Molecular evidence that *Carex songorica* Kar. & Kir. and *C. gotoi* Ohwi (Cyperaceae) are distinct species

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Abstract. *Carex songorica* and *C. gotoi* have similar appearance, but different distributions that almost do not overlap. Some authors considered *C. gotoi* as a subspecies of *C. songorica*. This opinion was based on the premise that *C. gotoi* differs from *C. songorica* only by slightly narrower utricles with thickened costate veins and somewhat longer beak. In order to clarify the extent of differences between these two taxa, we performed a molecular genetic study based on specimens from Asian Russia. We also re-examined herbarium specimens in order to clarify the correctness of identification and the differences between *C. songorica* and *C. gotoi*. Our results suggest that these taxa can be considered distinct species.

1 Introduction

The genus *Carex* L. (Cyperacea Juss.) is one of the most species-rich and widespread genera in the world flora. It includes over 2000 species [1, 2, 3].

The section *Tumidae* Meinsh. contains about 17 species from Europe, moderate zone of Asia, east of North America, south of South America, Northern Africa Australia, and New Zealand [2]. Species of this section inhabit banks of rivers and lakes, swamps, wet and swampy meadows, from the sea level to the middle, sometimes high mountain zones.

Carex songorica Kar. et Kir was described in 1842 by G.S. Karelin and I.P. Kirillov based on collections from Central Asia from the Lepsa river (LE). This species was found to be widespread in the Caucasus, southeastern Turkey, Iran, Afghanistan, West Pakistan, Turkmenistan, Uzbekistan, Kyrgyzstan, Tajikistan, Kazakhstan, Russia (Siberia), northern and eastern Mongolia, China (Dzungaria).

C. *gotoi* Ohwi was described in 1930 from the Korean Peninsula (KYO). In 1931 V.I. Kreczetovicz described *C. sukaczovii* V.I. Krecz. from Chita oblast (Russia), which he later on synonymized with *C. gotoi*. *C. gotoi* was subsequently found in Russia (Eastern Siberia and the Russian Far East), eastern Mongolia, northeastern China and the Korean Peninsula.

C. songorica and *C. gotoi* have similar appearance, but different distributions that almost do not overlap. Some authors [2, 4, 5] considered *C. gotoi* as a subspecies of *C. songorica*, *C. songorica* subsp. *gotoi*. This opinion was based on the premise that *C. gotoi*

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differs from *C. songorica* only by slightly narrower utricles with thickened costate veins and somewhat longer beak.

In order to clarify the extent of differences between these two taxa, we performed a molecular genetic study based on specimens from Asian Russia. We used three markers: a fragment of the plastid *matk* gene, and two fragments of the ribosomal RNA cluster, the external transcribed spacer (ETS) and the internal transcribed spacer 2 (ITS2). These sequences were successfully used in many studies on sedges, e.g., for the closely related section *Vesicariae* [6]. We also re-examined herbarium specimens in order to clarify the correctness of identification and the differences between *C. songorica* and *C. gotoi*.

2 Materials and methods

Herbarium specimens were taken from the NSK Herbarium. For *C. songorica*, we sampled 10 specimens from distant geographic locations and for *C. gotoi*, 4 specimens. Details on the specimens are given in Table 1.

Table 1. Sampling points. Reg., region; v., village

Specimen no.	Sampling locality	Collection date	Collectors
C. songorica			
C250	Khakasia Rep., Bograd d., Beregovoye v.	19.07.1969	G. Pavlova, V. Baranova
C251	Irkutsk oblast, southern shore of Baikal, Slyudyanka	04.08.1974	A. Kiseleva
C 267	Tuva Rep., Piy-Khem reg., Eastern Sayan, Saigara v.	29.07.1973	M. Lomonosova, N. Iskakova
C268	Krasnoyansk krai, Shirokino reg., Sukino v.	19.07.1966	Maskayev, Dyatlova
C270	Altai Rep., Kosh-Agach reg., Kurai v.	28.08.1985	L. Malyshev
C279, C280	Khakasia Rep., Askiz reg., N. Uzhungul v.	18.07.1971	A. Koroleva, G. Gileva
C282	Khakasia Rep., Askiz reg., N. Uzhungul v.	26.07.1971	A. Koroleva, T. Martynova
C260	Altai Rep., Ongudai reg., Talda v.	07.07.2007	I.N. Shekhovtsova
C062	Altai Krai, Terekta reg., Kastakhta river	12.08.1984	L. Malyshev
C. gotoi			
C252	Irkutsk oblast, Bayandai reg. Khuty ulus	08.07.1956	Frolova, Khilova, Radkevich
C253	Buryatia Rep., Eravna reg., Telemba v.	16.07.1953	A. Kurban-Talieva
C264	Chita oblast., Aleksandrovskii zavod v.	02.07.1963	Peshkova, Martynova
C265	Chita oblast, Olovyannaya reg., Khara-Byrka v.	21.07.1961	G. Peshkova

