Asian Journal of Conservation Biology, July 2016. Vol. 5 No. 1, pp. 45-50 AJCB: FP0072
ISSN 2278-7666 ©*TCRP* 2016

Phylogenetic relationships of the Genus *Tamarix* L. (Tamaricaceae) from Iran based on nuclear and plastid DNA sequences

Reza Arianmanesh [1]*, Iraj Mehregan [1], Mostafa Assadi [2] and Taher Nejadsattari [1]

[1] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2] *Research Institute of Forest & Rangelands, Tehran, Iran*

(Accepted June 24, 2016)

ABSTRACT

***Tamarix* L. with almost 54 species is the largest genus of the Tamaricaceae. This study was carried out on the species of *Tamarix* that growing in Iran. Plastid trnS-trnG sequences were obtained for 16 samples recognized by recent taxonomic treatments from Iran. In addition, we used 13 previously trnS-trnG sequences from GenBank to test the monophyly of *Tamarix* in Iran. Phylogenetic analysis were conducted using Bayesian inference. In this study we use DNA sequence data to identify species of *Tamarix* that growing in Iran, and to determine if the molecular data are congruent with the morphological distinctions that currently segregate taxa. We also test congruence of morphologically based sectional classifications and our molecular gene trees. The results indicate that *Tamarix* species from Iran constituted a monophyletic group. Data analysis indicates the three taxonomic sections based on morphology (Baum 1978) are not supported by the molecular analyses and to determine the evolution of *Tamarix* use of morphological characteristics coupled with molecular data will be most effective.**

Key words: Iran, *Tamarix*, phylogeny, trnS-trnG, ITS.

INTRODUCTION

Tamarix is an old world genus, but many of its species have become naturalized and invaded other parts of the world (Gaskin and Schaal, 2003). The genus *Tamarix* is characterised by considerable morphological and ecological similarity among its species, making it one of the more taxonomically challenging genera among the angiosperms (Baum, 1978) and it remains one of the few in the Tamaricaceae family that can grow in different climatic and edaphic conditions; hence the species exhibit considerable phenotypic variation (Di Tomaso, 1998). Many taxa are almost indistinguishable in the vegetative state (Crins, 1989), making their morphological identification difficult. Hybridization between the various species also plays a role in the taxonomic confusion in *Tamarix* (Wilken, 1993). *Tamarix* is one of five genera in the family Tamaricaceae and is represented by 55 species world-wide (Heywood *et al*., 2007). The family Tamaricaceae grows most successfully in temperate and sub-tropical regions. The Tamaricaceae family has previously been placed as a sister family to Frankeniaceae because they share characters such as secondary chemistry, gland structure, and scale-like leaves (Kubizki, 2003). Among the genera of the Tamaricaceae, *Tamarix* is the largest with 55 species (Heywood *et al*., 2007). *Tamarix* are native to the Mediterranean countries, southern Europe, China, India, Mongolia, North Africa and south western Africa (Baum, 1978; Heywood *et al*., 2007). *Tamarix* plants are shrubs, semi-shrubs and tall trees that can grow up to 18 m in height. They are adaptable halophytic or xerophytic plants mostly with multiple stems and slender branches (Brotherson and Winkel, 1986). Young branches are reddish brown in colour, sometimes black with light-green coloured leaves. Leaves of *Tamarix* are taxonomically useful as their shape and attachment modes vary according to species, e.g. sessile vs. vaginate (Baum, 1978). They are scale-like, about 3 mm in length (Baum, 1978) and usually contain salt glands (Bredenkamp and Phepho, 2008).

