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Species delimitation and inter-specific gene flow in *Tamarix* L. (Tamaricaceae)

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Key words: *Tamarix*, ITS, HGT tree, Species delimitation.

Ključne besede: *Tamarix*, ITS, drevo HGT, razmejitev vrst.

Abstract

Tamarix L. play important role in preventing deforestation in Iran. Tamarix species exhibit wide range of morphological variation therefore, the species delimitation become difficult. This is further complicated due to similarity of morphological characters in closely related species and the occurrence of inter-specific hybridization. The present study was performed to identify Tamarix species and their potential hybrids in Semnan Province of Iran. We used ITS and ISSR and 42 morphological characters for our investigation. Molecular phylogeny of the studied species and their relationship was not in agreement with the species tree of morphological characters and with taxonomic treatment of the genus. HGT tree of ITS and morphological data obtained revealed the occurrence of inter-specific hybridization or introgression between Tamarix species.

Izvleček

Vrste rodu *Tamarix* so v Iranu pomembne za preprečevanje krčenja gozdov. Zanje je značilna široka morfološka variabilnost, s katero so sposobne preživeti v različnih ekoloških razmerah, zato je razmejitev vrst težavna. Dodatne težave predstavljajo podobni morfološki znaki pri ozko sorodnih vrstah in prisotnost medvrstnega križanja. V članku želimo določiti vrste rodu *Tamarix* in njihove potencialne križance iz province Semnan v Iranu. V raziskavi smo uporabili ITS in ISSR molekulske markerje in 42 morfoloških znakov. Molekularna filogenija obravnavanih vrst in njihova razmerja niso bili v skladu z dendrogramom morfoloških znakov in s taksonomsko členitvijo rodu. Z HGT dendrogramom podatkov iz ITS in morfološko analizo smo pokazali obstoj medvrstnega križanja oziroma introgresije med vrstami rodu *Tamarix*.

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1. Introduction

The genus *Tamarix* L. contains about 54 species that mainly grow in saline areas of deserts and semi-deserts in Asia, Europe, North-east and South-west of Africa. *Tamarix* species play important role in preventing deforestation in Iran (Sheidai et al. 2018). *Tamarix* species have limited benefits to human but have been used as ornamental plant in gardens or public plantations. For example, *T. gallica* L., *T. chinensis* Lour., *T. ramosissima* Ledeb. are frequently used as ornamental plants for their feathery appearance and their catkin-like inflorescences (Gaskin 2003). *Tamarix* species are good for windbreak (for example *T. aphylla* (L.) Karst. and *T. Africana* Poir.) or for erosion control and easily grow in poor soils (Baum 1967, Gaskin and Schaal 2002, Ijbari et al. 2014).

Tamarix species hybridize and may form different taxonomic forms due to inter-specific hybridization and introgression (Gaskin & Schaal 2003, Gaskin & Kazmer 2019, Mayonde et al. 2019). Due to different degree of gene flow among *Tamarix* species, plants with variable morphological characters occur in the same area. Therefore, high gene flow in *Tamarix* species caused by inter-specific hybridization resulting in phenotypic variations have rendered taxonomic classification of the genus problematic (Baum 1978, Ijbari et al. 2014). *T. tetrandra* Pall. ex M.Bieb., *T. gallica* L., *T. chinensis*, *T. ramosissima*, and *T. parviflora* DC. are considered to be one variable species or hybridizing group, designated by the hybrid name *T. pentandra* Pall. (Sudbrock 1993). *T. chinensis* and *T. ramosissima* are morphologically alike and differ only in some microscopic characters and genetically distinct in Asia (Mayonde et al. 2016). However,

Figure 1: Distribution map of *Tamarix* species in Iran. **Slika 1:** Karta razširjenosti vrst rodu *Tamarix* v Iranu.

they have been proven to be genetically different and are known to hybridize in North America and South Africa (Gaskin & Schaal 2002, Gaskin & Kazmer 2009, Mayonde et al. 2016).

Morphological characteristics are important in *Tamarix* species delimitation. *Tamarix* leaves are taxonomically useful and they show variation in shape and attachment modes in different species (Baum 1978). *Tamarix* flowers are bisexual, rarely unisexual and plants are either monoecious or dioecious. The flowers either have five or four sepals with corresponding number of petals. Flowers have five or numerous stamens that are free or fused and are inserted into a fleshy, glandular, hypogynous disc (Obermeyer 1976, Baum 1978). The presence of bisexual flowers and cross-pollination in *Tamarix* lead to the occurrence of high genetic diversity and hybrid formation in these species (Gaskin & Schaal 2002, Gaskin & Kazmer 2009, Gaskin et al. 2012, Mayonde et al. 2015, 2016). The intraspecific genetic variability may be used for local adaptation and also prevents homozygosity and genetic extinction of the studied *Tamarix* taxa (Ijbari et al. 2014).

Thirty-five *Tamarix* species occur in Iran as reported by Schiman-Czeika (1980). These species have been used in plantation to prevent deforestation in Iran. However, our general survey and extensive collections in different provinces may suggest the occurrence of more number of species/ sub-species in the country. The correct identity of our *Tamarix* collections can be verified at population level through detailed taxonomic investigations using both morphological and molecular approaches (Arianmanesh et al. 2014, Ijbari et al. 2014).

