88) from southern Tibet (Xizang Province, China) and *I. ensata* var. *chinensis* from the outskirts of Beijing. Later, *I. ensata* f. *tibetica* Dykes (1916: 194), was described from the mountains of eastern Qinghai Province, western China. It was assumed (Dykes 1916, Farrer 1916) that the plants collected by R. Farrer and W. Purdom from Qinghai Province could be a distinct species. Unfortunately, botanists did not pay attention to this fact, and *I. ensata* f. *tibetica* was forgotten. In his final work, Dykes (1924) identified the plants from the mountains of western China (*I. ensata* f. *tibetica*) as *I. ensata* var. *grandiflora*. Both the phylogenetic and morphological analyses performed here have confirmed that the plants from southern Tibet (*I. ensata* var. *grandiflora*) belong to *I. lactea*, and those from the eastern Qinghai Province form a genetically isolated group. A comparison of the original material of *I. ensata* f. *tibetica* (E00711784!, E00711785!) with five specimens from Gansu and Qinghai (as GAS, QHA, QHU, QIH, and QIN in the molecular study) showed that they are morphologically identical but are different from *I. lactea* and *I. oxypetala*. Therefore, we came to the conclusion that the plants collected at the border between Gansu and Qinghai belong to a separate species, which we named *I. tibetica*. The morphological features of this species were studied by us using the herbarium specimens listed in Appendix 1. Some authors (Maximowicz 1880, Grubov 1970, Peschkova 1975, Rodionenko 2006) suggested that the most important character for identifying the *I. ser. Lacteae* species is the shape of the falls. We also suggest using this feature, along with other ones (the morphology of rhizome, the number of flowers, the standard width, the bracts-ovary/perianth length ratio, and the fruit apex length), to identify species of this group.

Our data agree best with Grubov’s view (1970) on the taxonomic composition and the species distribution of *I. ser. Lacteae*. The geographical range of *I. lactea* extends from eastern Kazakhstan, northern Kyrgyzstan, and Siberia to Mongolia and northern China. The main part of the *I. oxypetala* range covers the central and eastern China provinces, as well as the Korean Peninsula. Some authors (Dykes 1924, Waddick & Zhao 1992) have stated that these irises have naturalized in many places where they had not been originally indigenous. In China, *I. oxypetala* is an invasive plant that colonizes waste places and lands that are no longer used for cultivation (Waddick & Zhao 1992). In northern India, they are considered (cited as *I. ensata*) alien species (Andrabi *et al.* 2015). Our study also shows that the distributions of *I. lactea* and *I. oxypetala* are geographically disjunctive, and that these species are not native to some parts of the range (in southeastern Kazakhstan, agricultural Fergana Valley, Pakistan, northern India, southern Xizang Province of China, Japan, and the Russian Far East). We are not inclined to believe that these are previously widespread species whose present distribution pattern was formed through fragmentation and extensive extinctions. In the above-mentioned regions, the plants occur within populated places, being associated with anthropogenic habitats, and have naturalized occasionally. The geographic distribution of *I. lactea* and *I. oxypetala* is associated to human activity, which was also noted by Dykes (1924). In northern India, these plants are used as forage and a source of fibre (Grisard 1888). In China, *I. oxypetala* is often used in traditional Chinese medicine (Kitagawa 1940, Waddick & Zhao

1992, Smith & Stuart 2003) and, apparently, has a history of cultivation of over 2000 years. We can safely assume that the pattern of *I. oxypetala* distribution in China, the Korean Peninsula, and the Russian Far East is a result of secondary species range expansion spreading over anthropogenic habitats. The economic value of these species and, at the same time, the ability to disperse have undoubtedly increased due to their great vitality. Their deep rooting system provides them with better survivability during long periods of drought than in any other irises (Dykes 1912). Anatomically, their leaves are also strongly adaptable to the arid climate (Wang & Gao 2008). Additionally, these species are resistant to saline and alkaline soils, and they can tolerate trampling, tamped soil, pests, and diseases (Dykes 1912, Waddick & Zhao 1992, Wang *et al.* 2012).

Taxonomic treatment

As a result of the revision of *I. ser. Lacteae*, three species are here accepted, for one of which a new combination is proposed. The list of accepted species and information on their distribution and habitats are provided below. In the distribution section, the Chinese provinces where the species have been found are detailed. Typifications of the names are proposed.

