

Article

Typha lepechinii Mavrodiev et Kapit. sp. nov. (Typhaceae Juss.)—A New Endangered Endemic Cattail in the Outmost East of European Russia[†]

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Abstract: *Typha lepechinii*, a new species from European Russia, is described. In terms of the external morphology, it is similar to *T. shuttleworthii* but differs from the latter by the shape of female inflorescence, wider leaf blades and geographical distribution. Like *T. shuttleworthii*, *T. lepechinii* is clearly distinct from all other cattails of *T. sect. Ebracteolatae* due to its short male inflorescence that is 2–4 times shorter than the contiguous female inflorescence. The spatial disjunction of this new species from the main distribution range of predominantly European *T. shuttleworthii* is significant: *T. lepechinii* is an endemic of the outmost East of the Russian Plain, in particular, the regions of Middle and Southern Cis-Ural region (basin of the Middle and Lower Kama). In terms of the political administrative borders, this new cattail was found in the Udmurt Republic, the Republics of Tatarstan and Bashkortostan, the parts of the Russian Federation. From the elementary comparative molecular standpoint, *T. lepechinii* is different from its sister *T. shuttleworthii* due to four single nucleotide positions and two indels of the *rpl32* gene and *rpl32-trnL* intergenic spacer (cpDNA). The new cattail is named after I. I. Lepechin (1737–1802)—a Russian scientist-encyclopedist whose primary botanical interest focused on the regions of Ural and Siberia.

Keywords: Cis-Ural region of Russia; endangered species; Poales; *Typha* sect. *Ebracteolatae* *rpl32* gene and *rpl32-trnL* intergenic spacer (cpDNA)

1. Introduction

At least seven species and hybrids from *Typha* L. sect. *Ebracteolatae* have been listed as the flora of European Russia [1]. One of the most interesting taxa from this section is *Typha shuttleworthii* WDJ Koch and Sond., which has been described from Northern Switzerland and, as is estimated today, is distributed from Western, Southern and Central Europe to the Mediterranean regions, European Russia, Poland and Belarus [1–5]. This species was also reported in Northwestern Iran and Eastern Turkey [6]. However, because *T. shuttleworthii* is a predominantly Western European plant [1,7], here we tend to agree with Ghahreman and Sanei Chariat-Panahi [8] that the proper name for the Northwestern Iranian populations of Shuttleworth's cattail is actually *T. persica* Ghahr and Sanei Chariat-Panahi. The findings of *T. shuttleworthii* previously documented from the regions of Russian

Krasnodar Territory, Crimea and some areas of Caucasus [1] must also be clarified from a taxonomic standpoint.

Despite its relatively wide distribution range, *T. shuttleworthii* (incl. *T. transsilvanica*) is not a common species. This cattail is considered extinct (EX) in Hungary, critically endangered (CR) in Slovakia, Bulgaria and the Czech Republic, endangered (EN) in Serbia, strongly threatened (ST) in Germany, and vulnerable (VU) in Switzerland and Western Ukraine [3].

This taxon is extremely rare in Belarus [2,9]. Current, well-documented records of *T. shuttleworthii* in Russia include only the populations of its outmost Eastern European parts [10]. The age of the youngest of three known herbarium specimens of this cattail that were collected in Central European Russia (Moscow and Kaluga regions) has now exceeded 70 years [2]. Thus, both the current distribution as well as the conservation status of *T. shuttleworthii* in Russia is unclear now. However, it is fairly likely that this cattail has gone extinct in Central Russia, as has happened in Hungary [3].

The populations of Shuttleworth's cattail from the outmost European East Russia ("Pre-Ural" or "Cis-Ural" region) are of our particular interest not only because of their high vulnerability [10,11] but also due to significant disjunction from the main range of *T. shuttleworthii* in Western and Central Europe [1,3,10]. It is also remarkable that the Eastern European plants of this cattail are different from the Western European plants from both morphological [10,11] and molecular standpoints. We would like to officially establish that, in our current opinion, the Shuttleworth's cattail from the outmost regions of Eastern Europe represents a separate species. The goals of this study include the formal description of this previously unrecognized species, as well as its justification within the framework of the most recent plastid (cpDNA) sequence data, currently available for *T. sect. Ebracteolatae* [12,13].

2. Materials and Methods

Methods of the field work are already given in Kapitonova et al. [10]. Specimens were collected following standard techniques and deposited in the IBIW, TOB, UDU, and UFA herbaria.

The cpDNA matrix of *Typha* sect. *Ebracteolatae* was reconstructed using the set of previously published GenBank numbers for the *rpl32* gene and *rpl32-trnL* intergenic spacer. All GenBank numbers have been taken from Guisinger et al. 2010 (GU195652) [14], Zhou et al. (MG430863-70) [12], and Volkova and Bobrov (MZ302924, MZ302927, MZ302929, MZ302931-42, MZ302948-50, MZ302953) [13]. The cpDNA matrix includes sequences of most of the species of sect. *Ebracteolatae*, which have been recognized in Mavrodiev and Kapitonova [1] (except for the presumable hybrid *T. intermedia* Schur and hybrid *T. × argoviensis* Hausskn. ex Asch. et Graebn.), as well as the recently described *T. yakutii* A. Krasnova and Chemeris [15]. In case of *T. lepechinii*, an analyzed sequence of the *rpl32* gene and *rpl32-trnL* was obtained right from the holotype of this species (Figure 1) [13].



Figure 1. *Typha lepechinii* sp. nov: holotype, IBIW (unique collection number: 52711). Photo: E. A. Belyakov.

To reconstruct this cpDNA sequence matrix, we used Seaview ver. 5.0.4 [16] and Mesquite ver. 3.70 [17]. Alignment was performed with MUSCLE ver. 2.0 [18] as implemented by Seaview [16].