Tamarix species occur in 21 provinces of Iran (Figure 1). Ecological and climatic differences may influence the morphological appearance of *Tamarix* but will not affect the identity of the species (Sheidai et al. 2018). Despite the phenotypic differences in our plant collection and the previous studies observed in different localities, we hypothesized that the identities are most likely to be the same. Due to co-occurrence of two or three *Tamarix* species in overlapping areas, it is very important to identify and delimit these species. Moreover, due to frequent gene exchange between species in the same area there is high probability of hybrid formation. Therefore, it is necessary to highlight gene flow among the species growing within each locality (Ijbari et al. 2014, Sheidai et al. 2018).

Molecular tools in systematic provide the means to investigate the identity of different plant species at the DNA level, showing genetic variation within and among populations, and can also detect introgression patterns between closely related species (Le Roux & Wieczorek 2008). ITS sequences are useful to construct phylogenies of angiosperms at lower taxonomic levels (Baldwin et al. 1995) and reveal polymorphisms (double base readings) within plant individuals (Campbell et al. 1997). Polymorphisms in some individuals can occur because concerted evolution is not fast enough to homogenize repeats of mutations among the multiple copies in the genome, and/or because of recent hybridization events (Campbell et al. 1997). ISSR molecular markers were shown to be informative for genetic diversity and population structure studies (see for example, Sheidai et al. 2012, 2013, Azizi et al. 2014).

Our study was conducted in the Semnan Province of Iran because species of this area have not been identified. After morphological identification of *Tamarix* species, their identification was also checked by BLAST using ITS (Internal transcribed sequences) of the nuclear DNA (nrDNA). Furthermore, the morphological and ITS analyses of identified species were carried to reveal the species delimitation and relationship (Sheidai et al. 2013, Minaeifar et al. 2016) and we carried out introgression patterns in populations of in *T. szowitsiana* Bge. and *T. androssowii* Litv. species by using Inter-simple sequence repeats (ISSR) and in *T. Androssowii*, *T. Meyerii* Boiss. and *T. Szowitsiana* by ITS molecular markers.

2. Materials and methods

2.1 Morphological investigation

Eighty plants were randomly collected from 22 geographically areas in Semnan Province in Iran and used for morphological investigations. The voucher specimens were deposited in Herbarium of Shahid Beheshti University (HSBU) (Table 1).

Morphological characters (Table 2) used are according to Ijbari et al. (2014). Morphological data were standardized (Mean = 0, Variance = 1) and used to estimate Euclidean distance. Grouping of the species was done by UPGMA (Unweighted paired group using average method) clustering and principal coordinate analysis (PCoA) (Podani 2000). These analyses were done using PAST ver. 2.17 (Hammer et al. 2012).

2.3 Molecular investigations

For molecular analyses, we used both the multilocus genome-wide markers (i.e. ISSR) and the single locus (i.e. ITS 1, 5.8S, ITS2) regions. Both markers were used for species diversity analysis and phylogeny (Weising et al. 2005, Sheidai et al. 2014).

Table 1: Geographic areas studied and ecological features.
Tabela 1: Obravnavana geografska območja in njihove ekološke značilnosti.

Number of locality	Province	Locality	Altitude (m)	Longitude	Latitude	Voucher number
1	Semnan	Bagh village	1109	36.12507	54.26741	1294
2	Semnan	10 km to Garmsar	1033	35.19760	52.60075	1394
3	Semnan	5 km to Damghan	1146	36.7152	54.15927	1494
4	Semnan	Sorkheh	1149	35.27208	53.10930	1594
5	Semnan	10 km to Semnan	1165	35.30488	53.17239	1694
6	Semnan	Bagh village	1109	36.12507	54.26741	1794
7	Semnan	5 km to Damghan	1146	36.7152	54.15927	1894
8	Semnan	50 km to Chesameh ali villag	1395	36.15214	54.9535	1994
9	Semnan	20 km to Chesameh ali villag	1400	36.15169	54.9289	2194
10	Semnan	Chesameh ali villag	1376	36.14671	54.10539	2294
11	Semnan	25 km to Garmsar	1033	35.19760	52.6075	2394
12	Semnan	20 km to Garmsar	995	35.18984	52.7327	2594
13	Semnan	10 km to Garmsar	844	35.14412	52.17773	2694
14	Semnan	20 km to Garmsar	995	35.18984	52.7327	2794
15	Semnan	Bagh village	1109	36.12507	54.26741	2894
16	Semnan	5 km to Damghan	1146	36.7152	54.15927	2994
17	Semnan	Amiriyeh villag	1144	36.6482	54.14472	3294
18	Semnan	Turan Protected Area	1007	36.28135	55.42987	3394
19	Semnan	Hadad village	1124	36.16662	54.44510	3494
20	Semnan	20 km to Shahrood	1114	36.12805	54.29208	3694
21	Semnan	10 km to Semnan	909	35.14338	52.24440	3794
22	Semnan	10 km to Sorkheh	903	35.14463	52.24709	3894

Table 2: Morphology characteristics in *Tamarix*.
Tabela 2: Morfološke značilnosti vrst rodu *Tamarix*.