Iris ser. ***Lacteae*** Doronkin (1990: 412)

= *I.* subg. *Eremiris* Spach (1846: 32) ≡ *Eremiris* (Spach) Rodionenko (2006: 1707)

= *I.* subsect. *Ensatae* Diels (1930: 502), *quoad descr.*, *typo excl.* [*quod ab ser. Laevigatae pertinet*] ≡ *I. ser. Ensatae* (Diels) Lawrence (1953: 362), *pro parte*, *typo excl.*

= *I. sect. Haloiris* Doronkin (1990: 412)

Type (designated by Doronkin 1990: 412):—*I. lactea* Pallas (1776: 713).

1. ***Iris lactea*** Pallas (1776: 713) ≡ *Eremiris lactea* (Pall.) Rodionenko (2006: 1708) ≡ *Limniris lactea* (Pall.) Peruzzi *et al.* (2014: 276)—*Iris ensata* auct., non Thunberg (1794: 328). **Protologue citation**:—“*Observauui in desertis aridis circa lacum Tarei Dauriae*”. **Type** (neotype, designated here by E.V. Boltenkov):—RUSSIAN FEDERATION. [Zabaykalsky Krai], ad Tarei Nor, [fl.], [June 1772], [*Pallas*] *s.n.* (BM000958418!, right-hand side specimen).

Distribution and habitat:—This species is widespread in southern Siberia, eastern Kazakhstan, Mongolia, China (Inner Mongolia, northern parts of Gansu, Heilongjiang, Hubei, Ningxia, Shaanxi, and Shanxi provinces, and northern and western Xinjiang Province), northern Kyrgyzstan (Chuy Valley); it has naturalized in southern Xizang Province of China and northern Pakistan. It is usually found in steppe and desert regions at elevations ranging from 200 to 2500 m and even up to 4270 m in Xizang Province. The plant grows mostly on saline soils of stony or sandy steppes, on rubbly slopes, and on grass meadows located in river valleys and on lake banks. In Xizang, it is very common along irrigation channels and waste lands, in parks and gardens, marshes, and on banks of streams.

2. ***Iris oxypetala*** Bunge (1833: 63) ≡ *Eremiris oxypetala* (Bunge) Rodionenko (2006: 1708)—*Iris ensata* auct., non Thunb. **Protologue citation**:—“*Hab. frequens in montosis et pratensibus [China boreali]*”. **Type** (lectotype, designated by Grubov 1970: 31, as “type”):—CHINA. [Beijing], China, 1831, *Bunge s.n.* (LE01011526!).

Distribution and habitat:—This species is distributed in China (Anhui, Beijing, Chongqing, southern Gansu, Hebei, southern Heilongjiang, Henan, Hubei, Hunan, Inner Mongolia, Jiangsu, Jilin, Liaoning, Ningxia, Qinghai, Shaanxi, Shandong, Shanxi, northern Sichuan, and Tianjin provinces) and the Korean Peninsula; it has naturalized in Russian Federation (Primorsky Krai: Ussuriysky and Khasansky districts), in Fergana Valley (eastern Uzbekistan, southern Kyrgyzstan, and northern Tajikistan), in northern India (Himachal Pradesh, Jammu and Kashmir, and Uttarakhand), southeastern Kazakhstan, northern and western Pakistan (Balochistan, Gilgit-Baltistan, Punjab, Khyber-Pakhtunkhwa, and Kurram Valley), and Japan. This plant usually occurs in sunlit areas under dry conditions at elevations ranging from sea level to 2500 m; in northern India, the species is distributed up to 3500 m. It is found growing on grass meadows with tamped soil and on open grassy hillsides, in dry steppe, on grassy lake banks and near rivers, as well as on sandy and clayey seashores. It is a common roadside weed, colonizing abandoned fields and pastures.

3. ***Iris tibetica*** (Dykes) Bolt. **comb. & stat. nov.** **Basionym**:—*I. ensata* f. *tibetica* Dykes (1916: 194). **Protologue citation**:—“... from Sining [Xining] throughout the Da-Tung Alps to 11,000 feet”. **Type** (lectotype, designated here by E.V. Boltenkov):—CHINA. [Qinghai Province], inordinately abundant on all the open loess plains and slopes from

Sining throughout the Da-Tung Alps, (*I. sp. "hyacinthina"*), up to 11000, [fl.], May–June [1915], [R. Farrer & W. Purdom] 496 (E00711784!).

