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A new species of *Tamarix* (*Tamaricaceae*) from Hormozgan Province, S Iran, supported by morphology and molecular phylogenetics

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Abstract: The genus *Tamarix* (*Tamaricaceae*) is a lineage of shrubs and trees with leaves reduced to scales and numerous species adapted to moist and often saline soils in arid and semi-arid climates. Extensive morphological variation and hybridization complicate species delimitation and identification. Based on both morphological and DNA sequence characters, *Tamarix humboldtiana* Akhani, Borsch & N. Samadi is described as a new species from S Iran. Phylogenetic analysis of plastid *rpl16* intron and *trnG-trnS* spacer sequences depicts a sister group relationship of its unique plastid haplotype to *T. tetrandra*, whereas nuclear ITS sequence data show close affinities to *T. kotschyi*. The new species differs from *T. kotschyi* by distinctly pedicellate, 5-merous flowers and vaginate-amplexicaul leaves. The stem and foliar anatomy and the epidermal micromorphology provide additional characters differentiating the new species from *T. kotschyi*. The gametic chromosome number of $n = 12$ reflects that of most of other species of the genus. *Tamarix humboldtiana* is a rare species living at freshwater riversides in S Iran and is according to current knowledge critically endangered.

Key words: *Caryophyllales*, endemic species, flora of Iran, molecular diagnosis, phylogenetics, reticulate evolution, species concept, *Tamaricaceae*, *Tamarix*

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Introduction

The genus *Tamarix* L. constitutes the most speciose lineage of *Tamaricaceae* and is widely distributed in arid zones of the Old World with major diversity in the Irano-Turanian area (Baum 1978; Qaiser 1981). The monograph of Baum accepted 54 species, a number that has increased to 62 by newly described species (Hernández-Ledesma & al. 2015; Villar & al. 2015b). However, there is great discrepancy in the circumscription of species, and regional accounts of *Tamarix* often arrive at different treatments in comparison to Baum (1978). Species of *Tamarix* are difficult to delimit because of high morphological plasticity and frequent hybridization (Rusanovich 1986). Several species invaded habitats outside their natural range (Gaskin & Schaal 2002), where they also introgress with native species (Mayonde & al. 2015).

In an ongoing research project on the evolution and diversity of the genus *Tamarix*, a population deviating morphologically from the widespread *T. kotschyi* Bunge was discovered in Hormozgan Province, S Iran. Molecular phylogenetic inference indicated that this entity

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belongs to a lineage comprising the Irano-Turanian *T. kotschyi* and the East Mediterranean and Caucasian *T. tetrandra* Pall., but the Hormozgan population differs markedly by morphological characters, in particular the number of floral parts and the shape and anatomy of the leaves. We then added further available material morphologically similar to *T. kotschyi* from the region to cover the variability of that species.

As a basis for describing a species new to science nowadays, a biological entity should be recognized that is inferred in an evolutionary context (Borsch & al. 2015). Phylogenetic analysis of molecular data are thus not only useful to identify relatives but also instrumental to examine species limits, although this depends on practical taxonomic research with the availability of plants in collections or the possibility to encounter several populations in the field. Molecular data obtained from the individual that has provided the type specimen have become an integral part of the diagnosis (González Gutiérrez & al. 2013). This is of particular importance in speciose genera with many morphologically similar taxa, where discrete molecular characters (SNPs, microstructural mutations) with well-established orthology can complement morphological characters in a diagnosis and will be helpful to robustly place the type in later evolutionary studies. The use of molecular data in addition to morphology is of particular importance in genera with frequent hybridization such as *Tamarix* to unravel individuals of hybrid origin (Gaskin & Schaal 2002, Mayonde & al. 2015).

The specific objectives of this paper are to clarify relationships and identity of the morphologically deviant *Tamarix* collection from Hormozgan Province in Iran (*Akhani 21693*), to provide diagnostic characters both from morphology and DNA and, based on this, describe this entity as a new species.

Material and methods

Field work and plant material

Plant materials were collected during two field expeditions in 2011 and 2013. The herbarium specimens and the type specimens are preserved in the herbarium of the Halophytes and C_4 plants Research Laboratory (herb. Akhani), housed at the School of Biology, University of Tehran and duplicated in the herbaria of Iranian Research Institute of Plant Protection (IRAN) and the Botanic Garden and Botanical Museum Berlin (B). The habitat of the species was studied by recording cover-abundance and vegetation data in a plot according to Braun-Blanquet (1964). The Electric Conductivity (EC) of the water in the river was measured by a commercial EC-meter Windaus (Model WinLab Data Line).

Morphology

The morphological descriptions are provided by detailed examination and measurements of the specimens. The details of floral parts were first photographed by an Olympus stereomicroscope (SZX 12) coupled with a digital camera DP 12. The details were then measured using the software Olysia Bioreport 3.2 (Build 670).

Karyotype

Young flowers were fixed in Pinar solution (ethanol: chloroform: propionic acid; 6:3:2) in the field in March 2013 for at least 48 hours at 4°C, and were then stored in 70% ethanol in a refrigerator. Slides were prepared by squashing anthers in 2% acetocarmin. All slides were examined under a Nikon OPTOPHOT-2 and photographed by a Moticam 2300 digital camera.

Anatomy

Segments of first-year stems (3–4 mm long) were fixed in AFE (acetic acid glacial- formalin- ethanol 70%, 1:1:9). They were dehydrated in an ethanol series and embedded in Araldite resin (TAAB, Berkshire, U.K.) based on Millonig (1976). Ultra-thin transverse sections (1.8 µm) were made using a glass knife and a Leica Ultracut UCT microtome (University of Tehran). Sections were stained using 0.5% (w/v) Toluidine blue in 1% (w/v) Na_2CO_3. Images were taken with DP12 Olympus digital camera mounted on an Olympus BX51 microscope.

For epidermal studies we followed slightly modified procedures in Bokhari (1971) and Akhani & al. (2013). Leaves were stored in KOH 10% plus a few drops of H_2O_2 for 12 hours, then were washed 3 times with distilled water and immersed in H_2O_2 + distilled water for two hours. After washing with distilled water, leaves were soaked in Javel water for an hour. Macerated epidermis was separated and stained with Toluidine blue for a minute. Permanent slides were prepared using Entellan mounting media (Merck, Darmstadt, Germany). Quantitative traits were measured using image processing software ImageJ (http://rsb.info.nih.gov/ij/).