No	Characters	No	Characters
1	leaf length	21	leaf shape
2	length of inflorescence	22	shape of leaf margin
3	width of inflorescence	23	leaf pile
4	ratio of leaflet size/ pedicel size	24	inflorescence
5	ratio of leaflet size/ calyx size	25	flower density
6	length of leaflet	26	leaflet attachment
7	width of leaflet	27	shape of leaflet
8	ratio of pedicel size/ calyx size	28	shape of leaf top
9	calyx segments	29	shape of internal calyx
10	length of internal calyx	30	shape of external calyx
11	length of external calyx	31	tip of internal calyx
12	width of internal calyx	32	tip of external calyx
13	width of external calyx	33	internal calyx naviculate
14	corolla segments	34	external calyx naviculate
15	corolla length	35	calyx pile corolla symmetry
16	corolla width	36	base of filament
17	stamen number	37	attachment of stamen to lobe
18	anther length	38	place of stamen extrusion
19	anther width	39	anther tip, anther
20	disc diameter stem pile		

2.3.1 DNA extraction

Fresh leaves were randomly collected from 5-10 *Tamarix* trees in each population. CTAB activated charcoal protocol was used to extract genomic DNA (Križman et al. 2006). The quality of extracted DNA was examined by running on 0.8% agarose gel.

2.3.3 ITS analysis

ITS region DNA was amplified with 0.2 µM primer ITS1 (5′ TCCGTAGGTGAACCTGCGG-3′, Bioron, Germany), and primer ITS4 (5'- TCC GCT TATTGA TAT GC -3') (Chen et al. 2010). PCR reactions were performed in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany), 20 ng genomic DNA and 3 U of Taq DNA polymerase (Bioron, Germany). The amplification reaction was performed in a Techne thermocycler (Germany) using the following parameters: 2 min initial denaturation step at 94 °C, followed by 35 cycles of 5 min at 94 °C; 1.30 min at 56 °C and 2 min at 72 °C. The reaction was completed by a final extension step of 7 min at 72 °C. PCR products were visualized on 2.5% agarose gels with GelRed™ Nucleic Acid Gel Staining.

Fragment sizes were estimated using a 100 bp size ladder (Thermo- Fisher Scientific, Waltham, MA USA).

ITS sequences obtained were aligned with MUSCLE (Robert 2004) implemented in MEGA 5. The molecular clock test was performed as implemented in MEGA 5 (Tamura et al. 2011). The test was done by comparing the ML value for the given topology with and without the molecular clock constraints under the Tamura and Nei (1993) model. Different phylogenetic trees were obtained from ITS data like UPGMA (Unweighted paired group using average), Neighbor Joining (NJ) and Maximum likelihood (ML) methods. Hundred times bootstrapping was used for final trees.

2.3.2 ISSR analysis

7 ISSR (inter simple sequence repeat) primers UBC810, UBC849, (CA) 7AC, (GA) 9T, (GA) 9A and (AGC) 5GG were used according to Ijbari et al. (2014) and were purchased from University of British Columbia, Canada. The polymerase chain reaction (PCR) reactions were performed in a 25µl volume containing 10 Mm Tris-HCl buffer at pH 8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP (Bioron, Germany), 0.2 µM of each primer, 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The polymerase chain reaction was performed in a Techne thermocycler (Germany) with the following parameters: 5 min initial denaturation step at 94 °C, followed by 40 cycles of 45s at 94 °C; 1 min at 55 °C and 1min at 72 °C. The reaction was completed with a 7 min extension step at 72 °C. The amplification products were visualized by running on 2% agarose gels. The fragment size was estimated using a 100 bp molecular size ladder (Fermentas, Germany). In order to identify reproducible bands, the experiment was replicated 3 times.

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0). Grouping of the plant specimens was done by different clustering and ordination methods such as UPGMA (Unweighted paired group using average), and MDS (Multidimensional scaling) (Podani 2000). These analyses were done in PAST ver. 2.17 (Hammer et al. 2012).

3. Results

3.1 Species identification based on morphological characters and its marker

Our preliminary identification based on selected morphological characters resulted in nine distinct species (Table 3).

Table 3: Identified species based on morphological characters.

Table 3: Vrste, določene na osnovi morfoloških znakov.

No.	Species identified with morphological characters	Localities in Table 1
1	*Tamarix arceuthoides* Bge.	11
2	*T. ramosissima* Ledeb.	8, 9 , 10
3	*T. karkalensis* Lour.	19
4	*T. szowitsiana* Bge.	12 , 13
5	*T. meyeri* Boiss.,	4,5,6, ,7
6	*T. androssowii* Litw.	14, 15, 16, 17, 18
7	*T. androssowii* var. *transcaucassica* (Bunge) Qaiser	20
8	*T. aucheriana* (Decne. ex Walp.) B. R. Baum.	1, 2, 3
9	*T. mascatensis* Bge	21, 22

One sample of any species ITS sequences were obtained and compared with available sequences in *Tamarix* species. The results are provided in Table 4. All identified species had at least 95% homology with the reported ITS sequence for the same taxa in NCBI (National Center for Biotechnology Information).