Distribution and habitat:—This is a narrow endemic species to China, distributed over the southern spurs of the Qilian Mountains, in Gansu Province (Dingxi: Lintao County; Gannan Tibetan Autonomous Prefecture: Jonê, Têwo, and Lintan counties; Lanzhou: Yongdeng and Yuzhong counties; Linxia Hui Autonomous Prefecture: Yongjing County; Wuwei: Tianzhu Tibetan Autonomous County; Zhangye: Shandan and Sunan counties) and eastern Qinghai Province (Hainan Tibetan Autonomous Prefecture: Gonghe and Xinghai counties; Haibei Tibetan Autonomous Prefecture: Menyuan Hui Autonomous County; Haidong: Hualong and Huzhu counties, Ledu District; Huangnan Tibetan Autonomous Prefecture: Jainca and Tongren counties; Xining: Datong, Huangzhong, and Huangyuan counties). It is found growing at high elevations only (2000–3800 m above sea level), predominantly in the subalpine zone. It is common on open grassy loess plains and moist slopes, along roadsides, on steep scrubby hillsides, gorge bottoms, and in cultivated valleys; also, it occurs in river valleys, particularly on open grassy meadows along right tributaries of the Yellow River (Baoku, Datong, Tao, and Wei). This plant prefers hard, poor soil in hot places, while in richer, moist places it tends to run to leaf and rarely blooms (Farrer 1917).

Identification key to *Iris* ser. *Lacteae*

1. Rhizome horizontal, creeping; flowers 1 or 2; bracts reach the middle of the first flower; falls obovate, with obtuse apex; standard width 1.5 cm; fruit apex abruptly narrowed to beak 0.1–0.4 cm in length *I. tibetica*
- Rhizome vertical or oblique; flowers more than 2; bracts often shorter than the first flower; standard width no more than 1.1 cm; fruit with elongated or short beak at apex 2
2. Falls oblanceolate, gradually narrowed to acute apex; fruit apex gradually narrowed to beak (0.5–) 0.7–1.1 (–1.4) cm in length *I. oxypetala*
- Falls oblong-elliptic or obovate, with rounded or obtuse apex; fruit apex narrowed to distinct beak 0.2–0.5 (–0.8) cm in length *I. lactea*

Acknowledgements

We are grateful to the curators of E, IRK, K, LE, MHA, MW, NS, NSK, TASH, TK, and VBGI for the access to the collections. Our special thanks are due to Irina Popova (Bishkek), Andrei Erst (NS), Tatiana Poluboyarova and Vladimir Doronkin (NSK), Alla Verhozina (IRK), Sun Guofeng (Beijing Botanical Garden, Institute of Botany, Chinese Academy of Sciences, Beijing, China) for providing specimens. Also, we acknowledge the support from the Royal Botanic Garden Edinburgh as a supplier of plant material and, especially, from John Mitchell and Peter Brownless (E), who rendered us assistance and gave helpful information. This study was supported by the Far Eastern Branch of the Russian Academy of Sciences (grant no. 15-I-6-030).

References

- Akhter, C., Khuroo, A.A., Malik, A.H. & Dar, G.H. (2012) A taxonomic appraisal of genus *Iris* L. (Iridaceae) in Kashmir Himalaya, India. *The Iranian journal of botany* 19: 119–126.
- Alekseeva, N.B. (2008) Genus *Iris* L. (Iridaceae) in Russia. *Turczaninowia* 11: 5–68. [in Russian]
- Al-Gabbiesh, A.H., Hassawi, D.S. & Afifi, F.U. (2006) Determination of genetic diversity among *Iris* species using random amplified polymorphic DNA analysis. *Biotechnology* 5: 173–179.
<https://doi.org/10.3923/biotech.2006.173.179>
- Andrabi, S.M., Reshi, Z.A., Shah, M.A. & Qureshi, S. (2015) Studying the patterns of alien and native floras of some habitats in Srinagar city, Kashmir, India. *Ecological Processes* 4: 2.
<https://doi.org/10.1186/s13717-014-0030-9>
- Artyukova, E.V., Kozyrenko, M.M., Ilyushko, M.V., Zhuravlev, Yu.N. & Reunova, G.D. (2001) Genetic variability of *Iris setosa*. *Molecular Biology* 35: 134–138.
<https://doi.org/10.1023/A:1004823422785>
- Baker, J.G. (1876) A synopsis of the known species of *Iris*. VIII. *The Gardeners' Chronicle, new series* 5: 323–324.