Molecular phylogeny

DNA isolation and sequencing of ITS followed the approach described in Malekmohammadi & al. (2017). PCR conditions for the *trnG-trnS* spacer were as described in Schäferhoff & al. (2009) with the primers trnS and trnG (Hamilton 1999) for amplification and sequencing and for the *rpl16* intron followed Sánchez-del Pino & al. (2012). The sequences were edited manually and aligned with a motif-based approach (Löhne & Borsch 2005) using PhyDE (Müller & al. 2012). Simple indel coding (Simmons & Ochoterena 2001) was implemented with SeqState (Müller 2005) to generate a presence-absence matrix that was added to the sequence matrix. Parsimony analysis was done with PAUP* v.4.0b10 (Swofford 2002), employing random stepwise addition with 1 tree held at each step and TBR branch swapping. Jackknifing employed 10,000 replicates with 36.788% of the characters deleted and one tree held at each replicate. MrBayes v. 3.2.5 (Ronquist & al. 2012) and a GTR+G

model was used for Bayesian inference (BI) as determined with jModeltest2 (Darriba & al. 2012). Four runs with four chains each were carried out for 10 million generations and the first 25% of the trees discarded as burn-in. This fitted with convergence diagnostics (standard deviation of split frequencies), effected sampling sizes and detection of stationarity based on log-likelihood plots. *Myricaria* Desv. and *Reaumuria* L. constituted the outgroups in both data sets. Trees were than illustrated with TreeGraph2 (Stöver & Müller 2012). Our phylogenetic analysis focuses on the samples relevant to the description of this new species but is excerpted from a larger data set generated in an ongoing phylogenetic analysis of the genus *Tamarix*. We have deliberately included several accessions of *T. kotschyi* to ensure that our new species is not just a hybrid or an aberrant population of this species.

Results and Discussion

Description of new species

Tamarix humboldtiana Akhani, Borsch & N. Samadi, **sp. nov.** – Fig. 1, 2.

Holotype: Iran, Hormozgan, c. 100 km ENE of Bandar Abbas, 2 km S of Bikah village, along Minab river near Deh Gel Kan village, 27°19'56"N, 57°12'07"E, 153 m, 8 Mar 2011, *H. Akhani 21693* (IRAN; isotypes: B [barcode B 10 0465441; stable identifier http://herbarium.bgbm.org/object/B100465441], herb. Akhani).

Morphological diagnosis — Based on molecular phylogenetic data, *Tamarix humboldtiana* is closely related to *T. kotschyi* and *T. tetrandra*. It differs from both species by its pentamerous flowers. It differs further from *T. kotschyi* by its clearly pedicellate (vs ± sessile) flowers. Anatomically, *T. humboldtiana* differs from *T. kotschyi* by the absence of ingrowing papillae that overarch the stomatal pores.

Molecular diagnosis — *Rpl16* intron, positions in the sequence of the type specimen, upstream of the large 3′ exon: nucleotide character states "C" in pos. 135, "G" in pos. 195, "C" in pos. 323, "G" in pos. 896. *TrnG-trnS* spacer, positions in the sequence of the type specimen, downstream of the *trnG* exon: nucleotide character state "T" in pos. 550.

Morphological description — Shrubs, to 2 m tall; *bark* reddish, glabrous; inflorescence parts indistinctly papillose, irregularly powdery on surface under high magnification (×100). *Leaves* subvaginate, 1.1–2 mm long; young stems 5–7 mm in diam., covered by amplexicaul leaves. *Racemes* appearing in winter, prior to development of leaves, ± perpendicular to slightly oblique to inflorescence axis, 1–2.5 × 0.6–0.7 cm, 7–20-flowered; *pedicels* of all flowers 1–1.5 mm long; *bracts* shorter than pedicels, amplexicaul-vaginate, triangular, 0.75–1 mm long, margin membranous, entire or irregularly crenate, apex acute. *Flowers* pentamerous. *Calyx* ovate, (1–)1.1–1.4(–1.5) × 0.8–1 mm, margin with a narrow, membranous band 0.18–0.23 mm wide, apex ± obtuse to ± acute. *Petals* white, elliptic, (1.6–)2–2.2 × 0.8–1.1 mm, apex obtuse. *Staminal disk* synlophic (peridiscal and lacking intermediate lobes), dark red, c. 0.8 mm in diam.; *filaments* 1.7–2.2 mm long; *anthers* c. 0.9 mm long, apex minutely apiculate. *Ovary* (including style) reddish in living state, brownish to yellowish when dry, conical, to 4.5 mm long; *stigmas* 3, 0.2–0.4 mm long. *Seeds* unknown.

Anatomical description — Leaves are dorsiventral (bifacial), attached with adaxial surface in direct contact with stem and abaxial surface exposed to sunlight (Fig. 5A). Epidermal cells are narrowly oblong, polygonal or irregular with straight anticlinal walls (Fig. 5C). Epidermal surface is dotted with stomata and 8–celled salt glands. Stomatal type is brachyparacytic. Stomatal pores are transversely arranged to course of vein. Both stomata and salt glands are sunken. Young stems include 6 or 7 discrete vascular bundles; outer parts contain massive sclerenchyma fibres (perivascular fibres) functioning as mechanical tissue (Fig. 5A). Pith cells are round or elliptic with narrow walls. Chlorenchyma tissue occurs in outer cortex of stem. Epidermis of sun-exposed stems has stomata and salt glands similar to leaves but fewer in number.

Karyological description — Meiotic study in this species shows a diploid chromosome number with $n = 12$, as in most other *Tamarix* species (see Samadi & al. 2013). Twelve bivalents are vividly distinguishable in diakinesis and metaphase I (Fig. 4).

Molecular description — Sequences describe the type specimen (code T282) and are available in EMBL/GenBank/DDBJ under accession numbers LR583807 (*rpl16* intron), LR584012 (*trnG-trnS* spacer) and LR583687 (nrITS).

Phenology — Flowers February–March; fruits March–April.