Table 4: *Tamarix* species identified and their ITS sequence homology to the reported species.

Tabela 4: Določene vrste rodu *Tamarix* in istorodnost njihovih ITS sekvenc z obravnavanimi vrstami.

Species	Homology %	Accession No.
T. aucheriana	100	AF484762
T. arceuthoides	95	AY452028
T. mascatensis	99	KT809493
T. ramosissima	98	KM657148
T. karkalensis	97	KJ377278
T. meyeri	96	KJ729661
T. androssowii	99	KT377273
T. androssowii var. *transcaucassiva*	–	–
T. szowitsiana	–	–

3.2 Relationship between species based on morphological studies

Different clustering methods (WARD, NJ and UPGMA dendrograms) based on 42 morphological characters in identical species produced similar results. Therefore, only UPGMA dendrogram is presented (Figure 2). Plants of

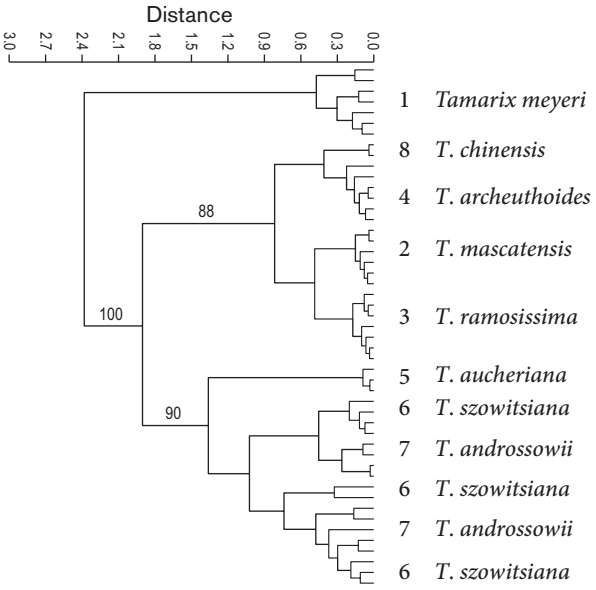

Figure 2: UPGMA dendrogram of *Tamarix* species based on morphological data.
Slika 2: Dendrogram UPGMA vrst rodu *Tamarix* na osnovi morfoloških podatkov.

each species were grouped together and formed a distinct cluster. Therefore, the studied species were delimited based on morphological characters.

In PCoA plot of the morphological characters (Figure 3), the species in the sect. *Tamarix* viz. *T. arceuthoides*, *T. mascatensis* and *T. ramosissima* and *T. karkalensis* were grouped in one cluster. Similarly, the species of the sect. *Oligadenia* viz. *T. meyerii*, *T. androssowii*, *T. szowitsiana* and were grouped together, while *T. auscheriana* of the sect. *polyadenia* was placed far from the other species. In addition *T. aucheriana* was also placed far from the other study species within the sec. *Polyadenia*. This is due to stamen number, width of inflorescence and disc diameter.

Within the sec. *Oligadenia*, *T. androssowii* and *T. szowitsiana* show close affinity due to its ratio of pedicel size/calyx size, inflorescence, length of inflorescence, length of external calyx and leaf length. While, *T. meyeri* is placed far from of them due to leaflet length, leaflet width, width of external and internal calyx.

3.2 Relationship between species based on its studies

UPGMA, NJ, and maximum likelihood (ML) methods in identical species produced similar results for ITS data. Therefore, only the NJ tree is presented (Figure 4). In general, the studied species from different sections were placed intermixed. Therefore, ITS data could not delimit the species according to the presumed sections in genus *Tamarix*. All the obtained clades had high bootstrap value (>80%). *Tamarix karkalensis* differed the most from the other species and formed a single clade. This was followed by *T. arceuthoides*. The samples identified as *T. androssowii* were placed close to each other.

3.3 Introgression evidenced

Molecular phylogeny of the studied species and their relationship was not in agreement with the species tree of morphological characters and with taxonomic classification of the genus.