First, the simple summaries of the single nucleotide polymorphic positions (SNPs) and indels were established as separate figures (see Results). Of the 28 sequences examined, 22 sequences represent broadly defined *T. latifolia* L. Due to the clear patterns in sequence variation and the fact that many sequences were identical, not all of these 28 sequences were included in future cladistic analyses, and the placeholders of the identical sequences have been used instead. We also determined that within the obtained alignment there are five patterns of variation within the *rpl32* gene and *rpl32-trnL* intergenic spacer sequences, and included from one to a few sequences of each of these five patterns as placeholders in future cladistic analyses.

The three-taxon statement (3TS) permutations of reduced molecular alignment were conducted with TAXODIUM ver. 1.2 [19]. *Typha laxmanni* Lepechin was *a priori* defined as an outgroup taxon for maximum parsimony analysis, as well as an operational outgroup for the 3TS permutations of the molecular data (Williams–Siebert (WS) representation of unordered multistate characters; reviewed and implemented in Mavrodiev and Madorsky [19]).

The molecular matrix was analyzed using standard Fitch parsimony [20], the three-taxon statement analysis (3TA) [21], and the Average Consensus (AC) method [22,23], as it applied to the array of the 3TSs *a priori* presented exactly as minimal rooted trees (3TS-ACA) [24,25].

Upon obtaining the aligned molecular matrix, the summary of indels was constructed in Mesquite [17] and manually recorded as a reduced binary (“presence–absence”) matrix following the assumption that the lack of indel is a plesiomorphic character-state. This binary matrix was analyzed using Wagner parsimony [20], the 3TA following fractional weights of the 3TSs [19,20], and the modified AC method [24,25].

The Fitch parsimony, Wagner parsimony, 3TA and 3TS-ACA were conducted in PAUP* ver. 4.0a [26] and Clann ver. 4.1.5 [27], as described in Mavrodiev and Madorsky [19] and Mavrodiev et al. [24,25].

3. Results

3.1. Taxonomic Treatment

Order Poales Small.
 Family Typhaceae Juss.
 Genus *Typha* L.
 Section *Ebracteolatae* Graebn.
Typha lepechinii Mavrodiev et Kapit. sp. nov.

3.2. Diagnosis

Typha lepechinii sp. nov. is similar to *T. shuttleworthii* but differs from the latter by the morphology of female inflorescence, wider leaf blades and geographical distribution.

Mature female inflorescence (9) 12–18 (29) cm long, slightly ashy at the base. Leaf blades 10–15 (20) mm wide. Endemic of the Cis-Ural region (the outmost East of the Russian Plain) *T. lepechinii* sp. nov.

Mature female inflorescence 5–15 (20) cm long, with an ashy, silvery shade. Leaf blades 5–10 (15) mm wide. Predominantly Western European plant *T. shuttleworthii*.

Like *T. shuttleworthii*, *T. lepechinii* sp. nov. clearly differs from all other cattails of sect. *Ebracteolatae* due to its short male inflorescence that is 2–4 times shorter than the contiguous female inflorescence.

3.3. Etymology

The species is named after a member of St. Petersburg's Academy of Science Ivan Ivanovich Lepechin (1737–1802)—a famous Russian scientist-encyclopedist, traveler, and naturalist, whose primary botanical interest focused on the regions of Ural and Siberia.

3.4. Material Examined

Type

RUSSIA—holotype: Udmurt Republic • City of Izhevsk, vicinity of the botanical garden of Udmurt State University, drainage channel; 56°55' N, 53°15' E; 25 July 2010; "O.A. Kapitonova; holotype: IBIW (n. 52711) (Figure 1), isotype: TOB (n 6538)".

Paratypes

1. RUSSIA—Udmurt Republic • Alnashsky district, five km south of the village Muvazhi; ~56°01' N, 52°42' E; shallow water of a small pond on river Golyushurminka; 1 July 2006; V. I. Kapitonov, O. A. Kapitonova (TOB, n 6540);
2. RUSSIA—Republic of Tatarstan • Mendeleev district, vicinity of village Izhevka (1.5 km N-NW from the village), small creek dam, in shallow riverside water and on the damp shore; 28 June 2006; V. I. Kapitonov, O. A. Kapitonova (TOB, n 6539);
3. RUSSIA—Republic of Bashkortostan • Miyakinsky district; 53°31'57.70" N, 54°50'28.73" E; on the damp shore along the stream Kurmanay, 1.9 km from the village Kurmanaibash; 23 July 2014; A. A. Muldashev (UFA, n 289).

Description

Perennial herb 1.1–1.5 (2) m tall, robust. Leaves linear, lamina (leaf blade) 44–78 (100) cm × 10–15 (20) mm, pale green, equal to or slightly longer than reproductive shoot. The width of the dry lamina is 2–4 mm less than that of living plants. The female inflorescence (Figure 2A) is cylindrical (9) 12–18 (29) × 0.7–2.4 cm; if mature it is brown and slightly ashy at its base, but green or dark green when blooming (Figures 2B and 3). Basal parts ("protuberances") of separate female spikelets equal to or shorter than 1 mm long, with an apex of 1–2 (3) abortive flowers (carpodia) and sometimes with a single poorly developed flower (Figure 4). Female flowers are 7.4–8.8 mm long. The gynophore's hairs are 7.1–8.5 mm long, slightly shorter, equal to, or slightly longer than the stigma, and are scabrous due to small spiny prongs on its outer cell walls (Figure 5). Stigma lanceolate pointed at the apex, 0.3–0.7 × 0.1–0.2 mm. Bracts absent. Carpodia 6.4–6.9 mm long, with upper (thickened) part 0.9–1.3 × 0.2–0.5 mm, slightly convex at apex, usually with a long cusp. Ovaries are 0.8–1.5 mm in length, seeds are 0.6–0.8 mm in length. The male inflorescence is 1.8–8 cm long, 2–4 times shorter than the female (Figures 2 and 3), with an axis covered by thin, but slightly thickened at the apex, narrow-lanceolate hairs. Male and female inflorescences are contiguous. Male flowers of 4–6 stamens (Figure 6), with anthers 1.6–1.9 mm long and filaments 2.1–3.3 mm long. Pollen in fertile tetrads. Flowering: late June–July; fruiting: August–September.