- Balbis, J.B. (1804) *Miscellanea Botanica*. [Torino], 68 pp.
- Bandelt, H.-J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.
<https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Blanco White, A. (2012) Series *Ensatae* (Diels) Lawrence. In: The Species Group of the British Iris Society (Ed.) *A guide to species irises: their identification and cultivation*. Cambridge University Press, Cambridge, pp. 202–203.
- Boltenkov, E.V., Artyukova, E.V. & Kozyrenko, M.M. (2016) Species divergence in *Iris* series *Lacteae* (Iridaceae) in Russia and adjacent countries based on chloroplast DNA sequence data. *Russian Journal of Genetics* 52: 507–516.
<https://doi.org/10.1134/S1022795416040037>
- Bonfield, J.K., Smith, K.F. & Staden, R. (1995) A new DNA sequence assembly program. *Nucleic Acids Research* 23: 4992–4999.
<https://doi.org/10.1093/nar/23.24.4992>
- Bunge, A.A. (1833) *Enumeratio Plantarum*. St. Petersburg, 73 pp.
- Corander, J., Marttinen, P., Sirén, J. & Tang, J. (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics* 9: 539.
<https://doi.org/10.1186/1471-2105-9-539>
- Cornman, R.S. & Arnold, M.L. (2007) Phylogeography of *Iris missouriensis* (Iridaceae) based on nuclear and chloroplast markers. *Molecular Ecology* 16: 4585–4598.
<https://doi.org/10.1111/j.1365-294X.2007.03525.x>
- Crespo, M.B., Martínez-Azorín, M. & Mavrodiev, E.V. (2015) 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* 232: 1–78.
<https://doi.org/10.11646/phytotaxa.232.1.1>
- Diels, L. (1930) Iridaceae. In: Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien*, ed. 2, 15a. W. Engelmann, Leipzig, pp. 463–505.
- Don, D. (1840) Descriptions of the Indian species of *Iris*. *Transactions of the Linnean Society of London* 18: 309–316.
<https://doi.org/10.1111/j.1095-8339.1838.tb00180.x>
- Doronkin, V.M. (1987) Iridaceae. In: Malyshev, L.I. & Peschkova, G.A. (Eds.) *Flora Sibiri [Flora of Siberia]: Araceae-Orchidaceae* 4. Nauka, Novosibirsk, pp. 113–125.
- Doronkin, V.M. (1990) The synopsis of Siberian species of the genus *Iris* (Iridaceae). *Botanicheskii Zhurnal* 75: 409–416. [in Russian]
- Doronkin, V.M. (2012) Iridaceae Juss. In: Baikov, K.S. (Ed.) *Konspekt flory Aziatskoi Rossii: sosudistye rasteniya (Synopsis of Flora of Asian Russia: Vascular Plants)*. Sibirskoye otdeleniye Rossiyskoy Akademii nauk, Novosibirsk, pp. 456–459. [in Russian]
- Dykes, W.R. (1910) Three new Chinese irises. *The Gardeners' Chronicle, series 3* 47: 418.
- Dykes, W.R. (1912) *The genus Iris*. Cambridge University Press, Cambridge, 245 pp.
- Dykes, W.R. (1916) Some Tibetan irises. *The Gardeners' Chronicle, series 3* 59: 194–195.
- Dykes, W.R. (1924) *A handbook of garden irises*. M. Hopkinson & Co., London, 250 pp.
<https://doi.org/10.5962/bhl.title.36836>
- Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.
<https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Farrer, R. (1916) Report of work in 1914 in Kansu and Tibet. *Journal of the Royal Horticultural Society* 42: 47–114.
- Farrer, R. (1917) Report of work in 1915 in Kansu and Tibet. *Journal of the Royal Horticultural Society* 42: 324–348.
- Fedtschenko, B.A. (1935) *Iris*. In: Komarov, V.L. (Ed.) *Flora of the USSR* 4. Izdatel'stvo Akademii nauk SSSR, Leningrad, pp. 511–557. [in Russian]
- Fischer, F.E.L. & Meyer, C.A. (1839) *Index quintus seminum, quae hortus botanicus Imperialis Petropolitanus pro mutua commutatione offert: accedunt animadversiones botanicae nonnullae* 5. Academiae Caesariae Petropolitanae, Petropoli, 43 pp.
- Goldblatt, P., Rodriguez, A., Powell, M.P., Davies, T.J., Manning, J.C., Van der Bank, M. & Savolainen, V. (2008) Iridaceae ‘Out of Australia’? Phylogeny, biogeography, and divergence time based on plastid DNA sequences. *Systematic Botany* 33: 495–508.
<https://doi.org/10.1600/036364408785679806>
- Gouy, M., Guindon, S. & Gascuel, O. (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27: 221–224.
<https://doi.org/10.1093/molbev/msp259>
- Grisard, J. (1888) Extraits des procès-verbaux des séances des sections. Cinquième section.—Végétaux. *Bulletin de la Société Nationale d'Acclimatation de France, série 4* 5: 698–700.
- Grubov, V.I. (1970) Critical remarks on the taxonomy and nomenclature of some species of the genus *Iris* L. of the URSS flora. *Novosti Sistematiki Vysshikh Rastenii* 6: 29–37. [in Russian]