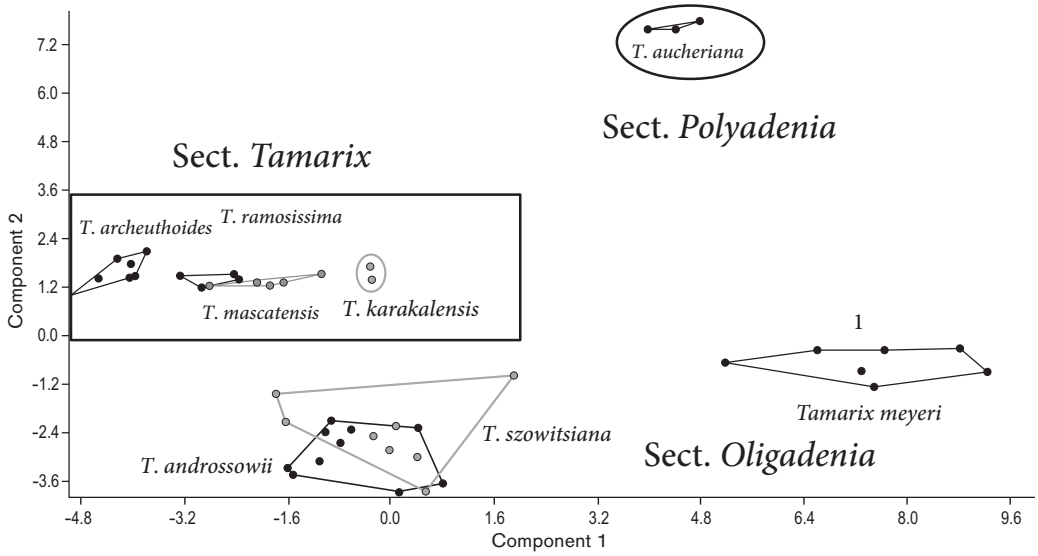

Figure 3: PCoA plot of *Tamarix* species based on morphological character.
Slika 3: Graf PCoA vrst rodu *Tamarix* na osnovi morfoloških podatkov.

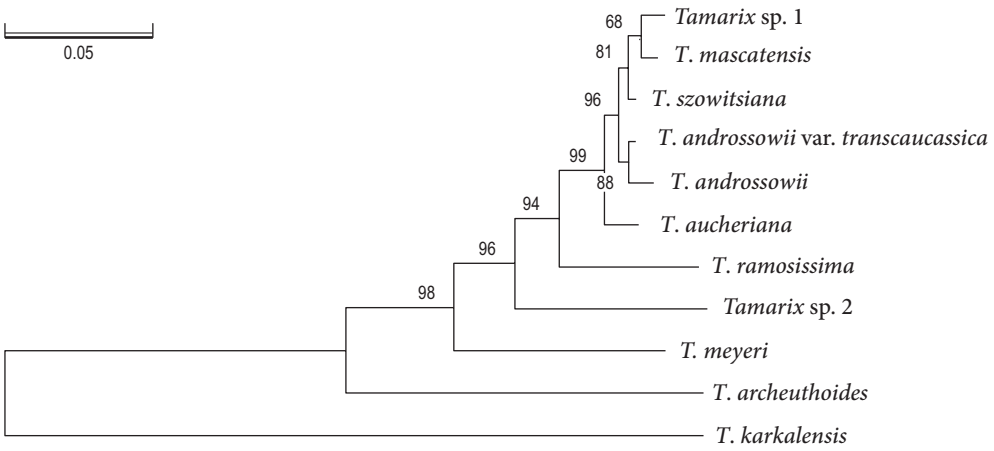

Figure 4: NJ tree of *Tamarix* species based on ITS sequences. Numbers above branches are bootstrap value.
Slika 4: Drevo združevanja NJ vrst rodu *Tamarix* na osnovi ITS sekvenc. Številke nad vejami prikazujejo število bootstrap ponovitev.

Therefore, the potential gene flow among the studied species was investigated by HGT (Horizontal Gene Transfer) analysis with the help of T-REX program (Figure 5). The HGT tree is based on both morphological and ITS tree of the studied species. The results revealed some degree of gene flow between *T. meyerii* and almost all the other studied species in the region. Moreover, *T. Karkalensis* had gene exchange with *T. arceuthoides*, while, *T. ramosissima* exchanged gene with *T. arceuthoides*. The plant named *Tamarix* sp1, was therefore, considered to be *T. androssowii* that was produced by gene flow between this species and *T. meyerii*. However, *Tamarix* sp2 was considered to be new variety of *T. szowitsiana*, formed by introgression between *T. szowitsiana* and *T. meyerii*.