3.5. Distribution and Ecology

Typha lepechinii sp. nov. is distributed in the East of the Russian (Eastern European) Plain, in the regions of Middle and Southern Cis-Urals (basin of the Middle and Lower Kama). In terms of the political administrative borders, the new cattail was found in the Udmurt Republic, the Republics of Tatarstan and Bashkortostan (Figure 7).



Figure 2. *Typha lepechinii* sp. nov.: male and female inflorescences (A) and general aspect (B). A. Russia, Udmurt Republic, City of Izhevsk, vicinity of the botanical garden of Udmurt State University, drainage channel; 56°55' N, 53°15' E; 25 July 2010 (*locus classicus*). B. Russia, Udmurt Republic, Alnashsky district, five km south of the village Muvazhi; ~56°01' N, 52°42' E; shallow water of a small oxbow on river Golyushurminka. Photos: V. I. Kapitonov, modified from Kapitonova et al. [10] with permission from the *American Journal of Plant Science*.



Figure 3. *Typha lepechinii* sp. nov.: general aspect. Russia, Republic of Bashkortostan, Miyakinsky district; 53°31'57.70" N, 54°50'28.73" E; on the damp shore along the stream Kurmanay, 1.9 km from village Kurmanaibash. The female inflorescences are young and green. Photo: A. A. Muldashev.

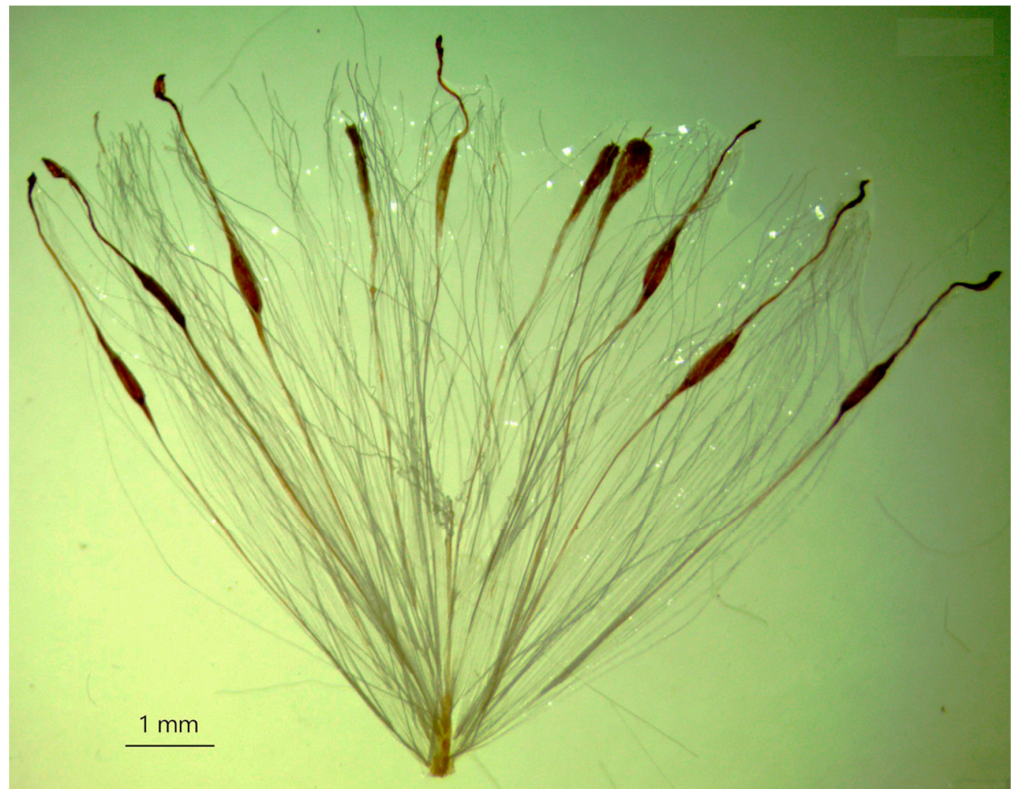


Figure 4. *Typha lepechinii* sp. nov.: female spikelet. Photo: O. A. Kapitonova.