Eponymy — The epithet "*humboldtiana*" commemorates the great German phytogeographer Alexander von Humboldt (1769–1859) whose 250th birthday is in the year of publication of this species. The name was also inspired by the Alexander von Humboldt Foundation having supported a research internship on diversity and phylogeny of the genus *Tamarix*.

Additional specimens examined — Iran: Hormozgan: c. 100 km ENE of Bandar Abbas, 2 km S of Bikah village, along Minab river near Deh Gel Kan village, 27°19'38"N, 57°12'05"E – 27°19'52"N, 57°12'10"E, 153 m, 21 Feb 2013, *H. Akhani, M. Dehghani, M. Doostmohammadi & A. Noormohammadi 23716 & 23717* (herb. Akhani).

Fig. 1. *Tamarix humboldtiana*. – A: habit and habitat along Minab river; plants grow on the river side and many of them are damaged by flooding; B: part of inflorescence with young flowers; C: part of inflorescence, showing whitish petals and reddish ovary and anthers. – All photographs taken at the type locality on 21 Feb 2013 by H. Akhani.

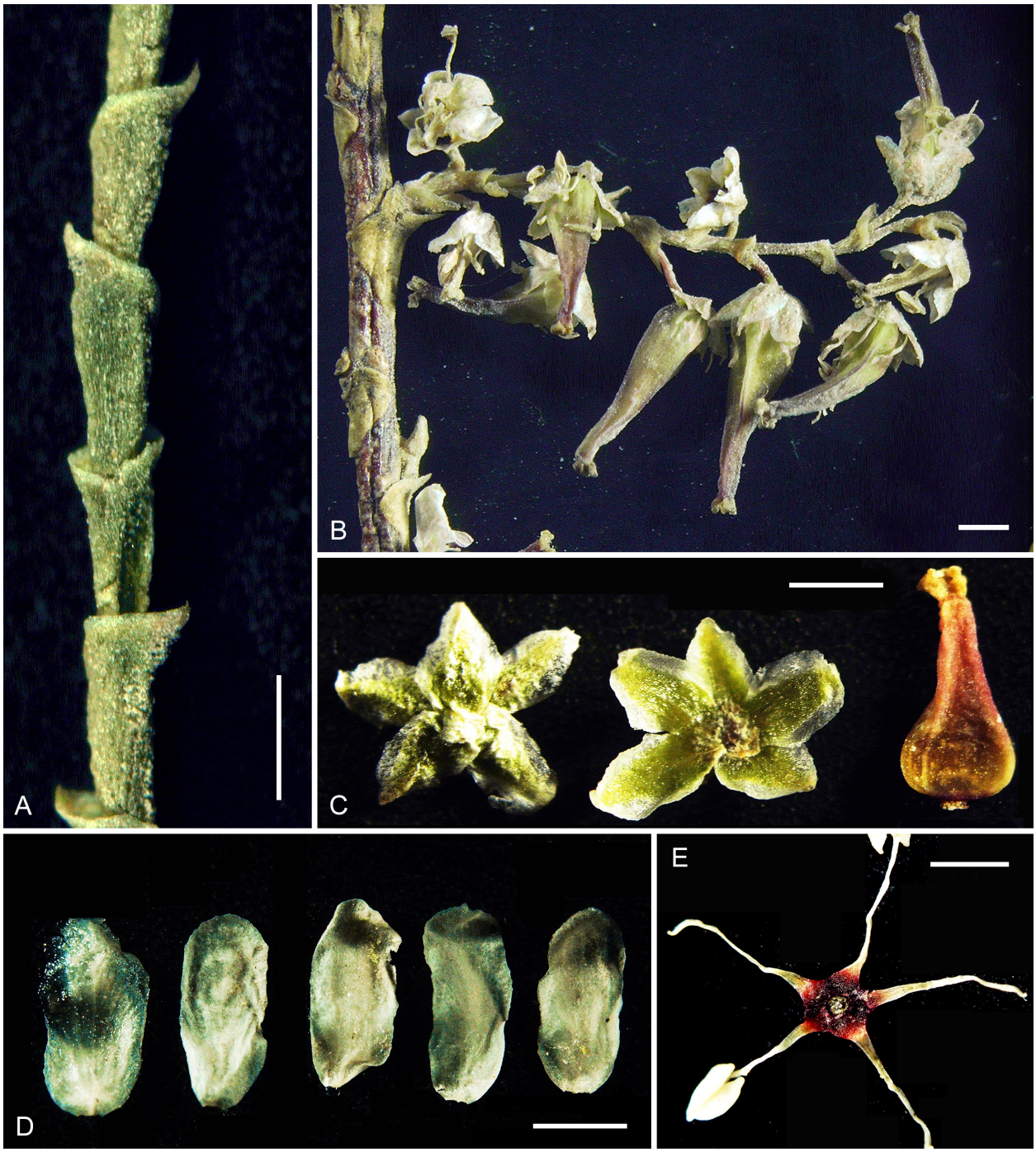

Fig. 2. Details of leaf, inflorescence and flower parts of *Tamarix humboldtiana*. – A: part of leafy young branch showing vaginate leaves; B: raceme, note long pedicels; C: calyx outside (left), calyx inside (centre), ovary and stigma (right); D: petals; E: staminal disk, showing 5 peridiscal filaments attached to synlophic disk. – Scale bars: A–E = 1 mm.

Sequence characters and phylogenetic relationships

The *trnG-trnS* spacer contained two poly A/T microsatellites, the latter of which was excluded from analysis as a mutational hotspot because of unclear homology of sequence elements in particular with respect to *Myricaria* and *Reaumuria*. Another highly variable poly AT/TA satellite-like region located approximately 700 positions downstream of *trnG* was also excluded because of unclear homology. Another long poly A/T microsatellite was found close to the *trnS* exon but the matrix was trimmed upstream because pherograms of the trnG primer after the poly A/T stretch were not readable and also pherograms of primer trnS were not reliable in the initial positions. The *trnG-trnS* matrix had 993 positions and contained 14% variable characters of which 5% were informative. Two mutational hotspots located 345 bp downstream of *trnG* and 692 bp downstream of *trnG* were excluded because of uncertain homology

Fig. 3. Distribution map of *Tamarix humboldtiana* (●).