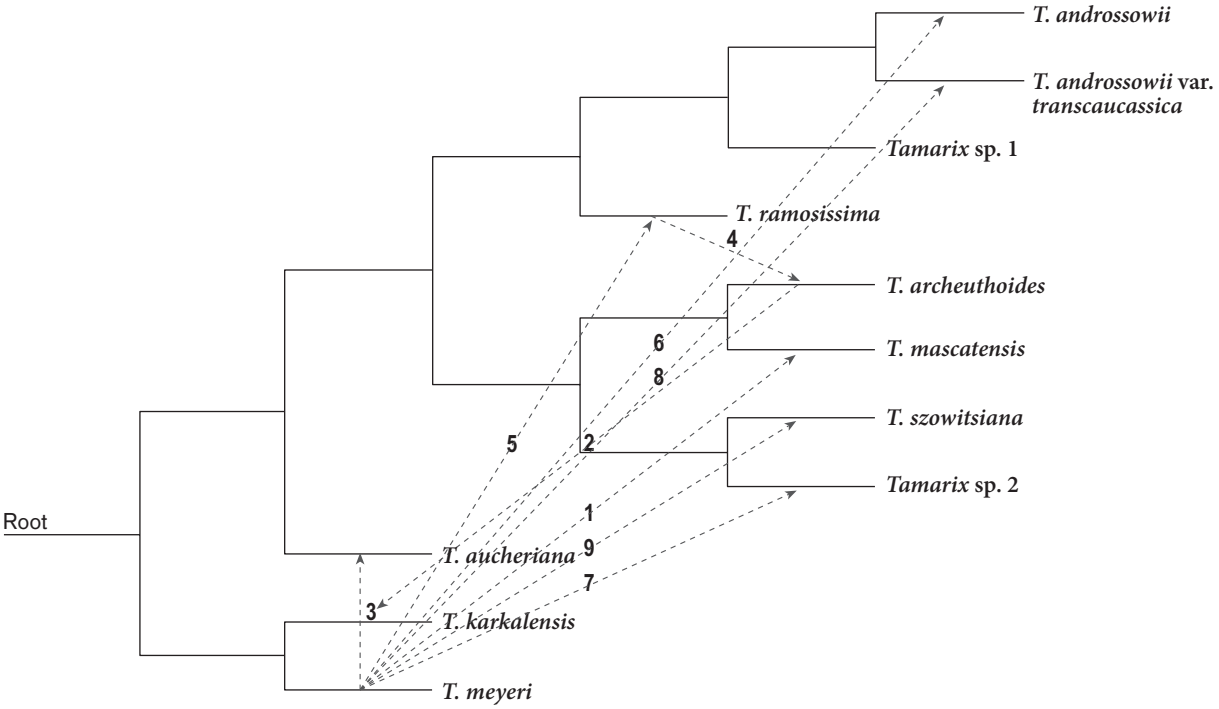

Figure 5: HGT tree of *Tamarix* species based on morphological and ITS data, showing gene flow among these taxa.
Slika 5: Drevo HGT vrst rodu *Tamarix* na osnovi morfoloških in ITS podatkov, ki prikazuje pretok genov med taksoni.

As evidenced in UPGMA tree of morphological characters (Figure 6), plants of *T. szowitsiana* and *T. androssowii* were placed intermixed due to variability and overlap in their morphological characters. Detailed morphological study of these plants in six geographical populations revealed that some plants have a two or more mixture of species characters. The same result was obtained by ISSR study.

UPGMA tree (Figure 6) and MDS plot (Figure 7) of ISSR data revealed admixture of samples in *T. szowitsiana* (coded 1), *T. androssowii* (coded 2) and the trees with mixture of characters from both species (coded 3).

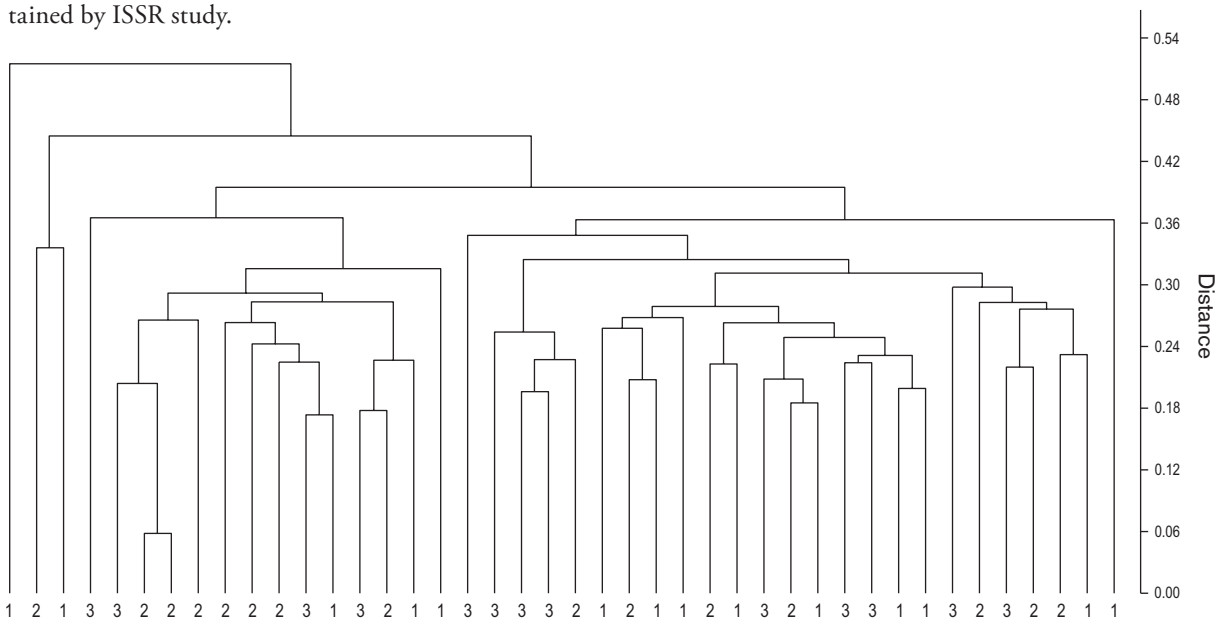

Figure 6: UPGMA tree of the studied samples based on ISSR data.
Slika 6: Drevo združevanja UPGMA preučevanih vzorcev na osnovi ISSR podatkov.