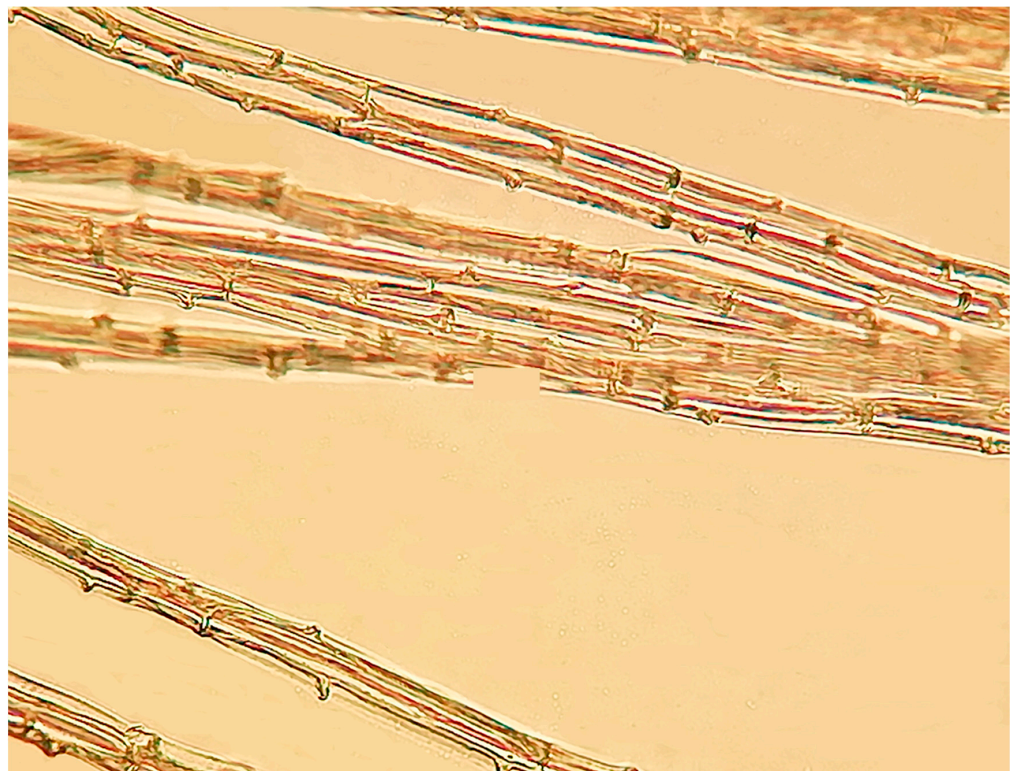


Figure 5. *Typha lepechinii* sp. nov.: gynophore's hairs. Photo: O. A. Kapitonova.

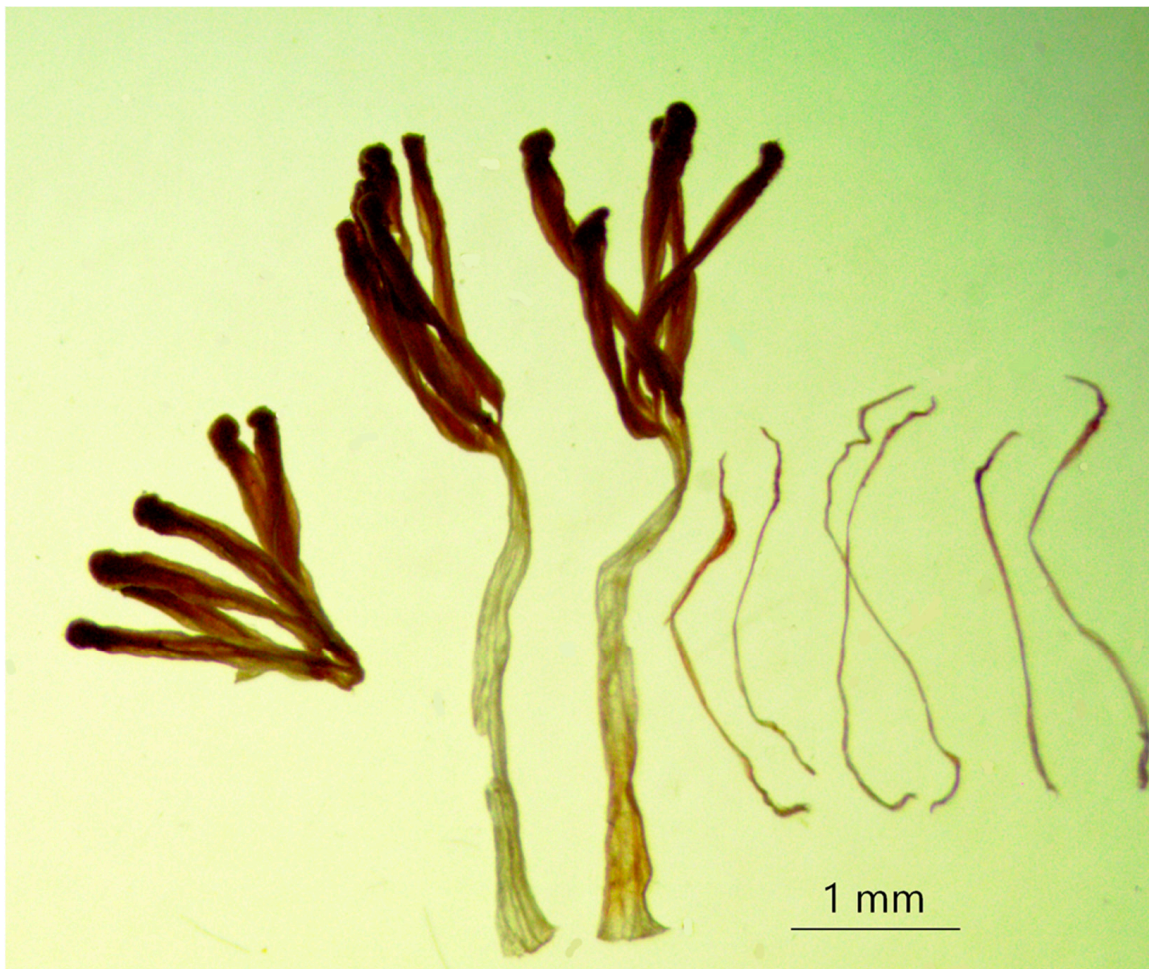


Figure 6. *Typha lepechinii* sp. nov.: Male flower (on the left) and the hairs of the axis (on the right). Photo: O. A. Kapitonova.

Typha lepechinii sp. nov. is a continental species [10,11]. It grows in shallow waters, damp and dry shores of small oxbows, ponds and reclamation canals, and along riversides and streams. Within the Udmurt Republic, *T. lepechinii* sp. nov. is now existing within secondary habitats, such as artificial ponds and drainage channels [10,11].

3.6. Conservation Status

Typha lepechinii sp. nov. was initially considered a highly vulnerable species (VU) [10] but under binomial '*T. shuttleworthii*' is currently listed in the Red List of the Republic of Bashkortostan with a first category of rarity (EN) [28].

3.7. Cladistic Analyses of cpDNA Sequence Data

The results of cladistic analyses of the cpDNA sequence data of *Typha lepechinii* sp. nov. and related species are summarized in Figures 8–11.