- Grubov, V.I. (1977) Iridaceae. In: Grubov, V.I. (Ed.) *Rasteniya Tsentral'noi Azii* [Plants of Central Asia] 7. Nauka, Leningrad, pp. 88–102. [in Russian]
- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
<https://doi.org/10.1093/bioinformatics/17.8.754>
- Kitagawa, M. (1940) Materials to the flora of Eastern Asia, III. *Report of the Institute of Scientific Research Manchoukuo* 4: 75–118.
- Koidzumi, G. (1925) Contributiones ad cognitionem florum Asiae orientalis. *Botanical Magazine (Tokyo)* 39: 299–318.
https://doi.org/10.15281/jplantres1887.39.468_299
- Kostrakiewicz, K. & Wróblewska, A. (2008) Low genetic variation in subpopulations of an endangered clonal plant *Iris sibirica* in southern Poland. *Annales Botanici Fennici* 45: 186–194.
<https://doi.org/10.5735/085.045.0303>
- Kozyrenko, M.M., Artyukova, E.V., Boltenkov, E.V. & Lauve, L.S. (2004) Somaclonal variability of *Iris pseudacorus* L. according to RAPD and cytogenetic analyses. *Biotechnology in Russia* 2: 11–22.
- Kozyrenko, M.M., Artyukova, E.V. & Zhuravlev, Yu.N. (2009) Independent species status of *Iris vorobievii* N.S.Pavlova, *Iris mandshurica* Maxim., and *Iris humilis* Georgi (Iridaceae): Evidence from the nuclear and chloroplast genomes. *Russian Journal of Genetics* 45: 1394–1402.
<https://doi.org/10.1134/S1022795409110143>
- Krylov, P.N. (1929) Iridaceae. In: Krylov, P.N. (Ed.) *Flora Zapadnoi Sibiri* [Flora of Western Siberia] 3. Tomskoye otdeleniye Russkogo Botanicheskogo obshchestva, Tomsk, pp. 660–672. [in Russian]
- Lamote, V., Roldán-Ruiz, I., Coart, E., De Loose, M. & Van Bockstaele, E. (2002) A study of genetic variation in *Iris pseudacorus* populations using amplified fragment length polymorphisms (AFLPs). *Aquatic Botany* 73: 19–31.
[https://doi.org/10.1016/S0304-3770\(02\)00006-2](https://doi.org/10.1016/S0304-3770(02)00006-2)
- Lawrence, G.H.M. (1953) A reclassification of the genus *Iris*. *Gentes Herbarum* 8: 346–371.
- Ledebour, K.F. (1833) Botanik: Enumeratio plantarum, quas in China boreali collegit Dr. Al. Bunge anno 1831. *Dorpaten Jahrbücher für Litteratur, Statistik und Kunst, besonders Russlands* 1: 218–222.
- Lee, W.T. (1996) *Lineamenta florum Koreae* 1. Academy Press, Seoul, 1688 pp. [in Korean]
- Lemaire, C. (1858) *L'illustration horticole, journal spécial des serres et des jardins, ou choix raisonné des plantes les plus intéressantes sous le rapport ornemental, comprenant leur histoire complète, leur description comparée, leur figure et leur culture* 5: t. 157.
- Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.
<https://doi.org/10.1093/bioinformatics/btp187>
- Lindley, J. (1840) *Iris fragrans*. *Edwards's Botanical Register* 26: t. 1.
- Link, H.F. (1820) Der botanische garten bei Berlin und die Willdenowsche kräutersammlung. *Jahrbücher der Gewächskunde* 1: 13–93.
- Link, H.F. (1821) *Enumeratio plantarum horti regii botanici Berolinensis altera* 1. G. Reimer, Berolini, 458 pp.
<https://doi.org/10.5962/bhl.title.66>
- Linnaeus, C. (1753) *Species Plantarum* 1. Laurentii Salvii, Stockholm, 560 pp.
<https://doi.org/10.5962/bhl.title.669>
- Lynch, R.I. (1904) *The Book of the iris*. J. Lane, London & New York, 214 pp.
<https://doi.org/10.5962/bhl.title.17677>
- Mathew, B. (1989) *The Iris*, ed. 2. Timber Press, Portland, 215 pp.
- Mavrodiev, E.V., Martínez-Azorín, M., Dranishnikov, P. & Crespo, M.B. (2014) At least 23 genera instead of one: The case of *Iris* L. s.l. (Iridaceae). *PLOS One* 9: e106459.
<https://doi.org/10.1371/journal.pone.0106459>
- Maximowicz, C.J. (1880) Diagnoses plantarum novarum asiaticarum. III. *Bulletin de l'Académie Impériale des Sciences de Saint-Petersbourg* 26: 420–542.
- Meerow, A.W., Gideon, M., Kuhn, D.N., Motamayor, J.C. & Nakamura, K. (2007) Genetic structure and gene flow among south Florida populations of *Iris hexagona* Walt. (Iridaceae) assessed with 19 microsatellite DNA loci. *International Journal of Plant Sciences* 168: 1291–1309.
<https://doi.org/10.1086/521692>
- Pallas, P.S. (1776) *Reise durch verschiedene Provinzen des russischen Reichs* 3. Kaiserlichen Akademie der Wissenschaften, St. Petersburg, 760 pp.
- Pavlova, N.S. (1987) Iridaceae Juss. In: Kharkevich, S.S. (Ed.) *Sosudistye rasteniya sovetskogo Dal'nego Vostoka* [Vascular plants of the Soviet Far East] 2. Nauka, Leningrad, pp. 414–426. [in Russian]
- Peruzzi, L., Conti, F. & Bartolucci, F. (2014) Novità nomenclaturali; Notulae: 2098–2099. *Informatore Botanico Italiano* 46: 276–277.
- Peschkova, G.A. (1975) About some species of the genus *Iris* L. from Central Siberia. *Novosti Sistematiki Vysshikh Rastenii* 12: 135–140.