Dried leaves (100–500 mg) were ground in a mortar to fine powder and transferred to an Eppendorf tube with 1 ml of CTAB buffer (3% CTAB, 1.4 M NaCl, 30 mM Tris-HCl (pH 8.0), 2 mM EDTA) for 4 h at 65 °C. After incubation, 1 ml of chloroform was added and mixed, and the tube was centrifuged for 5 min at 16 000 g. The supernatant was transferred to a new tube and mixed with an equal volume of isopropanol, incubated for 5 min and centrifuged for 10 min at 16 000 g. The resulting pellet was dissolved in distilled

water and purified on BioSilica columns (Russia) to remove residual polyphenols interfering with PCR reactions.

DNA amplifications were performed using commercial PCR mix (Biolabmix, Russia). A fragment of the plastid *matk* gene was amplified using universal primers matK-1 (5′-TTCAA-ATCCT-TCAAT-GCTGG-3′) and matK-3 (5′-TGAGA-GGAAG-GACTG-GAACT-AA-3′) from Shekhovtsov et al. (2012). For the external transcribed spacer (ETS), the universal primers ETS-1F (5'-CTGTG-GCGTC-GCATG-AGTTG-3') and 18S-R (5'-AGACA-AGCAT-ATGAC-TACTG-GCAGG-3') from Starr et al. (2003) were used. The complete internal transcribed spacer 2 (ITS2) with flanking sequences were amplified using primers CITS2-F2 (5'-CAACG-GATAT-CTCGG-CTCTC-3') and CITS2-R2 (5'-GATTC-GCTCG-CCGTT-ACTAT-3') from Shekhovtsov et al. [6]. All DNA fragments were sequenced from both ends.

DNA chromatograms were edited using Chromas v.2.6.6 (Technelysium Pty Ltd). Phylogenetic trees were constructed for the concatenated dataset using MEGA X [7]. For the Maximum Parsimony (MP) algorithm, the Subtree-pruning-regraphing search with 1000 bootstrap repetitions was performed.

3 Results and discussions

3.1 Phylogenetic Analyses

Table 2. Table 2. Nucleotide substitutions in the studied DNA markers. M stands for C/A; K, for G/T; R, for A/G; Y, for C/T. Numbers indicate positions in the alignment.

Specimen	ITS2				ETS									
	178	225	249	311	98	147	235	255	266	293	358	454	479	521
C. gotoi														
C252	C	C	G	A	C	C	C	G	A	A	G	C	A	T
C264	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C265	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C253	.	.	.	.	.	.	.	.	.	.	.	.	R	.
C. songorica														
C267	T	M	K	.	T	A	T	A	C	.	R	.	G	C
C250	T	A	T	.	T	A	T	A	C	.	R	.	R	.
C282	T	A	T	G	T	A	T	A	C	R	A	Y	G	.
C062	T	A	T	G	T	A	T	A	C	R	A	.	G	.
C251	T	A	T	G	T	A	T	A	C	R	A	.	G	.
C260	T	A	T	G	T	A	T	A	C	R	A	.	G	.
C268	T	A	T	G	T	A	T	A	C	G	A	.	G	.
C270	T	A	T	G	T	A	T	A	C	.	A	T	G	.
C279	T	A	T	G	T	A	T	A	C	.	A	T	G	.
C280	T	A	T	G	T	A	T	A	C	.	A	T	G	.

For all studied specimens, we obtained sequences of *matk* (591 bp), ITS2 (438 bp), and ETS (593 bp). No indels were found in any of the genes. The plastid *matk* sequences of *C. songorica* and *C. gotoi* were identical. Four polymorphic sites were found in ITS2, and nine, in ETS (Table 2). Most of these positions delimited *C. songorica* from *C. gotoi*.