(variable polyA microsatellite and AT-rich sequence elements in other genera; variable AT satellite-like elements). The *rpl16* intron data set began approximately 70 bases downstream of the small *rpl16* 5′ exon. Sequences were completely alignable and yielded a matrix of 1121 positions of which 9.4% were variable and 3.3% informative. A total of 59 indels were coded, of which 12 were informative. The ITS pherograms were without polymorphic sites except the sequence of the type specimen of *Tamarix humboldtiana*, which had a double signal of "A" and "G" in position 3 of ITS2 downstream of 5.8S.

The phylogenetic trees based on combined plastid *rpl16* and *trnG-trnS* sequence data (Fig. 6A) as well as nuclear ITS (Fig. 6B) both show that *Tamarix humboldtiana* belongs to a well-supported clade with *T. kotschyi* and *T. tetrandra*. The trees from the different genomic compartments are incongruent regarding the position of the new species. In the plastid tree, *T. humboldtiana* is sister to *T. tetrandra* (0.9 PP), and this lineage again is sister to a well-supported monophyletic group of all individuals of *T. kotschyi* (1.0 PP, 95% JK). To the contrary, the ITS topology depicts sequences of *T. humboldtiana* and *T. kotschyi* in an also well-supported clade (0.97 PP, 97% JK). The ribotype obtained from the type specimen of *T. humboldtiana* even appears nested among *T. kotschyi* ribotypes. However, this position is not well supported and is based on very few sequence differences. Since deviant ITS sequences of two different ancestral species can also evolve in a pattern that is biased toward one parent (Wendel & al. 1995; Winterfeld & al. 2009), the observed incongruent gene trees are in line with a case of hybrid speciation. Interestingly, the only polymorphic site identified in the pherograms of the *T. humboldtiana* sample exhibits both "A" and "G". The "A" is the common state in *Tamarix*, whereas "G" occurs in *T. meyeri* Boiss. and *T. tetrandra*. This would be in line with a scenario of hybrid speciation including ancestors of *T. kotschyi* and *T. tetrandra.* Nevertheless, there are many more differences between the ITS sequences of *T. kotschyi* and the new species, suggesting largely biased ITS evolution towards the *T. kotschyi* ribotype. Further

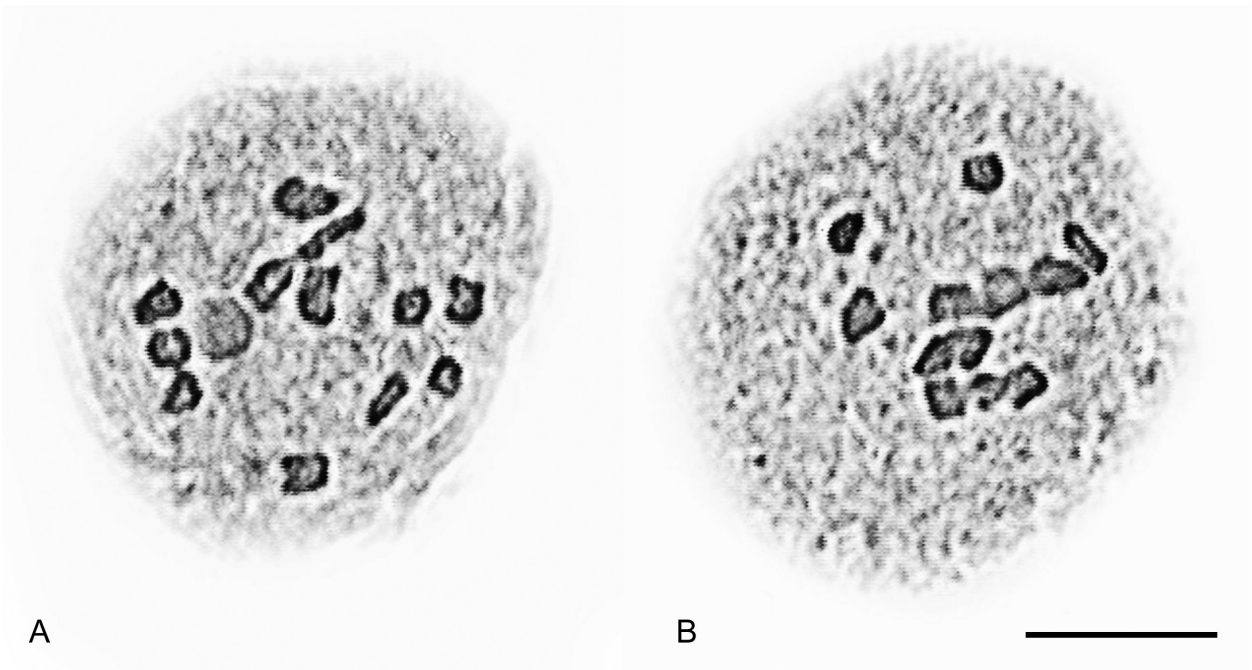

Fig. 4. Meiosis in *Tamarix humboldtiana*, *n* = 12. – A: diakinesis; B: metaphase I. – Scale bar = 10 µm.

phylogenomic analysis and the inclusion of more samples will likely shed more light on this in the future. Villar & al. (2019) sampled neither *T. kotschyi* nor *T. tetrandra*. The rather distant cp haplotype of sample *Akhani 21693* (the type specimen of *T. humboldtiana*) was not found in more extensive plastid analyses of *Tamarix* (Akhani & Borsch, pers. comm.), indicating that the maternal parent remains unknown and possibly extinct. A further difference of the *rpl16* sequence of the sample constituting the type in comparison with the other sequences is a longer polyA-stretch upstream of the large 3′ exon in positions 496–504 with 9 A/Ts only in the sample of the type specimen. Nevertheless, we did not use it as diagnostic character because microsatellites are highly variable, making their diagnostic potential at species level questionable (González Gutiérrez & al. 2013).