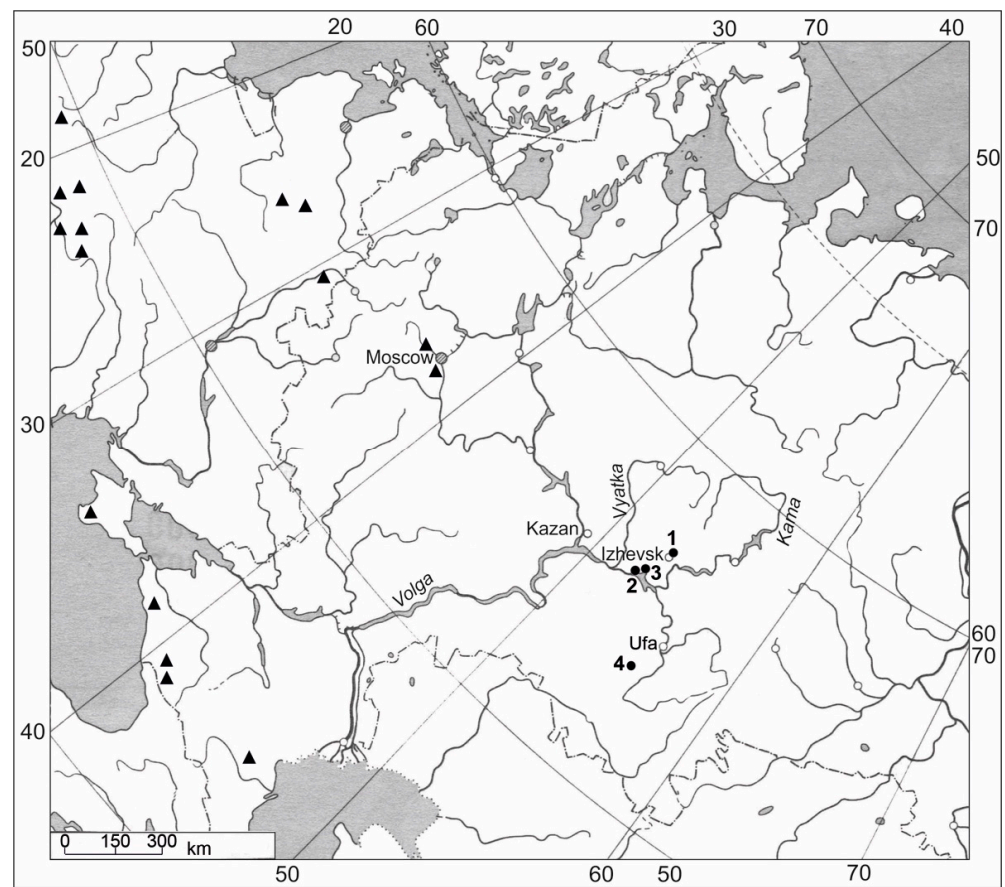


Figure 7. *Typha lepechinii* sp. nov. (points 1–4): distribution area. 1—Russia, Udmurt Republic; city of Izhevsk, vicinity of the botanical garden of Udmurt State University (56°55' N, 53°15' E); 2—Russia, Republic of Tatarstan • Mendeleev district, vicinity of village Izhevka, 3—Russia, Udmurt Republic, Alnashsky district, five km south of the village Muvazhi, river Golyushurminka (~56°01' N, 52°42' E); 4—Russia, Republic of Bashkortostan, Miyakinsky district; on the damp shore along the stream Kurmanay, 1.9 km from the village Kurmanaibash (53°31'57.70" N, 54°50'28.73" E). The distribution sites for *T. shuttleworthii* have been drawn in triangles. Drawing: O. A. Kapitonova. Map modified from Kapitonova et al. [10] with permission of the *American Journal of Plant Science*.

3.7.1. Standard cpDNA Matrix (No Indels Included)

The total number of characters in the final trimmed molecular alignment of *T. sect. Ebracteolatae* with no Outgroup added/with included Outgroup (*Typha laxmannii*) was 745/745, consisting of 728/720 invariable characters, and 17/25 variable characters (all mutually congruent), 13/13 of each were informative for standard maximum parsimony analysis. After the number of taxa was reduced to 10, this matrix implies 816 3TSs (again, all mutually congruent and informative) if permuted relatively *T. laxmannii*.

Manually analyzing the obtained cpDNA matrix, we found five distinct patterns in sequence variation that differed from each other by 1–12 SNPs (Figure 8).

While Patterns I (*T. lepechinii* sp. nov.) and II (*T. shuttleworthii*) each contain a single sequence, the other patterns contain more than one sequence. Pattern III contains 17 sequences (one sequence of *T. elata*, *T. incana*, *T. yakutii* and 14 sequences of *T. latifolia*), Pattern IV is of five sequences (one of *T. caspica* and four of *T. latifolia*), Pattern V is of four sequences of *T. latifolia*.

Each pattern of variation is clearly congruent to the geography of the sequenced samples (Figure 8). Pattern I is represented by *T. lepechinii* sp. nov., a narrow endemic of the outmost East of the European Russia (see above).