In Iran, 25 species of *Tamarix* are present (Assadi, 1988; Boissier, 1879; Mobayen, 1996; Parsa, 1949). *Tamarix* species that distribution in different regions of Iran, showed in Table 1. The purpose in this study were to test the naturalness of *Tamarix* species classification that are present in Iran and to test the congruence of morphological and molecular data at sectional and species level. In this paper, we assess the systematic position of *Tamarix*, analysing nucleotide sequences of plastid and nuclear DNA in a phylogenetic framework. The plastid sequences analysed was trnS-trnG and the nuclear region analysed was the internal transcribed spacer region of the nuclear ribosomal DNA (Gaskin and schaal, 2003). We were particularly interested in clarifying the relationships among species of *Tamarix* to and discussing its morphological peculiarities in light of its inferred phylogenetic position.

MATERIALS AND METHODS

Specimen were collected in the field and dried in silica gel or preparation from herbaria in Iran (TARI, IAUH). Phylogenetic reconstruct were carried out in 16

**Corresponding Author's E-mail: dr.r_arianmanesh@yahoo.com*

Table 1. List of Iranian taxa investigated in our analysis and herbaria where the vouchers are deposited *TARI= Herbarium of Research Institute of Forests and Rangelands, IAUH= Islamic Azad University Avicennia Herbarium*

Species	Origin, voucher
T. octandra	Iran: Prov. West Azerbaijan; Uromia lake, 1330m, Assadi and Shirdelpour, (12011 TARI).
T. passerinoides var macrocarpa	Iran: Prov. Ghom; West of Namak lake, 950m, Assadi and Bazgosha, (56601 TARI).
T. korolkowii	Iran: Prov. Khorasan; 6km south of Sabzehvar, 1000m, Assadi and Massoumi, (55891 TARI).
T. arceuthoides	Iran: Prov. Golestan; Atrak river, 180m, Assadi and Massoumi, (55407a TARI).
T. hispida var karelinii	Iran: Prov. Isfahan; Zavareh, 992m , Arianmanesh. (IAUH 000014836)
T. ramosissima	Iran: Prov. Isfahan; Varzaneh,1479m , Arianmanesh. (IAUH 000014842)
T. tetragyna var meyeri	Iran: Prov. Isfahan; Isfahan,1578m , Arianmanesh. (IAUH 000014843)
T. tetragyna var deserti	Iran: Prov. Isfahan; Varzaneh,1481m , Arianmanesh. (IAUH 000014841)
T. rosea	Iran: Prov. Yazd; between Bafgh and Ravar, 2200m, Assadi and Bazgosha, (56068 TARI).
T. aralensis	Iran: Prov. Isfahan; 20 km Meymeh to Delijan, 2113m , Arianmanesh. (IAUH 000014840)
T. mascatensis	Iran: Prov. Fars; Kazeroon, Parishan lake, 1970m, Arianmanesh. (IAUH 000014848)
T. aphylla	Iran: Prov. Isfahan; between Ardestan and Zavareh, 1089m , Arianmanesh. (IAUH 000014837)

specimen of *Tamarix* presented from Iran. Tables 1 and 2 lists all taxa used in this study and summarizes sources, voucher specimen data and GenBank accession numbers. Total DNA was extracted using the DNeasy Plant Mini kit (Qiagen, Germany). We amplified the trnS-trnG region of the plastid DNA using primer combinations trnS and trnG primers: a forward primer trnS annealing, 5'-GCCGCTTTAGTCCACTCAGC-3', and a reverse primer trnG annealing, 5'- GAACGAATCA-CACTTTTACCAC -3'. The PCR protocol for trnS-trnG region included: 30 cycles of 2 min denaturation (95°C), 1 min annealing (55°C), and 2 min elongation (72° C), with two additional seconds elongation per cycle. We amplified the ITS region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA using primer combinations ITSTX4F and ITSTX4R primers: a forward primer ITSTX4F annealing, 5'-ACT TGTTCACCGAAACACGG-3', and a reverse primer ITSTX4R annealing, 5'-TAAGGCGCACGGCGTGATCC -3'. The PCR protocol for ITS region included: 30 cycles of 2 min denaturation (95°C), 1 min annealing (55°C), and 2 min elongation (72° C), with two additional seconds elongation per cycle (Gaskin and schaal, 2003).