Interestingly, none of the closely related putative species or their close ancestors occurred sympatrically with the new entity found along the Minab river. The observed sympatric species were *Tamarix mascatensis* Bunge and *T. stricta* Boiss., both of which are very distantly related (Akhani & Borsch, unpubl. data). *Tamarix mascatensis*

Table 1. Morphological, micromorphological and anatomical comparison of *Tamarix humboldtiana* and *T. kotschyi*. The morphometry of anatomical data of *T. kotschyi* are the average of five specimens (*Akhani & al. 21834, 21977, 22265, 22267* and *22395*). The leaf and stem anatomical measurements of *T. humboldtiana* refer to *Akhani & al. 23716* and the epidermis of *Akhani 21693*.

Characters	***Tamarix humboldtiana***	***Tamarix kotschyi***
Life form	shrub	shrub
Leaves	vaginate-amplexicaul	semi-amplexicaul to amplexicaul
Pedicel length	conspicuous, 1–1.5 mm	very small, 0.5–0.75 mm
Bracts length	0.5–1 mm	1.5–2.5 mm (membranous, diaphanous)
Flowers	5-merous	4-merous
Sepals length	1–1.5 mm	0.75–1.5 mm
Petals length	2–2.5 mm	2–2.25 mm
Disk	synlophic	synlophic (parasynlophic)
Stigmas	3	3
Epidermal cells shape	elongate oblong, polygonal, irregular	oblong, polygonal, irregular
Epidermal cells length	(23) 34.45 ± 6.75 (49)	(18) 24.21 ± 4.07 (35)
Epidermal cells width	(15) 20.27 ± 3.08 (26)	(13) 18.74 ± 2.86 (25)
Stomata type	brachyparacytic	brachyparacytic + laterocytic
Stomata density in 1 square mm	(109) 149 ± 25 (175)	(89) 102 ± 14 (117)
Subsidiary cell ingrowing papillae	no	yes
Chromosome number	*n* = 12	*n* = 12
Distribution	S Iran restricted to Hormozgan Province	C and S Iran and Afghanistan
Ecology	freshwater riversides	freshwater riversides

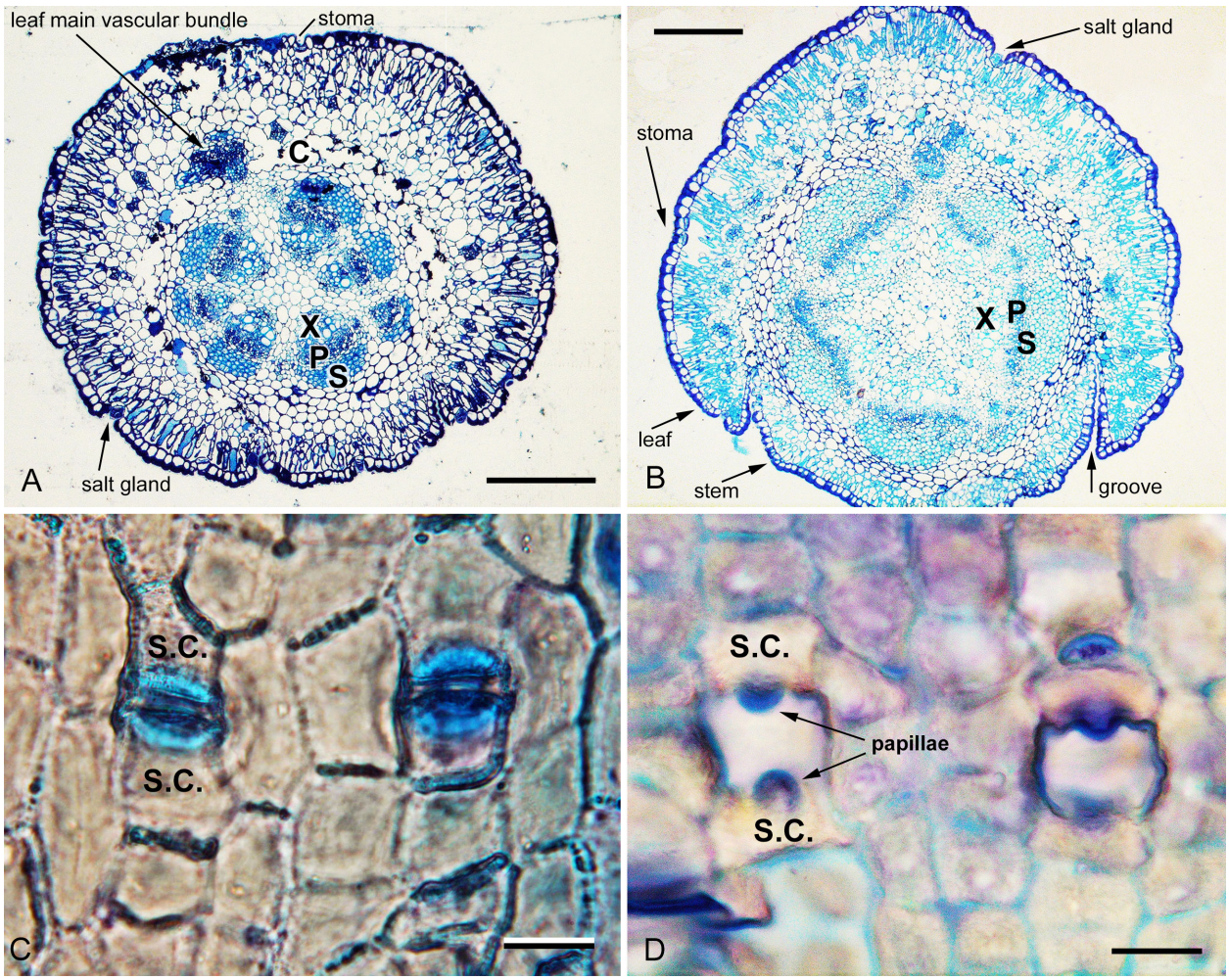

Fig. 5. Cross-section of stem and surrounding leaf and epidermal micromorphology of *Tamarix humboldtiana* and *T. kotschyi*. – A: stem and surrounding leaf of *T. humboldtiana.* The vaginate part of leaf and stem tissues can be distinguished by the collapsed parenchyma cells, which can be distinguished from the grooves on the lower corners of the section, which separate leaf and stem; note the slightly sunken stomata and salt glands; B: stem and surrounding leaf of *T. kotschyi*; note that leaf encircles more than half of the stem in both species but in *T. humboldtiana* much larger parts of the stem are covered; C: leaf adaxial epidermis of *T. humboldtiana* showing brachyparacytic stomata and subsidiary cells lacking ingrowing papillae; D: *T. kotschyi*, brachyparacytic stomata characterized by two ingrowing papillae. – Abbreviations: S = sclerenchyma; S.C. = subsidiary cells; P = phloem; X = xylem. – Scale bars: A, B = 200 µm; C, D = 20 µm.

appears close to *T. meyeri* in the plastid tree (Fig. 6A). The phylogenetic trees therefore reject any close relationship between our new species and *T. mascatensis*, although both have pentamerous flowers.