Variable positions of <i>rpl32</i> gene and <i>rpl32-trnL</i> intergenic spacer																					
Species	24	32	45	177	195	205	221	372	416	438	447	517	540	569	605	664	665	Pattern	Location	Predominant distribution	GenBank #
<i>Typha lepechinii</i> sp. nova	C	C	G	T	G	A	A	G	G	A	G	A	A	G	T	A	G	I	Russia: Udmurtia	Outmost E of Europe	MZ302950
<i>T. shuttleworthii</i> W.D.J.Koch & Sond.	G	C	G	T	T	A	A	G	G	T	G	A	T	G	T	A	G	II	Germany: Bavaria	C and W Europe	MG430863
<i>T. elata</i> Boreau	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Tver region	Eurasia, N America	MZ302927
<i>T. incana</i> Kapit. & Dyukina	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Udmurtia		MZ302929
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Tyumen region - Khanty-Mansiisk		MZ302931
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yaroslavl region		MZ302937
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yaroslavl region		MZ302949
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: C Sakhalin		MZ302938
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yakutia		MZ302934
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yakutia		MZ302932
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yakutia		MZ302933
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yakutia		MZ302939
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Magadan region		MZ302942
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	USA: VA		MG430869
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	USA: AK		MG430870
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	USA: AZ		GU195652
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Germany: Bavaria		MG430868
<i>T. latifolia</i> L.	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	UK: England		MG430867
<i>T. yakutii</i> Krasnova & Chemeris	C	T	T	-	G	A	C	G	A	T	G	T	T	T	G	T	T	III	Russia: Yakutia		MZ302953
<i>T. caspica</i> Pobed.	C	C	T	A	G	A	A	A	A	T	G	T	T	T	G	T	G	IV	Russia: Krasnodar region	Africa, Europe (mostly S Russia), Caucasus, S Asia, C Asia and its nearest neighboring areas of Western Siberia	MZ302924
<i>T. latifolia</i> L.	C	C	T	A	G	A	A	A	A	T	G	T	T	T	G	T	G	IV	Russia: Omsk region		MZ302935
<i>T. latifolia</i> L.	C	C	T	A	G	A	A	A	A	T	G	T	T	T	G	T	G	IV	Russia: Kurgan region		MZ302936
<i>T. latifolia</i> L.	C	C	T	A	G	A	A	A	A	T	G	T	T	T	G	T	G	IV	China: Xingjiang		MG430866
<i>T. latifolia</i> L.	C	C	T	A	G	A	A	A	A	T	G	T	T	T	G	T	G	IV	Kenya		MG430864
<i>T. latifolia</i> L.	C	C	T	A	G	C	A	A	A	T	T	T	T	T	G	T	G	V	Russia: outmost S of Sakhalin	E Asia (E China, outmost S of Sakhalin, Kuril Islands)	MZ302948
<i>T. latifolia</i> L.	C	C	T	A	G	C	A	A	A	T	T	T	T	T	G	T	G	V	Russia: Kuril Islands - Yurii		MZ302940
<i>T. latifolia</i> L.	C	C	T	A	G	C	A	A	A	T	T	T	T	T	G	T	G	V	Russia: Kuril Islands - Kunashir		MZ302941
<i>T. latifolia</i> L.	C	C	T	A	G	C	A	A	A	T	T	T	T	T	G	T	G	V	China: Heilongjiang		MG430865

Figure 8. The summary of five (I–V.) patterns in variation of the plastid (*rpl32* gene—*rpl32-trnL* intergenic spacer) sequence data matrix of all analyzed species of *Typha* sect. *Ebracteolatae* were conducted with a special focus on *T. lepechinii* sp. nov.

Indels of <i>rpl32</i> gene and <i>rpl32-trnL</i> intergenic spacer											
Species	1	2	3	4	5	6	7	Pattern	Location	Predominant distribution	GenBank #
<i>Typha lepechinii</i> sp. nova	90–98	Ns	N	172–176	Ns	Ns	351–356	A	Russia: Udmurtia	Outmost E of Europe	MZ302950
<i>T. shuttleworthii</i> W.D.J.Koch & Sond.	Ns	Ns	N	Ns	Ns	Ns	351–356	B	Germany: Bavaria	C and W Europe	MG430863
<i>T. elata</i> Boreau	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Tver region	Eurasia, N America	MZ302927
<i>T. incana</i> Kapit. & Dyukina	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Udmurtia		MZ302929
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Tyumen region: Khanty-Mansiisk		MZ302931
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yaroslavl region		MZ302937
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yaroslavl region		MZ302949
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: C Sakhalin		MZ302938
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yakutia		MZ302934
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yakutia		MZ302932
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yakutia		MZ302933
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yakutia		MZ302939
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Magadan region		MZ302942
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	USA: VA		MG430869
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	USA: AK		MG430870
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	USA: AZ		GU195652
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Germany: Bavaria		MG430868
<i>T. latifolia</i> L.	Ns	135–170	171	172–176	177–184	Ns	351–356	C	UK: England		MG430867
<i>T. yakutii</i> Krasnova & Chemeris	Ns	135–170	171	172–176	177–184	Ns	351–356	C	Russia: Yakutia		MZ302953
<i>T. caspica</i> Pobed.	Ns	Ns	171	172–176	Ns	Ns	Ns	D	Russia: Krasnodar region	Europe (mostly S Russia), Caucasus, S Asia	MZ302924
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	Kenya	Africa,	MG430864
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	China: Xingjiang	C Asia and its	MG430866
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	Russia: Kurgan region	nearest neighboring areas	MZ302936
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	247–257	351–356	E	Russia: Omsk region	of Western Siberia	MZ302935
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	Russia: outmost S of Sakhalin	E Asia	MZ302948
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	Russia: Kuril Islands - Yurii	(E China, outmost S of Sakhalin, Kuril Islands)	MZ302940
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	Russia: Kuril Islands - Kunashir		MZ302941
<i>T. latifolia</i> L.	Ns	Ns	171	172–176	Ns	Ns	351–356	F	China: Heilongjiang		MG430865

Figure 9. The summary of six patterns of variation of indels within plastid (*rpl32* gene–*rpl32-trnL* intergenic spacer) sequence data matrix of all analyzed species of *Typha* sect. *Ebracteolatae* were conducted with a special focus on *T. lepechinii* sp. nov. Numbers indicate missing entities, letter N means “aNy nucleotide”, letters A–F assigned for the different patterns of variation of the indels.