[in Russian]

- Poljakov, P.P. (1950) De *Iride ensata* Thunb. nota critica. *Botanicheskie Materialy Gerbariya Botanicheskogo Instituta Imeni V. L. Komarova Akademii Nauk SSSR* 12: 88–90. [in Russian]
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
<https://doi.org/10.1093/bioinformatics/14.9.817>
- Rodionenko, G.I. (2006) *Eremiris*, a new genus of the family Iridaceae. *Botanicheskii Zhurnal* 91: 1707–1712. [in Russian]
- Royle, J.F. (1839) *Illustrations of the botany and other branches of the natural history of the Himalayan Mountains and of the flora of Cashmere* 1. Wm. H. Allen & Co., London, 472 pp.
- Schanzer, I.A. (2013) Phylogeny and taxonomy of recently diverged groups as exemplified by the genus *Rosa*. *Proceedings of the Zoological Institute of the Russian Academy of Sciences, supplement 2* 317: 202–216 [in Russian]
- Sergievskaia, L.P. (1972) *Flora Zabaykal'ya [Transbaikalia Flora]* 4. Izdatel'stvo Tomskogo universiteta, Tomsk, 70 pp. [in Russian]
- Sims, J. (1822) *Iris pallasii* var. *chinensis*. Pallas's chinese iris. *Curtis's Botanical Magazine* 49: t. 2331.
- Sims, J. (1825) *Iris longispatha*. Long-spathed flag. *Curtis's Botanical Magazine* 52: t. 2528.
- Smith, F.P. & Stuart, G.A. (2003) *Chinese medicinal herbs: a modern edition of a classic sixteenth-century manual*. Dover Publications, New York, 508 pp.
- Spach, E. (1846) *Histoire Naturelle des Végétaux. Phanerogames* 13. Librairie encyclopédique de Roret, Paris, 444 pp.
<https://doi.org/10.5962/bhl.title.44839>
- Swofford, D.L. (2003) *PAUP*: Phylogenetic analysis using parsimony (*and other methods): version 4.04*. Sinauer Associate, Sunderland.
- Tausch, I.F. (1823) *Hortus Canalius seu plantarum rariorum quae in horto botanico illustrissimae, ac excellentissimae Josephi Malabaila comitis de Canal coluntur, icones et descriptiones* 1. T. Haase, Pragae, without pagination.
- Thiers, B. (2017 continuously updated) *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (accessed 22 August 2017)
- Thunberg, C.P. (1794) Botanical observations of the flora Japonica. *Transactions of the Linnean Society* 2: 326–342.
<https://doi.org/10.5962/bhl.title.3681>
- Tillie, N., Chase, M.W. & Hall, T. (2000) Molecular studies in the genus *Iris* L.: A preliminary study. *Annali di Botanica, new series* 58: 105–112.
<http://dx.doi.org/10.4462/annbotrm-9068>
- Treviranus, L.C. (1821) *Indicis seminum quae in horto botanico Wratislaviensi prostant, appendix III*. Breslau, without pagination.
- Tscherneva, O.V. (1971) *Iris* L. In: Kovalevskaja, S.S. (Ed.) *Opredelitel' rastenij Srednej Azii (Conspectus florum Asiae Mediae)* 2. Izdatel'stvo Fan, Tashkent, pp. 124–132. [in Russian]
- Vahl, M. (1805) *Enumeratio plantarum vel ab aliis, vel ab ipso observatarum, cum earum differentiis specificis, synonymis selectis et descriptionibus succinctis* 2. Hauniae, 423 pp.
- Waddick, J.W. & Zhao, Y.-T. (1992) *Iris of China*. Timber Press, Portland, 192 pp.
- Wang, J. & Gao, Y.-K. (2008) Study on the relationship between leaf anatomical structure and drought resistance of four species of *Iris*. *Heilongjiang Agricultural Sciences* 5: 101–104. [in Chinese]
- Wang, K., Kang, J., Zhou, H., Sun, Y., Yang, Q., Dong, J. & Meng, L. (2009) Genetic diversity of *Iris lactea* var. *chinensis* germplasm detected by inter-simple sequence repeat (ISSR). *African Journal of Biotechnology* 8: 4856–4863.
- Wang, W.-Y., Yan, X.-F., Jiang, Y., Qu, B. & Xu, Y.-F. (2012) Effects of salt stress on water content and photosynthetic characteristics in *Iris lactea* var. *chinensis* seedlings. *Middle-East Journal of Scientific Research* 12: 70–74.
- Wilson, C.A. (2009) Phylogenetic relationships among the recognized series in *Iris* section *Limniris*. *Systematic Botany* 34: 277–284.
<https://doi.org/10.1600/036364409788606316>
- Wright, C.H. (1907) *Iris wilsoni*. *Bulletin of Miscellaneous Information* 26: 321.
- Wróblewska, A. & Brzosko, E. (2006) The genetic structure of the steppe plant *Iris aphylla* L. at the northern limit of its geographical range. *Botanical Journal of the Linnean Society* 152: 245–255.
<https://doi.org/10.1111/j.1095-8339.2006.00568.x>
- Zhao, Y.-T. (1980) An investigation on “Malien”. *Bulletin of Botanical Laboratory of North-Eastern Forestry Institute, Harbin* 9: 75–79.
- Zhao, Y.-T., Noltie, H.J. & Mathew, B. (2000) Iridaceae. In: Wu, Z.-Y. & Raven, P.H. (Eds.) *Flora of China: Flagellariaceae through Marantaceae* 24. Missouri Botanical Garden Press, St. Louis, pp. 297–313.