Our plastid and nuclear trees show the *Tamarix aphylla* clade (including *T. aphylla* (L.) H. Karst. and *T. usneoides* E. Mey), which was also found by Villar & al. (2015a, 2019) based on combined *trnG-trnS*, *ndhF-rpl32* and *trnQ-rps16* spacers with high support. They also both reveal a clade including several species (e.g. *T. meyeri*) to which *T. ramosissima* Ledeb. is congruently resolved as sister. This clade represents most of the diversity of *Tamarix* in this study, but its position is inconsistent between the ITS and plastid trees (Fig. 6). The earliest studies of Gaskin & Schaal (2003) also found *T. usneoides* as sister to the remainder of the genus based on ITS, but this position in their tree based on sequences of the *trnG-trnS* spacer was not supported. Further work is therefore needed to establish a robust plastid tree for *Tamarix* and to test for possible deep reticulations.

Morphological and anatomical characters

Superficially, *Tamarix humboldtiana* is also similar to *T. mascatensis* but differs clearly by having different chloroplast and nuclear sequences, subvaginate leaves (not only amplexicaul leaves), and pedicellate flowers. Both *T. humboldtiana* and *T. kotschyi* have a brachyparacytic stomatal type, although the laterocytic type rarely occurs in *T. kotschyi*. The subsidiary cells in *T. kotschyi* are associated with ingrowing papillae that overarch the stomatal pores, which might have a role in minimizing water loss (Fig. 5C). This character is absent in *T. humboldtiana* (Fig. 5D). Additional differences in epidermal cells and stomatal density between *T. humboldtiana* and *T. kotschyi* are given in Table 1.

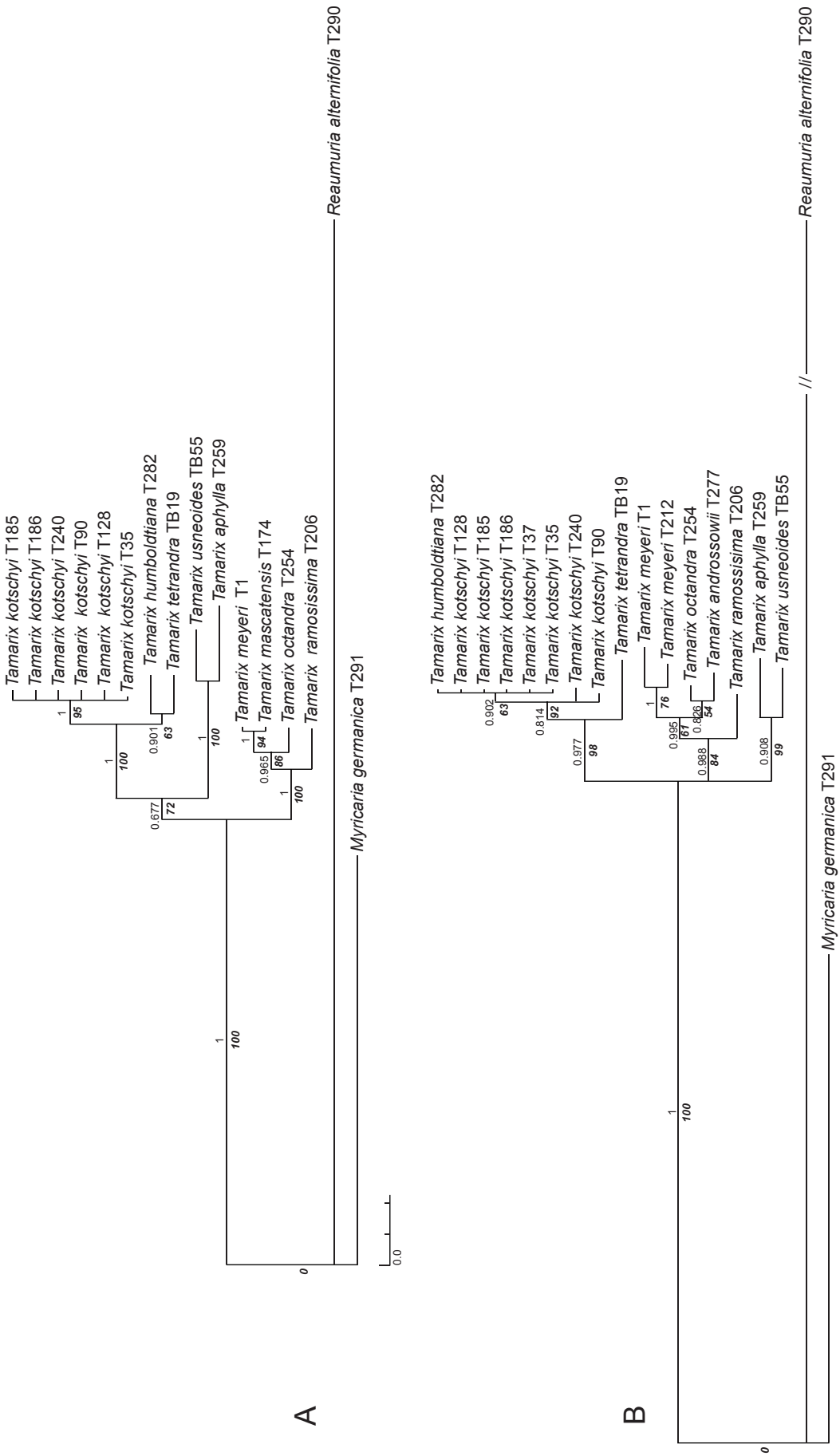

Fig. 6. Trees showing the position of the type specimen of *Tamarix humboldtiana*. – A: majority rule phylogram from Bayesian analysis of combined plastid *rpl16* intron and *trnG-trnS* spacer sequences; B: of nrITS sequences. Values above branches are bootstrap posterior probabilities, and below branches are jackknife percentages. Note that plastid haplotypes of *T. kotschyi* are resolved as monophyletic, whereas the sequence of *T. humboldtiana* is sister to *T. tetrandra*. In the tree inferred from nuclear ITS, *T. humboldtiana* appears among the sequences of *T. kotschyi*.