Taxa	Indels	1	2	3	4	5	6	7
<i>Typha lepechinii</i>		1	0	0	1	0	0	1
<i>T. shuttleworthii</i>		0	0	0	0	0	0	1
<i>T. elata</i>		0	1	1	1	1	0	1
<i>T. incana</i>		0	1	1	1	1	0	1
<i>T. latifolia</i> MZ302931		0	1	1	1	1	0	1
<i>T. yakutii</i>		0	1	1	1	1	0	1
<i>T. caspica</i>		0	0	1	1	0	0	0
<i>T. latifolia</i> MZ302935		0	0	1	1	0	1	1
<i>T. latifolia</i> MZ302941		0	0	1	1	0	0	1
<i>T. latifolia</i> MZ302936		0	0	1	1	0	0	1
<i>T. latifolia</i> MG430865		0	0	1	1	0	0	1
Outgroup		0	0	0	0	0	0	0

Figure 10. Similar to Figure 9, but simplified and established as a binary (“presence–absence”) matrix under the assumption that the presence of an indel is an apomorphic character state.

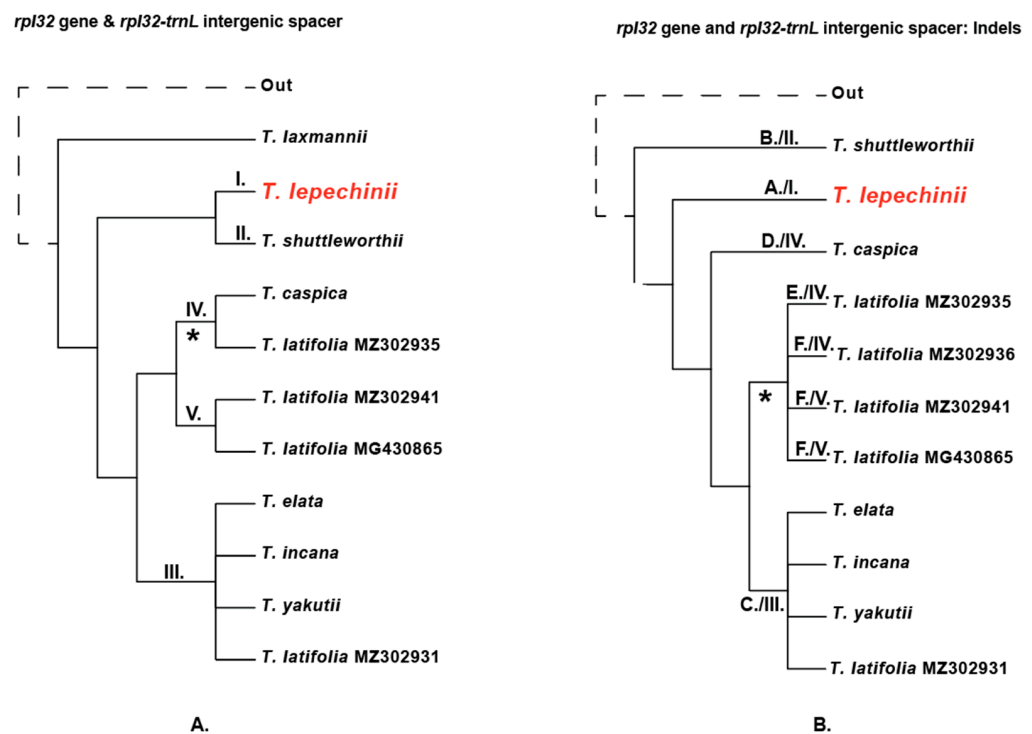


Figure 11. (A) The results of comparative Cladistic analysis (3TS-ACA) of five patterns of variation (I–V.) of cpDNA (*rpl32* gene–*rpl32-trnL* intergenic spacer) sequence data of *Typha* sect. *Ebracteolatae* (Figure 8). The hierarchy of patterns is given as a strict consensus of 15 average consensus trees with a score of 0.17160. Fitch parsimony and 3TA yielded similar results, but with Pattern IV (indicated by an asterisk) defined as a polytomy by both analyses. (B) The results of comparative Cladistic analysis (3TS-ACA) of six patterns of variation of indels (A.–F.) within plastid (*rpl32* gene–*rpl32-trnL* intergenic spacer) sequence data matrix. The hierarchy of patterns is given as a strict consensus of 225 average consensus trees with a score of 0.41648. Wagner parsimony and 3TA yielded similar results, but with pattern IV and pattern V (indicated by an asterisk) defined as polytomy by both analyses.

Pattern II is represented by *T. shuttleworthii*, a predominantly Western and Central European taxon. The exact sequence data of *T. shuttleworthii* that was included in the comparisons and cladistic analyses was obtained from a sample that had been collected in Bavaria [12], the closest neighboring German region of Switzerland.

We can continue this logic, even while keeping in mind the high invasiveness of the broad-leaf cattail.

Pattern III includes species that are broadly distributed across Eurasia and North America, but are predominantly found in northern and temperate areas.

The predominant distribution of Pattern IV includes Europe (mostly Southern Russia), Caucasus, Southern Asia, Central Asia and its nearest neighboring areas of Western Siberia, as well as Africa (Kenya). Contrary to Pattern III, Pattern IV clearly gravitates towards the South, not the North.

Finally, the remarkably distributed Pattern V includes the sequence data obtained from its plants, collected solely in the Far East of China (province Heilongjiang), in the outmost South of Sakhalin Island (Russia) as well as in the Russian Kurils (islands Yurii and Kunashir). Fitch parsimony analysis (the single most parsimonious tree of length 25, CI = RI = 1.000), 3TA (the single most parsimonious tree of length 816, RI = 1.000) and 3TS-ACA (strict consensus of 15 trees of the score 0.17160) yielded the similar results (Figure 11A):

- a. *Typha* sect. *Ebracteolatae* appeared as monophyletic [12];
- b. *Typha lepechinii* sp. nov. (Pattern I) is sister of *T. shuttleworthii* (Pattern II);
- c. Clade *T. lepechinii* sp. nov. plus *T. shuttleworthii* defined as a sister of all the remaining analyzed taxa of *T. sect. Ebracteolatae*;
- d. The clade that corresponds to Pattern III, is a sister of the clade that includes sequences of Patterns IV and V, but Pattern IV is defined as a polytomy by Fitch parsimony as well as by 3TA.
- e. In all the analyses *T. latifolia* appears as clearly polyphyletic.