PHYLOGENETIC ANALYSIS

Matrices were analyzed with PAUP4.0b10, with the following options: heuristic search with 1,000 random-addition-sequence replicates; tree bisection-reconnection (TBR) branch swapping; "collapse zero length branches;" saving all most parsimonious trees. Character state changes were treated as equally weighted. Nonoverlapping parsimony informative indels were coded as binary characters and added to the end of the data matrix. Relative clade support was estimated using 1,000 bootstrap, replicates in PAUP via "full heuristic" searches and simple taxon addition. The best-fitting substitution model (GTR+I+G) was determined under the Akaike

Table 2. List of Iranian and non-Iranian taxa used in present analysis from GenBank with accession number

Species	Genbank accession numbers (trnS-trnG)	Genbank accession numbers (ITS)
Myricaria alopecuroides	AF490774	KJ808603
T.aphylla	AY099923	AF484767
T.usneoides	KM657186	KM657172
T.canariensis	AF490780	AF484808
T.chinensis	AF490804	AF484776
T.dalmatica	AF490822	AF484794
T.elongata	AF490792	AF484777
T.rosea	AF490779	AF484751
T.laxa	AF490784	AF484756
T.nilotica	AF490777	AF484749
T.parviflora	AF490826	AF484810
T.aralensis	AF490781	AF484799
T.pycnocarpa	AF490791	AF484763
T.hohenackeri	AF490807	AF484779
T.meyeri	AF490800	AF484772
T.octandra	AF490787	AF484759
T.aucheriana	AF490790	AF484762
T.smyrnensis	AF490801	AF484773
T.arborea	AF490808	AF484780
T.androssowii	AF490785	KT783528
T.arceuthoides	KT809499	KT809489
T.hispida	KT809500	KT809490
T.korolkowii	KT809501	KT809491
T.mascatensis	KT809502	KT809493
T.octandra	KT809503	KT809494
T. passerinoides	KT809504	KT809495
T.ramosissima	KT809505	KT809496
T.tetragyna var deserti	KT809506	KT809497
T.tetragyna var meyeri	KT809507	KT809498