Table 2. List of voucher specimens used in phylogenetic reconstruction and relationships of *Tamarix humboldtiana*.

Taxon	Voucher specimen	DNA no.	GenBank accession numbers		
			ITS	*trnG-trnS*	*rpl16*
Myricaria germanica (L.) Desv.	Iran, West Azerbaijan: c. 40 km NW of Urmia toward Turkish border, Sero, along river, 37°42'43"N, 44°44'02"E, 1512 m, riverside dominated by *Tamarix* spp. and *Salix* sp., EC = 600 µm, 3 Jun 2011, *H. Akhani, A. Noormohamadi & N. Samadi 22637* (herb. Akhani, IRAN)	T291	LR583689	LR584014	LR583809
Reaumuria alternifolia (Labill.) Britten	Iran, West Azerbaijan: 12 km S of Khoy, near Khoy Industrial Town, gypsum salty hills, 38°25'28"N, 44°54'49"E, 1190–1266 m, 3 Jun 2011, *H. Akhani, A. Noormohamadi & N. Samadi 22679* (herb. Akhani, IRAN)	T290	LR583688	LR584013	LR583808
Tamarix androssowii Litw.	Iran, Tehran: around Pardisan Nature Park, along ring of Hemmat and Yadegar freeway, 34°44'53"N, 51°20'54"E, 1350 m, 8 Apr 2011, *H. Akhani 22303* (herb. Akhani, IRAN)	T277	LR583686	–	–
Tamarix aphylla (L.) H. Karst.	Iran, Khuzestan: Hendijan, along Zohre river dominated by *Tamarix* thickets, 30°13'25"N, 49°42'19"E, sea level, 3 Nov 2010, *H. Akhani 21640* (herb. Akhani, IRAN)	T259	LR583685	LR583806	LR584011
Tamarix humboldtiana Akhani, Borsch & N. Samadi	Iran, Hormozgan: c. 100 km ENE of Bandar Abbas, 2 km S of Bikah village, along Minab river near Deh Gel Kan village, 27°19'56"N, 57°12'07"E, 153 m, 8 Mar 2011, *H. Akhani 21693* (herb. Akhani, B 10 0465441, IRAN)	T282	LR583687	LR584012	LR583807
Tamarix kotschyi Bunge	Iran, Fars: c. 10 km S of Chenar Shahijan (Ghaemieh) toward Kazeroon, Pole Tole Kooshk (bridge), along river, 29°45'45"N, 51°33'06"E, 770 m, 15 Mar 2011, *H. Akhani, N. Samadi & A. Noormohammadi 21834* (herb. Akhani, IRAN)	T35	LR583690	LR584015	LR583810
Tamarix kotschyi	Iran, Fars: c. 10 km N of Nurabad-e Mamasani, Pole Fahlyan, along river, 30°10'57"N, 51°32'24"E, 923 m, 15 Mar 2011, *H. Akhani, A. Noormohammadi & N. Samadi 21836* (herb. Akhani, B 10 0465443, IRAN)	T37	LR583691	–	–
Tamarix kotschyi	Iran, Kerman: between Anar and Rafsanjan, near Kashkoyeh, along man-made elevated zone, 30°31'59"N, 55°35'46"E, 1500 m, 28 Mar 2011, *H. Akhani, A. Noormohammadi & N. Samadi 21977* (herb. Akhani, B 10 0465444, IRAN)	T128	LR583678	LR584004	LR583799
Tamarix kotschyi	Iran, Kerman: 47 km W of Baft toward Sirjan, 29°18'08"N, 56°13'01"E, 2082 m, 3 Apr 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22267* (herb. Akhani, IRAN)	T186	LR583680	LR584007	LR583802
Tamarix kotschyi	Iran, Kerman: 30 km NW of Jiroft on road toward Baft, near Delfard, 28°56'02"N, 57°39'58"E, along river, 1442 m, 2 Apr 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22265* (herb. Akhani, B 10 0465442, IRAN)	T185	LR583679	LR584006	LR583801
Tamarix kotschyi	Iran, Qom: c. 55 km N of Qom toward Tehran, along Tehran-Qom highway, 35°08'38"N, 50°58'30"E, 1164 m, 22 Apr 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22395* (herb. Akhani, IRAN)	T90	LR583692	LR584016	LR583811
Tamarix kotschyi	Iran, West Azerbaijan: 32 km NNW of Urmia, junction with Nazlu Chaei river, near Nazlu, 37°40'22"N, 45°58'27"E, 1323 m, EC = 303–329 µm, 3 Jun 2011, *H. Akhani, A. Noormohamadi & N. Samadi 22635* (herb. Akhani, IRAN)	T240	LR583683	LR584009	LR583804