APPENDIX 1. Specimens of *Iris tibetica* studied.

Iris tibetica:—CHINA. **Gansu**: near Pingfan, moist gorge bottom, 2350–2800 m, [fl.], 12–20 July 1923, *R.C. Ching* 476 (E!); vicinity of Choni [Jonê], moist soil, 3100–3300 m, [fr.], 7–15 September 1923, *R.C. Ching* 993 (E!); Choni, T'ao River basin, grassy meadow, [fl.], May 1925, *J.F. Rock* 12111 (E!, PE01012929); Choni, T'ao River basin, common along roadside in Tao River valley, forming clumps, 8500 ft., [fl.], June 1925, *J.F. Rock* 12323 (E!, K!, PE00034011); Upper Tebbu [Têwo] county, in meadows along Tao river, 8500 ft., [fr.], September–October 1925, *J.F. Rock* 13637 (E!); Lintan County, on weedy slope, 3000 m, [fr.], 20 July 1936, *T.P. Wang* 5160 (PE01012830); Lintao County, Yuzhong way, [fr.], 22 July 1956, *s.coll.* 1688 (KUN00360424, PE01012846); Yuzhong County, [fr.], 31 August 1956, *s.coll.* 3318 (PE01012847); Jonê County, Tao River, hillside and roadside, 2760 m, [fl.], 31 May 1957, *s.coll.* 3259 (KUN00360426, PE01012831, PE01012839, WUK00091427); Jonê County, 2700 m, [fl.], 5 June 1957, *s.coll.* 3275 (KUN00360425); Jonê County, Tao River south bank, 2500 m, [fl.], 12 June 1957, *Pan et al.* B0008 (IBSC00628840); Sunan Yugur Autonomous County, Qilian Mountains, [fl.], 30 June 1959, *Y. He* 3541 (PE01012820); Tianzhu Tibetan Autonomous County, Anyuanzhen, 2700 m, [fr.], 10 July 1959, *Y. He* 4617 (PE01012819); Tianzhu Tibetan Autonomous County, 2500 m, [fr.], 14 July 1959, *Y. He* 4867 (PE01012827); Lanzhou, Xinglong Mountain, 2350 m, [fr.], 4 September 1959, *Y. He* 5614 (PE01012825); Yuzhong County, [fl.], 10 June 1990, *S. Liu* 3660 (HNWP No. 160169); Yongdeng County, Tuo Lu, 2200 m, [fr.], 10 July 1991, *T. He* 1956 (HNWP No. 166985); Yongjing County, Tulu Ditch, 2300 m, [fr.], 10 July 1991, *R. Huang* 2297 (HNWP No. 167194 & 0232230); Yongdeng County, Liancheng, 2000 m, [fr.], 12 July 1991, *R. Huang* 2799 (HNWP No. 167262); Tianzhu Tibetan Autonomous County, 2910 m, [fl.], 15 July 1991, *R. Huang* 2556 (HNWP No. 167505); Shandan County, 2800 m, [fr.], 24 July 1991, *R. Huang* 2992 (HNWP No. 100562); Zhangye, Sunan Yugur Autonomous County, Mati Temple, 2500–2800 m, [fl., fr.], 28 July 1991, *T. He* 2876 (HNWP No. 166734); *ibid.* *R. Huang* 3192 (HNWP No. 0218496); road from Jonê along Tao He River valley, steep scrubby hillside, 2485 m, [fl.], 16 June 2000, *s.coll.