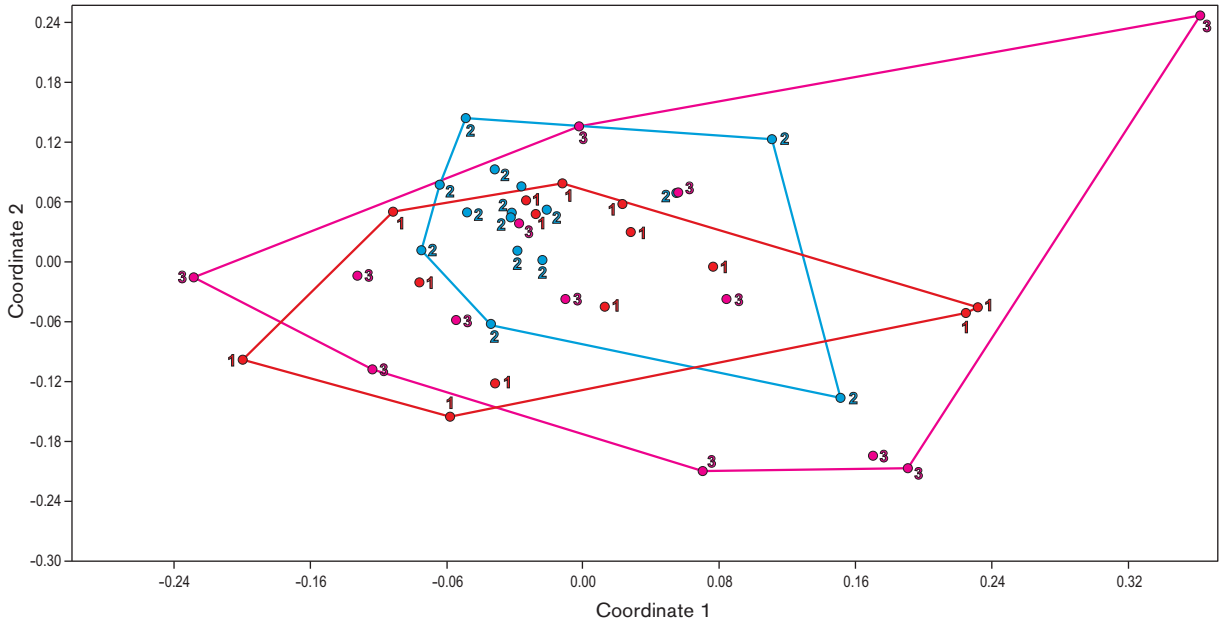

Figure 7: MDS plot of the studied samples based on ISSR data.
Slika 7: Graf MDS preučevanih vzorcev na osnovi ISSR podatkov.

4. Discussion

Tamarix species act against deforestation in Iran, therefore identification of these species and their hybrids throughout the country is crucial for conservation strategy. *Tamarix* species grow in different geographical populations in the country and face diverse environmental conditions. It is usually expected that species that grow in different geographical populations, show genetic and morphological variability (Sheidai et al. 2018). The same holds true for *Tamarix* species (Ijbari et al. 2014, Sheidai et al. 2018). Moreover, *Tamarix* species are known to form frequent inter-specific hybrids (Sheidai et al. 2018). Due to morphological overlaps in *Tamarix* species as a result of inter-specific gene flow, identification of *Tamarix* species is problematic and needs to have clear cut differentiating morphological features. The present study revealed that, it is better to start morphological identification of *Tamarix* species, first by considering 4-merous flowers versus 5-merous flowers. Secondly, characters like shape of disks and leaves should be considered for *Tamarix* identification (Obermeyer 1978, Bredenkamp & Phepo 2008).

However, we may still some degree of overlap even in these characters, therefore, it is better to accompany morphological identification with molecular data support. Combination of both morphological and molecular results provide a more reliable and consistent method of identifying *Tamarix* species.

Our second main objective in this study was to reveal gene flow or hybridization within *Tamarix* species. Molecular tools also provide the means to investigate the genetic diversity within and among populations, and can also detect hybridization and introgression patterns between closely related species (Le Roux & Wieczorek 2008).

Hybridization is a driving force of invasion, when new species are introduced into a new region, they may meet closely related species or genotypes and form hybrid. These hybrid individuals have high genotypic fitness in the newly-invaded habitat (Gaskin & Kazmer 2009). Hybridization followed by introgression (natural back-crossing between hybrids and parental lineages) can provide necessary genetic variability for *Tamarix* species to cope with environmental condition they face (Schierenbeck & Ellstrand 2009). These hybrids may survive in extreme habitats that are not suitable for either of the parent taxa, as was reported in *Helianthus* (Riesberg et al. 2003). The present study revealed the occurrence of inter-specific hybridization or introgression between *T. meyeri, T. szowitsiana*, and *T. androssowii*. The ecotypes formed show the separation of a single lineage into separate lineages that act as the initial stage of genetic divergence, which in some cases may lead to speciation (Schaal et al. 2003).

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5. References

Arianmanesh, R., Mehregan, I., Nejadsatari, T. & Assadi, M. 2015: Molecular phylogeny of *Tamarix* (Tamaricaceae) species from Iran based on ITS sequence data. European Journal of Experimental Biology 5: 44–50.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S. & Donoghue, M. J. 1995: The ITS region of nuclear ribosomal DNA-A valuable source of evidence on Angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247–277.