Almost no genetic diversity was detected within *C. gotoi*; only the C253 had one degenerate position in ETS. For *C. songorica*, on the contrary, many specimens had positions with the variants characteristic for *C. gotoi*.

Both ITS2 and ETS are parts of the ribosomal RNA cluster, and are represented as hundreds of tandemly repeated copies in the genome. Thus degenerate nucleotides represent not two alleles of a single locus located on different chromosomes, as we would normally expect, but the ratio of sequences with different variants within the cluster.

On the phylogenetic tree (Fig. 1) one can see that both taxa form two reciprocally monophyletic groups. As seen in Table 2, *C. songorica* differs from *C. gotoi* by as much as four positions in ITS2 and eight, in ETS. This is higher than for many species of sedges. E.g., the species *C. vesicaria* and *C. vesicata* differ by one and four substitutions in these loci, respectively (our data, not shown).

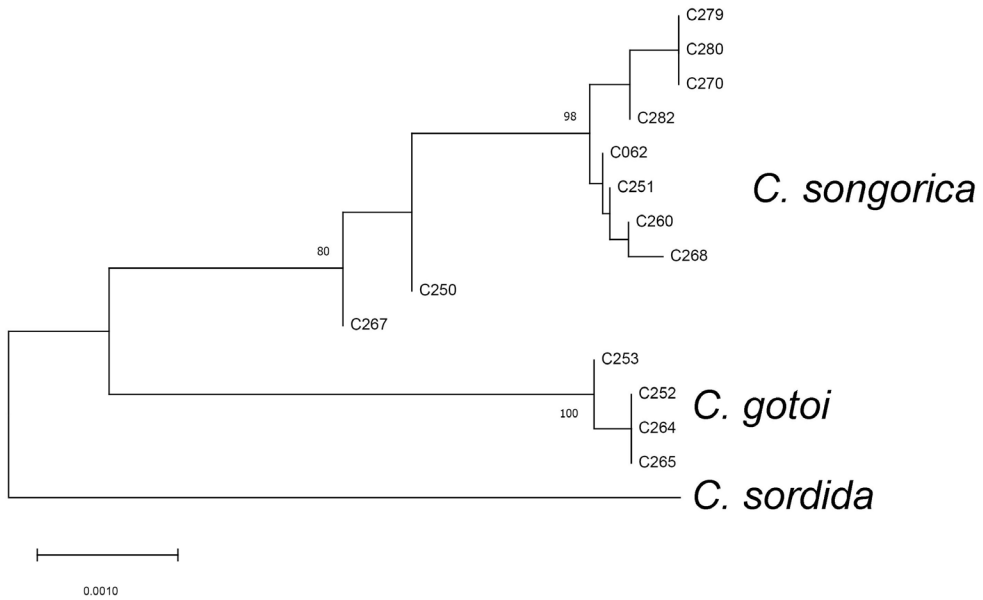

Fig. 1. Minimum Evolution phylogentic tree of C. songorica and C. gotoi. Number near the branched indicate bootstrap support.

3.2 Morphological comparison

C. songorica differs from *C. gotoi* by having orange utricles with fine veins and shorter beak 0.5-0.7 mm long. *C. gotoi* is characterized by dark purple utricles with thicker costate veins and longer beak (0.8–1.2 mm).

Specimen C252, reported as *C. songorica* from Irkutsk oblast (Bayanday region, v. Khuty) in the "Flora of Siberia" [5] and in the "Check-list of the Vascular Flora of the Irkutsk region" [8]. Our molecular data indicates that it belongs to C. gotoi. Its morphology is also closer to the latter species.

The Siberian Flora [5] reports *C. gotoi* from Khakasia (Bidzha river). We consider this to be the result of an erroneous identification, because our specimens from Khakasia (C279, C280, 282, C250) from adjacent regions belong to *C. songorica*.

We can thus conclude that *C. gotoi* и *C. songorica* are characterized by morphological and genetic differences and have different distributions. Although many botanists [2, 4, 5]

referred to *C. gotoi* as a subspecies of C. songorica, our results suggest that these taxa can be considered distinct species.

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