* 100 (E00293332!). **Qinghai**: [Haidong: Huzhu Tu Autonomous County], inordinately abundant on all the open loess plains and slopes from Sining throughout the Da-Tung Alps, (*I. sp.* “*hyacinthina*”), up to 11000, [fl.], May–June [1915], [*R. Farrer & W. Purdom*] 496 (E00711784!, lectotype); one specimen only, in wolves den, among *F496* of which it is probably a unique variety, (*I. sp.* “*kelaina*”), [fl.], June 15 [1915], [*R. Farrer & W. Purdom*] 501 (E00711785!, syntype); Datong Hui and Tu Autonomous County, Guanghui Temple, 1940 m, [fr.], 28 July 1936, *K.-M. Liou* 6099 (PE01012853); Huangyuan County, on the road to Haiyan, 2600 m, [fl.], 27 June 1958, *B. Zhong* 8205 (HNWP No. 002936, HNWP No. 002941, PE01012932, PE01012933); Ledu District, 2700 m, [fr.], 12 September 1959, *s.coll.* 1728 (HNWP No. 002944); Menyuan Hui Autonomous County, Xian Mi, 2500–2600 m, [fl.], 14 June 1960, *s.coll.* 2513 (PE01012852); Xinghai County, 3250 m, [fl.], 9 May 1966, *Z. Wu* 4 (HNWP No. 17781); western Tongren County, 2450, [fl.], 2 June 1970, *L. Zhou & L. Sun* 570 (HNWP No. 21846); Jainca County, 2080 m, [fl.], 4 June 1970, *S. Liu* 968 (HNWP No. 25948); Tongren County, 2800 m, [fr.], 26 July 1970, *S. Liu* 1610 (HNWP No. 26460); Tongren County, 2600 m, [fr.], 26 July 1970, *L. Zhou & L. Sun* 1188 (HNWP No. 22442); Qinghai Lake, [fl.], 8 June 1984, *X. Hu* 19408 (HNWP No. 108457); Huzhu Tu Autonomous County, Beishan National Forest Park, 2300–2600 m, [fr.], 29 August 1988, *J. Pan et al.* 024 (HNWP No. 151318); Datong Hui and Tu Autonomous County, Laoye Mountain, 2900 m, [fl.], 30 June 1989, *Z. Yang* 0550 (HNWP No. 59399); Xining, 2600 m, [fr.], 26 July 1989, *Z. Zhang et al.* 5128 (HNWP No. 158719); Xining, 2293 m, [fl.], 16 June 1990, *Z. Zhang et al.* 5669 (HNWP No. 160449); Huzhu Tu Autonomous County, Long stone ditch, [fl.], 3 July 1991, *R. Huang* 1870 (HNWP No. 168947); Yushu Zang, near Gonghe, common, almost weedy in dry areas around houses, ca. 2550 m, 18 August 1996, *s.coll.* *s.n.* (E!); Xining City, Xining to Ta Er, 7 km from Ta Er, cultivated valley, roadside banks with *Populus*, 2510 m, [fl.], 29 June 1997, *D.G. Long et al.* 3 (E00141055!); Huangyuan County, Riyue Xiang, open grassy meadows, 2905 m, [fl.], 30 June 1997, *D.G. Long et al.* 60 (E00141053!, E00141054!); Hualong Hui Autonomous County, Qingsha Shan, under *Populus*, 2875 m, [fl.], 13 June 2000, *s.coll.* 34 (E00287841!).