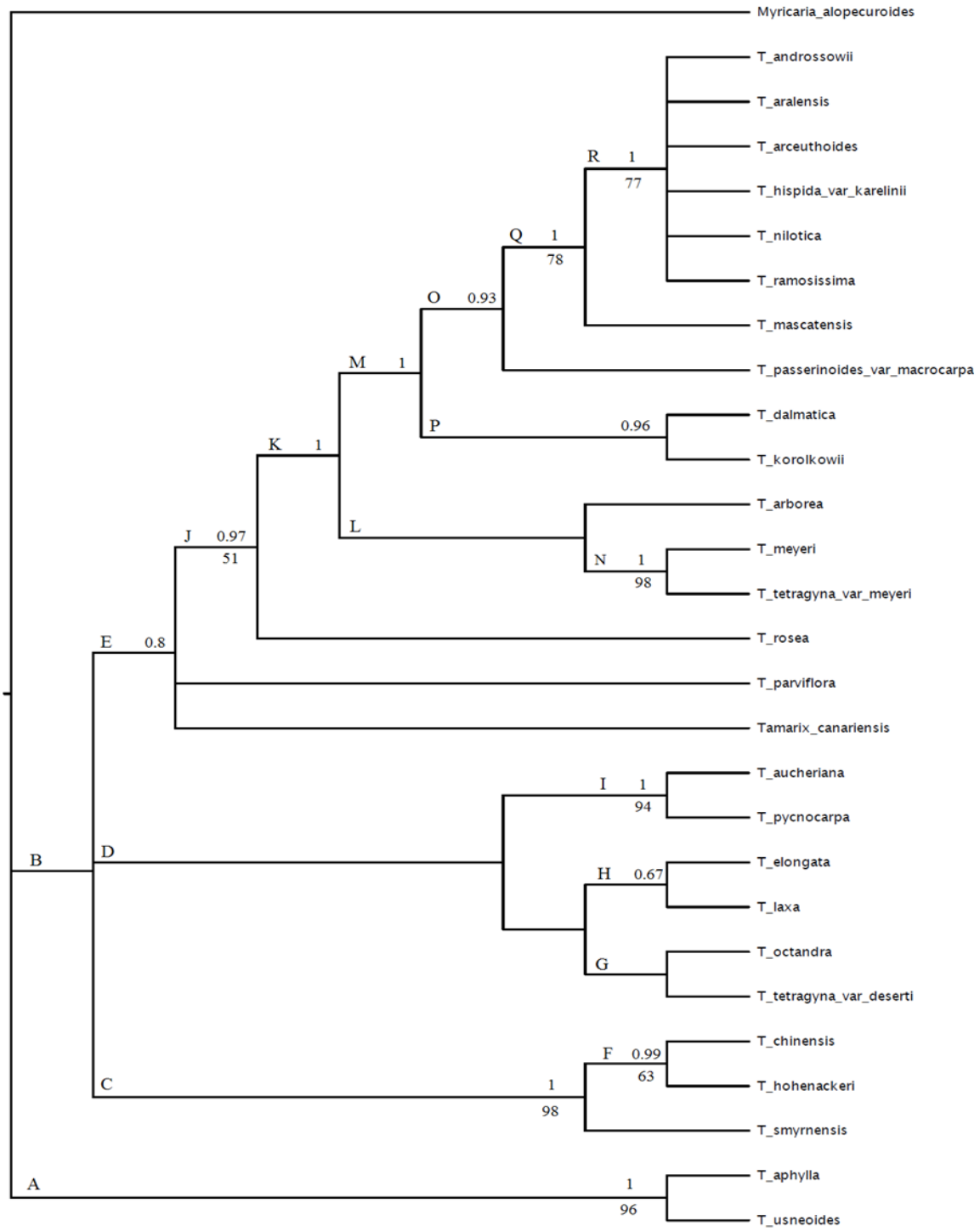

Figure 1. Phylogenetic relationships of 26 sampels of *Tamarix* (13 sampels of *Tamarix* from Iran and 13 sampels of *Tamarix* non from Iran) based on combined trnS-trnG and ITS sequence data
Numbers above branches are Bayesian posterior probabilities. Numbers below branches are percentage bootstrap values

Information Criterion (AIC) in Model selected (Posada, 2008). BI was performed in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). A 50% majority-rule consensus tree with Bayesian posterior probabilities (PPs) of clades was calculated after removing the first 10% generations as burn in.

RESULTS AND DISCUSSION

Phylogenetic reconstruct were carried out in 13 specimen of *Tamarix* presented from Iran. In this study we used the trnS-trnG and ITS sequence of 13 species of *Tamarix* from GenBank. List of Iranian and non-Iranian taxa used in our analysis with GenBank accession numbers showed in Table 2. We also used the trnS-trnG sequence of *Myricaria alopecuroides*, from GenBank as the outgroup (Gaskin and schaal, 2003). The combined data set of the trnS-trnG and ITS region included 1378 characters. Of these characters, 939 were constant, 199 variable and 63 of them potentially parsimony informative. The parsimony analysis of the trnS-trnG and ITS sequence data of the 27 specimens resulted in 10000 trees (maximum limit set) of 189 steps with a Retention Index (RI) of = 0.802.