3.7.2. Indel-Based Binary Matrix

The obtained cpDNA matrix contains seven indels (Figure 9). From these seven, five are parsimony-informative.

Wagner parsimony analysis of the indel-based binary matrix with all-zeros Outgroup included (Figure 10) (the single most parsimonious tree of length eight, CI = 0.8750; RI = 0.900), the 3TA of the same matrix (the single most parsimonious tree of length 79.384, RI = 0.9553), as well as the 3TS-ACA of this matrix (strict consensus of 225 trees of the score 0.41648), again yield similar results (Figure 11B), which are, however, slightly different from the results of the cladistic analyses of the molecular dataset with no indels included (see above):

- a. *Typha shuttleworthii*, not the clade (*T. shuttleworthii* + *T. lepechinii* sp. nov.), is defined as a sister of all the remaining taxa of *T. sect. Ebracteolatae*;
- b. *Typha lepechinii* sp. nov. is a sister of all the remaining analyzed taxa of sect. *Ebracteolatae*, excluding *T. shuttleworthii*;
- c. The clade that corresponds to Pattern III, is a sister of the clade that includes most sequences of Patterns IV (but not *T. capsica*) and V, but both Patterns are again defined as polytomies in all analyses;
- d. Clade (Pattern IV + Pattern V) is defined as a polytomy by Fitch parsimony as well as by 3TA;
- e. Pattern IV appears as paraphyletic due to the sisterhood of *T. capsica* and the clade (Patterns III + IV + V);
- f. Pattern IV is also the most variable in terms of the presence/absence of indels (see the indel-based patterns A–F (Figures 9 and 11B), which are not all necessary informative from a cladistic standpoint;
- g. In all the analyses of the indel-based binary matrix, *T. latifolia* appears as clearly polyphyletic (Figure 11B).

A simple visual comparison of the cpDNA sequence data shows that *T. lepechinii* sp. nov. is different from the sister *T. shuttleworthii* by four SNPs [13] and two indels (Figures 8 and 9).

4. Discussion

William Whewell (1794–1866) pointed out that the essence of Natural Classification can be understood from this simple proposition: “. . . natural arrangements obtained from different sets of characters, must *coincide* with each other” (summarized in Williams and Ebach [29]). This is what we see in the case of *Typha lepechinii* sp. nov.—from a Cladistic point of view, this species is nothing but the original relationship, obtained from cladistic analyses of the cpDNA sequence data. However, simultaneously, it also differs from all other cattails of *T. sect. Ebracteolatae* in external shape and geographical distribution. In other words, the recognition of new species is not solely the result of the cladistic analyses of cpDNA sequence data. The *congruence* between different sources of evidence (including molecular data) is the main argument in the taxonomic recognition of *T. lepechinii* sp. nov.

Even if the power of the *rpl32* gene and *rpl32-trnL* intergenic spacer is not sufficient to discriminate against *T. incana* and *T. elata* [13], this molecular marker is useful for the molecular studies at low taxonomic levels [30], in particular for the cpDNA-based systematic studies of the cattails of *T. sect. Ebracteolatae*. For example, the obtained trees based on the *rpl32* gene and *rpl32-trnL* spacer cpDNA sequence data (Figure 11) are not only sufficient to discern *T. lepechinii* sp. nov. from *T. shuttleworthii*, but also to show the polyphyly of the genetically heterogeneous *T. latifolia*, and also to clearly differentiate between morphologically related *T. elata* and *T. caspica* [1].

Again, the patterns in variation of this molecular marker are congruent with predominant geographical distributions of individual plants whose cpDNA was sequenced, and thus the predictive power of the marker is essential, even if it is still not the most variable region of the plastid genome of *Typha* [14]. Keeping in mind the results of the current comparative analyses of cpDNA sequence data (Figure 11), it is easy to predict, for example, that the populations of *T. latifolia* from the outmost South of Sakhalin, Russian Kuril Islands and the Far East of China (Pattern V), actually represent species from *T. sect. Ebracteolatae*, previously unknown for floras of China and Russia.

Unrooted phylogenetic analyses, especially if combined with a priori selected lumping style of treating diversity within *Typha* [13], as well within any other group of flowering plants, may have dangerous environmental protection connotations. In this paper it is enough to stress that just the taxon (e.g., species), not the “haplotype” or any semantically similar formal construct from the language of biosystematics or the phylogeography, is an actual object of protection in any Red List of Plants., for example, in the European Red List of Vascular Plants [31]. Due to this, the status of vulnerable or endangered plants must be accurately established within a clear taxonomic framework.

The communities with a dominance of *T. lepechinii* are normally moderate in size and typically have low taxonomic diversity [10]. General projective cover of such habitats varies between 50–100%; the overabundance of *T. lepechinii* itself changes from two to four if estimated following the Braun-Blanquet scale, with the ratio of the number of “generative” to “vegetative” shoots close to 2:3 [10]; the competitive potential of populations of *T. lepechinii* relative to *T. latifolia* is fairly low. As we already mentioned above, the new species was found either in natural areas and frequently, in secondary habitats [10]. Thus, *T. lepechinii* may be viewed as an illustration of the phenomenon of “extinction debt” [32]. From that, the secondary landscapes may be considered as a kind of temporary refuge, as they are in a way still helping this endangered cattail to survive within the current biota [32,33].

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