Taxon	Voucher specimen	DNA no.	GenBank accession numbers		
			ITS	*trnG-trnS*	*rpl16*
Tamarix mascatensis Bunge	Iran, Sistan va Baluchestan: between Zahedan and Bam, Nosratabad, in the town (29°51'26"N, 59°58'57"E, 1108 m) and Tamarix forest N of the town (29°52'09"N, 59°58'25"E, 1103 m), 1 Apr 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22220* (herb. Akhani, B 10 0465482, IRAN)	T174	–	LR584005	LR583800
Tamarix meyeri Boiss.	Iran, Golestan: 8 km W of Tangoli, 37°23'29"N, 54°34'52"E, 10 m, 5 May 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22441* (herb. Akhani, IRAN)	T212	LR583682	–	–
Tamarix meyeri	Iran, Khuzestan: W of Shoosh, *Tamarix* woodlands along Karkheh river, 32°11'43"N, 48°12'45"E, 76 m, 12 Mar 2011, *H. Akhani, N. Samadi & A. Noormohammadi 21720* (herb. Akhani, B 10 0465508, IRAN)	T1	LR583677	LR584003	LR583798
Tamarix octandra Bunge	Iran, West Azerbaijan: 7 km S of Evoghli toward Marand, along river and surrounding salty areas, EC (river) = 15.3 mS, 38°37'47"N, 45°15'20"E – 38°37'29"N, 45°15'07"E, 986–988 m, 6 Jun 2011, *H. Akhani, A. Noormohamadi & N. Samadi 22845* (herb. Akhani, IRAN)	T254	LR583684	LR584010	LR583805
Tamarix ramosissima Ledeb.	Iran, Mazandaran: near Neka, along river, 36°38'45"N, 53°18'36"E, 47 m, 4 May 2011, *H. Akhani, A. Noormohammadi & N. Samadi 22404b* (herb. Akhani)	T206	LR583681	LR584008	LR583803
Tamarix tetrandra Pall.	Cyprus, Kidhasi (Paphos): lit du Dhiarizos, 400 m, lit de rivière et prairies attenantes, 27 Apr 1991, *G. Alizar & al. 1648* (Optima 4th Iter Mediterraneum) (B 10 0471386)	TB19	LR583693	LR584017	LR583812
Tamarix usneoides E. Mey. ex Bunge	SW Africa: Bethanien, Warmbad, river in and around Kl. Ai-Ais, 5/6 Oct 1977, *H. Merxmüller & W. Giess 32510* (M)	TB55	LR583694	LR584018	LR583813

Table 3. Vegetation characteristics and species composition of a stand of *Tamarix humboldtiana* at the type locality.

Plot area: 10 × 20 m	
Total cover: 45%	
Shrub cover: 30%	
Grass cover: 20%	
Vegetation height: 2 m	
Tamarix humboldtiana Akhani, Borsch & N. Samadi	3
Tamarix mascatensis Bunge	1
Tamarix stricta Boiss.	1
Phragmites australis (Cav.) Steud.	2
Saccharum sp.	2
Juncus sp. (sterile)	1
Nerium oleander L.	1

Morphologically and geographically *Tamarix humboldtiana* shows some similarity to *T. kermanensis* Baum (Baum 1967), but *T. humboldtiana* has subvaginate leaves, shortly pedicellate flowers, and white petals. *Tamarix kermanensis* occurs commonly on sandy dunes and near saline rivers in Kerman, Hormozgan, Sistan and Baluchestan Provinces in Iran and adjacent Pakistan (Qaiser 1981). In contrast to *T. humboldtiana*, *T. kermanensis* has distinctly vaginate leaves, subsessile flowers, red petals, and triploid as well as tetraploid chromosome compliments (Samadi & al. 2013). Villar & al. (2019) found *T. kermanensis* in a clade together with *T. nilotica* (Ehrenb.) Bunge and *T. senegalensis* DC. in ITS trees and sister to *T. canariensis* Willd. in their plastid trees, albeit without statistical support. Our own unpublished data point to relationships of *T. kermanensis* to *T. arceuthoides* Bunge and *T. ramosissima* (H. Akhani & T. Borsch, pers. comm.), which are completely different lineages than the *T. kotschyi* clade depicted here (Fig. 6).

In his monograph of *Tamarix*, Baum (1978) synonymized the pentamerous *T. leptopetala* Bunge with the tetramerous *T. kotschyi*, a view accepted by Villar & al. (2015a). The type locality of *T. leptopetala* from the Lar valley in the Alborz Mountains (in valle Loura in montium Elbrus, 1813, *Kotschy 728*, GOET!, W!) has been examined in various seasons during our project. The only species found in the area is *T. ramosissima*. However, the specimen matches well *T. arceuthoides*

and *T. mascatensis*. The presence of a few pentamerous flowers, as exhibited by the type specimen of *T. kotschyi*, is very rare in natural populations of this species, unless it hybridizes with its sympatric species *T. arceuthoides* and *T. mascatensis*. Whereas the type specimen of *T. leptopetala* clearly differs from *T. humboldtiana* by having leaves not subvaginate, flowers without distinct pedicels, and moreover a completely different distribution and ecology. However, a definite answer regarding the conspecifity of *T. leptopetala* with *T. kotschyi* can only be given through DNA sequence data from the type.

Habitat and conservation

A small thicket of *Tamarix humboldtiana* was found along the margin of the Minab river on sandy-gravelly soils (Fig. 1A). This patch grows on the innermost vegetation zone of the very wide river bed. *Tamarix mascatensis* and *T. stricta* are the most common species in the area, particularly at some distance from the river margin. In dense stands dominated by these two species *T. humboldtiana* is rare. The river is a freshwater river with an electric conductivity (EC) of 1644 µs and 0.8 salinity. The habitat of the species is very fragile, because it occurs in the innermost vegetation zone of the riverside subjected to frequent floods (Fig. 1A). The associated species with *T. humboldtiana* are *T. mascatensis*, *T. stricta*, *Phragmites australis* (Cav.) Steud., *Saccharum* sp., *Juncus* sp. and *Nerium oleander* L. (Table 2).

Most species of *Tamarix* are locally very common. They are successful plants by both vegetative and generative reproduction. The riparian habitat and the very tiny, pappus-bearing seeds are advantages for effective dispersal. The question is, therefore, if the species is really as rare as the currently available data suggest. There are many rivers in the area that need to be searched for this species. Our propagation experiments showed that production of adventitious roots via propagation is very poor in *T. humboldtiana* in comparison with many other species, such as the co-occurring *T. mascatensis* and *T. stricta*. Several of the propagated branches died off after we transferred them into soil. These results and the restriction of the patches of *T. humboldtiana* to the fragile zone of the river indicate that the populations are driven by other competitive species. Therefore, we could suggest that natural impacts play a major role in threatening the survival of this species. These impacts are accelerated by the current water abstraction in the area and frequent flooding in the fragile marginal habitat along the rivers. Based on *T. humboldtiana* being known only from one small site, it meets the criteria B1ab(iii) of the category Critically Endangered (CR) of the IUCN (2012) for assigning threat categories. We strongly recommend further research and implementation of measures to ensure *in situ* conservation and cultivation activities for *ex situ* conservation of the species.

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