The Consistency Index (CI) was 0.772 excluding uninformative characters. The strict consensus tree of the combined trnS-trnG and ITS sequences is shown in Figure 1. The monophyly of *Tamarix* was well supported. The strict consensus tree including 26 *Tamarix* samples has two main clades (A and B, Figure 1). *T. aphylla* and *T. usneoides* are grouped together in clade A with strong support (PP = 1; BS = 96%). Just as their morphological similarity suggests that these two species are closely related, their almost identical trnS-trnG and ITS sequence data confirms this relationship. Within Clade B, 24 specimens have grouped together. Unlike clade A, clade B has some resolution (branching) with the specimens grouping into three sub-clades (C, D and E). Subclade C is well supported with a bootstrap value and posterior probabilities (PP = 1; BS = 98%) includes *T. chinensis*, *T. hohenackeri* and *T. smyrnensis*. Within subclades D and E, there is remarkably little resolution. There are six specimens that group together in sub-clade D. Sub-clade E within clade B, groups sixteen specimens with posterior probability of 0.8. Best support was occurred in clade A between *T. aphylla* and *T. usneoides* (PP = 1; BS = 96%), in clade I between *T. aucheriana* and *T. pycnocarpa* (PP = 1; BS = 94%) and in clade N between *T. meyeri* and *T. tetragyna* var. *meyeri* (PP = 1; BS = 98%).

In this study we provide the first phylogenetic analysis about the genus *Tamarix* from Iran. At first the monophyly of *Tamarix* on the 26 species exponent the three sections of Baum's classification, proposed by Gaskin and Barbara (2003). This analysis include 13 species of *Tamarix* from Iran and 13 species of non Iranian *Tamarix* . In this molecular phylogeny study, according to our obtained data, the genus *Tamarix* in Iran was determined as a monophyletic group.

The infrageneric taxonomy of Iranian Tamarix

The infrageneric taxonomy of *Tamarix* has undergone many revisions. Bunge divided the genus into sections on the basis of seasonality of flowering and used numbers of floral parts, raceme and vegetative morphology, filament insertion patterns, petal persistence, and capsule and style morphology as the basis for further subdivisions. Howere, Baum (1964) and others have shown that seasonality of flowering is unreliable and that many species have both vernal and aestival (or continuous) anthesis. One of the most useful sets of characters derives from disc morphology. The three sections recognized by Baum (1978) are characterized by features of the disk, as well as by raceme width, petal lenght, and stamen number and position.

Species of sect. *Tamarix* generally have racemes less than 5 mm broad, petals 1-2.25 mm long, and five antesepalous stamens. Series within this section differ in vegetative features such as the peresence or absence of papillae and leaf morphology. Species of Iranian *Tamarix* belong to sect. *Tamarix* include: *T. dioica*, *T. aphylla*, *T. ramosissima*, *T. arceuthoides*, *T. indica*, *T. korolkowii*, *T. karakalensis*, *T. aralensis, T. smyrnensis, T. mascatensis*, *T. serotina* and *T. hispida.*

Species of sect. *Oligadenia* have broader vernal racemes, some tetrandrous members, and disks with nectariferous lobes. The series within this section differ in bract length relative to pedicel length, petal shape and length, and raceme morphology. Species of Iranian *Tamarix* belong to sect. *Oligadenia* include: *T. rosea*, *T. kotschyi*, *T. androssowii*, *T. tetragyna*, *T. octandra*, *T. leptopetala* and *T. szowitsiana*.

Species of sect. *Polyadenia* also have broad racemes but have more stamens and disks that lack nectariferous lobes. Its two series differ in the number of antepetalous stamens arising from the disk. Species of Iranian *Tamarix* belong to sect. *Polyadenia* include: *T. stricta*, *T. dubia* and *T. passerinoides*.