Baum, B. R. 1967: Introduced and naturalized tamarisks in the United States and Canada [Tamaricaceae]. Baileya 15: 19–25.

Baum, B. R. 1978: The Genus *Tamarix*. Jerusalem, Israel: Israel Academy of Sciences and Humanities.

Bredenkamp, C. & Phepho, N. 2008: Hybridization of *Tamarix usneoides* and *Tamarix ramosissima*. Unpublished. South African Botanical Institute.

Enninful, E. K. &Torvi, D. A. 2008: A variable property heat transfer model for predicting soil temperature profiles during simulated wildland fire conditions. International Journal of Wildland Fire 17: 205–213.

Brotherson, J. D. & Winkel, V. 1986: Habitat relationships of saltcedar (*Tamarix ramosissima*). The Great Basin Natural 46: 535–541.

Campbell, J. Y., Lo, A. & Mackinlay, A. C. 1997: The Econometrics of Financial Markets Princeton University Press, Princeton, N.J.

Gaskin, J. F. 2003: Tamaricaceae. In: Kubitzki, K & Bayer, C. (eds.). The Families and Genera of Vascular Plants. Springer. pp. 363–368.

Gaskin, J. F. & Kazmer, D.J. 2009: Introgression between invasive saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA. Biological Invasions 11: 1121–1130.

Gaskin, J. F. & Schaal, B. A. 2002: Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proceedings of the National Academy of Sciences of the United States of America 99: 11256–11259

Gaskin, J. F. & Schaal, B.A. 2003: Molecular Phylogenetic Investigation of U. S. Invasive *Tamarix*. Systematic Botany 28: 86–95.

Hammer, Ø., Harper, D. & Ryan, P. D. 2012: PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4: 1–9.

Heywood, V. H., Brummitt, R. K., Culham, A. & Seberg, O. 2007: Flowering Plant Families of the World. Kew: Royal Botanic Gardens.

Ijbari, H., Sheidai, M., Mehrabian, A. R., Noormohammadi, Z. & Ghasemzadeh-Baraki, S. 2014: K-means clustering and STRUCTURE analyses of genetic diversity in *Tamarix* L. accessions. Turkish journal of botany 38: 1080–1094.

Križman, M., Jakše, J., Baričević, D., Javornik, B. & Prosek, M., 2006: Robust CTAB activated charcoal protocol for plant DNA extraction. Acta agriculturae Slovenica 87: 427–433.

Le Roux, J. & Wieczorek, A.M. 2008: Molecular systematic and population genetics of biological invasions: towards a better understanding of invasive species management. Annals of Applied Biology 157: 1–17.

Mayonde, S. G., Cron, G. V., Gaskin, J. F. & Byrne, M. J. 2015: Evidence of *Tamarix* hybrids in South Africa, as inferred by nuclear ITS and plastid trnS–trnG DNA sequences. South African Journal of Botany 96: 122–131.

Mayonde, S. G., Cron, G. V., Gaskin, J. F. & Byrne, M. J. 2016: *Tamarix* (Tamaricaceae) hybrids: the dominant invasive genotype in southern Africa. Biological Invasions 18: 3575–3594.

Mayonde, S, Cron, G.V, Glennon, K. L & Byrne, M. J. 2019: Genetic diversity assessment of *Tamarix* in South Africa – Biocontrol and conservation implications. South African Journal of Botany 121: 54–62.

Obermeyer, A. A. 1976: Tamaricaceae. In: Ross, J.H. (ed.): Flora of southern Africa. Botanical Research Institute, Department of agricultural technical services, Pretoria.

Podani, J. 2000. Introduction to the Exploration of Multivariate Data. Backhuyes, Leiden, 407 pp.

Schaal, B. A., Gaskin, F. & Caicedo, A. L. 2003: Phylogeography, haplotype trees, and invasive plants. Journal Heredity 94: 197–204.

Rieseberg, L. H., Raymond, O., Rosenthal, D. M., Lai, Z., Livingstone, K., Nakazato, T., Durphy, J. L., Schwarzbach, A. E., Donovan, L. A. & Lexer, C. 2003: Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301: 1211–121.

Schierenbeck, K. A. & Ellstrand, N. C. 2009: Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions 11: 1093–1105.

Schiman-Czeika, H. 1964: Flora Iranica. Graz: Akademische Druck-u. Verlagsanstalt, Vol. 4.

Sheidai, M., Zanganeh, S., Haji-Ramezanali, R., Nouroozi, M., Noormohammadi, Z. & Ghsemzadeh-Baraki, S. 2013: Genetic diversity and population structure in four *Cirsium* (Asteraceae) species. Biologia 68: 384–397.

Sheidai, M., Ziaee, S., Farahani, F., Talebi, S.M., Noormohammadi, Z. & Hasheminejad Ahangarani Farahani, Y. 2014: Infra-specific genetic and morphological diversity in *Linum album* (Linaceae). Biologia 69: 32–39.

Sudbrock, A. 1993: Tamarisk control-fighting back: an overview of the invasion, and a low-impact way of fighting it. Restoration and Management. Notes 11: 31–34.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2012: MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739.