Congruence of morphological and molecular data

Phylogenies from both nuclear ribosomal ITS and chloroplast trnS-trnG intergenic spacer sequence data were constructed. Portions of the final phylogenies presented in Figure 2 illustrate incongruence with earlier taxonomic understanding of the genus. For example, note that *T. dalmatica* and *T. korolkowii*, thought to belong in different sections of the genus (sects. *Oligadenia* and *Tamarix*, respectively), have identical placement (in clade P, Figure 1). Additionally, the most recent sectional classification of the genus (Baum, 1978) was not significantly similar to either the chloroplast or nuclear topologies found in Gaskin and Schaal (2003). The study shows that morphology within *Tamarix* is often misleading as a means of identifying specimens. Also, though not all species could be distinguished with molecular data. The three taxonomic sections based on morphology (Baum, 1978) are not supported by the molecular analyses. Thus, the morphological characters used to define the sections of *Tamarix* should be reevaluated.

Identification of species

Two species *T. hohenackeri* and *T. smyrnensis* are known synonymous by Baum (1978) but Qaiser (1983) knows *T. hohenackeri* independent of *T. smyrnensis*, also in Flora of Iran, both species *T. ramosissima* and *T. smyrnensis* are known synonymous by Assadi (1987). The major difference in the literature as to the separation of the two species is in the shape of petals. In *T. smyrnensis*, petals are ovate to suborbicular, strongle keeled especially in their lower part but in *T. ramosissima*, petals are obovate and not keeled. In according to this study, *T. smyrnensis* and *T. hohenackeri* located in clade C and *T. ramosissima* located in clade R. The results revealed that three species with little morphological difference have distinct DNA sequences and synonym of *T. ramosissima* and *T. smyrnensis*, as well as, synonym of *T. hohenackeri* and *T. smyrnensis* is not confirmed.

T. aralensis and *T. ramosissima* can be identified by their sessile leaves, pentamerous flowers and hololophic androecial disks. *T. aralensis* is distinguished from *T. ramosissima* by its caducous petals at the time of seed maturation (Baum, 1967). Crins (1989) claims that their morphology is similar, and that it is difficult to recognize these two taxa as different species. In this cladogram, molecular evidence support *T. ramosissima* and *T. aralensis* are distinct species.

Based on morphological characteristics, it is difficult to distinguish the two species *T. arceuthoides* and *T.*

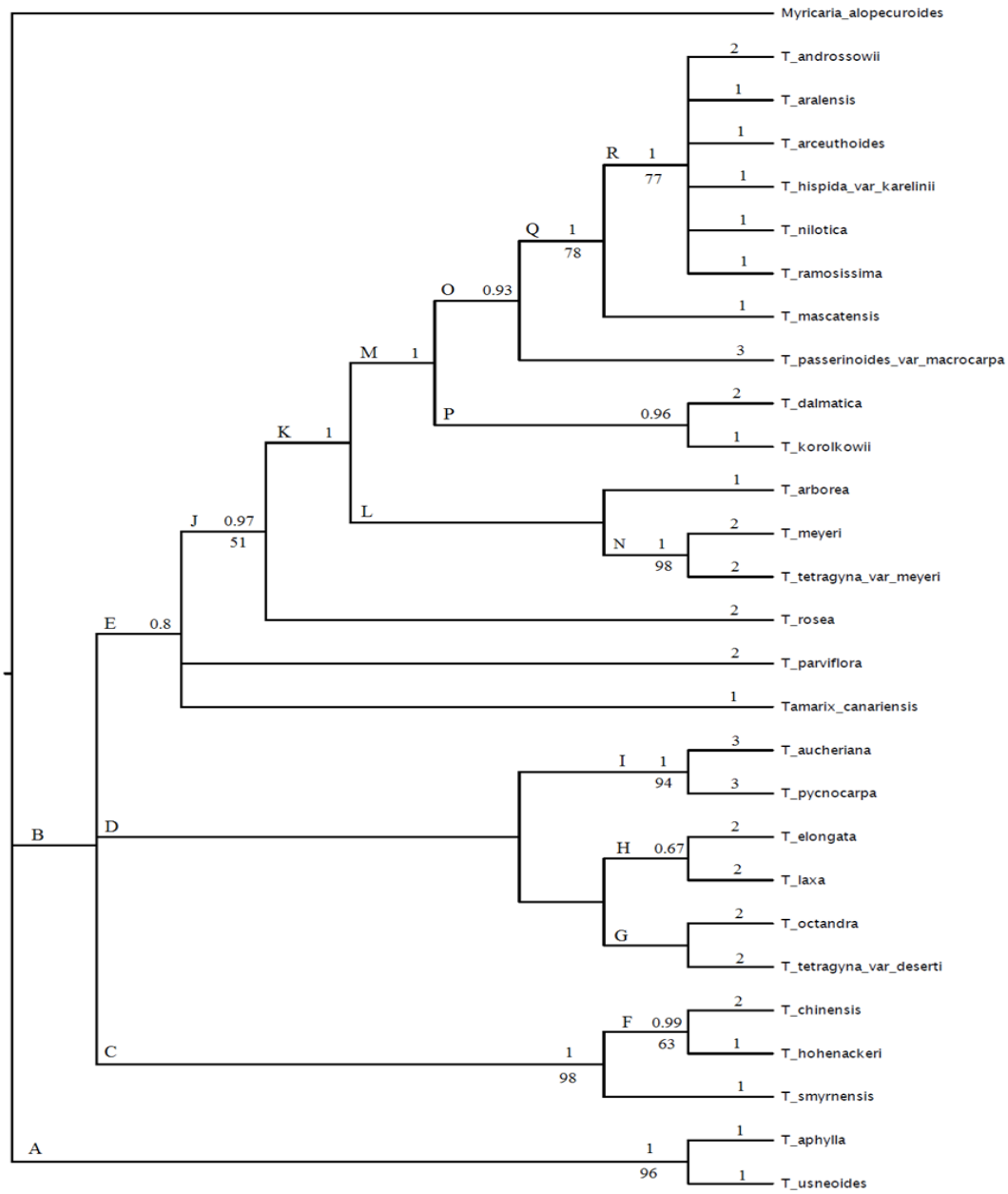

Figure 2. Section Category of the genus *Tamarix* on maximum parsimony consensus tree based on combined trnS-trnG and ITS sequence data. Section *Tamarix* = 1 , Section *Oligadenia* = 2 , Section *Polyadenia* = 3.

korolkowii. In *T. arceuthoides*, raceme is no dense and flowers are placed at a distance, in addition in the observed sample , the flower color is red but in *T. korolkowii*, raceme is dense and in the observed sample, the flower color is white. Assadi (1987) said, further researches may prove that the two types of species are the same as each others. In according to our research, they have located in different clades and have distinct DNA sequences.

Two species *T. meyeri* and *T. tetragyna* are known as distinct species by Baum (1978) but Assadi (1987), for *T. tetragyna*, has identified two varieties: var. *meyeri* and var. *deserti*. In this cladogram, *T. meyeri* and *T. tetragyna var. meyeri* with high bootstrap support (98%) were put together in clade N, thus the specific status of *T. meyeri* is not accepted.

CONCLUSION

The results indicate that *Tamarix* species from Iran constituted a monophyletic group. Despite the existence of a fairly recent monograph of the genus (Baum, 1978) *Tamarix* remains an exceedingly complex genus. Most species can not be identified without flowers and intermediate states exist for several morphological characters (and can even vary on a single individual or from season to season). Finally, DNA sequence data are in part incongruent with morphological distinctions currently used to segregate taxa. In conclusion, this analysis reveals that morphology within *Tamarix* does not always correlate with DNA sequence data. Baum's (1978) sectional classification of the genus is not statistically similar to DNA sequence data, and future subgeneric classification of *Tamarix* must include molecular data.

ACKNOWLEDGMENT

This article is extracted from my Phd thesis. We have finally thank from Islamic Azad University -Tehran Science and Research Branch for providing the facilities necessary to carry out the work.

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