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POLLEN MORPHOLOGY OF SOME *TAMARIX* (TAMARICACEAE) SPECIES OF IRAN

T. Shagholi[1], M. Keshavarzi[1]* and M. Sheidai[2]

[1]*Plant Science Dept., Alzahra University, Vanak, Tehran, Iran*
*E-mails: *m.keshavarzi@alzahra.ac.ir, corresponding author; neshat112000@yahoo.com*
[2]*Faculty of Life Sciences & Biotechnology, Shahid Beheshti University, Tehran, Iran*

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Tamarix L. (Tamaricaceae) is a halophytic shrub in different parts of Asia and North Africa. Taxonomy and species limitation of *Tamarix* is very complex. This genus has three sections as *Tamarix*, *Oligadenia*, and *Polyadenia*, which are mainly separated by petal length, the number of stamens, the shape of androecial disk and attachment of filament on the androecial disk. As there was no palynological data on pollen features of *Tamarix* species of Iran, in the present study 12 qualitative and quantitative pollen features were evaluated to find diagnostic ones. Pollen grains of 8 *Tamarix* species were collected from nature. Pollen grains were studied without any treatment. Measurements were based on at least 50 pollen grains per specimen. Light and scanning electron microscopes were used. Multivariate statistical methods were applied to clarify the species relationships based on pollen data. All species studied showed monad and tricolpate (except some individuals of *T. androssowii*). Some *Tamarix* species show a high level of variability, in response to ecological niches and phenotypic plasticity, which make *Tamarix* species separation much more difficult. Based on the results of the present study, pollen grains features are not in agreement with previous morphological and molecular genetics about the sectional distinction.

Key words: Iran, pollen grains, species relationship, *Tamarix*

INTRODUCTION

Tamarix L. (Tamaricaceae) is a genus with about 50–60 species throughout the world. These are halophytic shrubs and dwarf trees of Asia and north of Africa (Baum 1978). Species delimitation in this genus is very complex (Baum 1978, Villar *et al*. 2014). Linnaeus (1753) used the name *Tamarix* for this genus and described two species as *T. gallica* and *T. germanica.* Willdenow (1816) wrote the first monograph for the same genus. Ehrenberg (1827) was the first one who tries to find a clear separation of *Tamarix* from other genera in the Tamaricaceae family (Baum 1978) and recognised eight species for the *Tamarix*. Decaisne (1835) was the first one who uses disk features as diagnostic ones after that many botanists used the androecium characters in species delimitations and identification keys. The most famous global revision of the genus *Tamarix* date back to 1978 and the Baum monograph, includes three sections as *Tamarix*, *Oligadenia*, and *Polyadenia* and some series. These are separated by petal length, the number of stamens, the shape of androecial disk and attachment of filament on the androecial disk. Sections are separated into nine series based on several floral features.

For pollen morphological study in this genus, the literature showed that Erdtman (1952) studied the pollen morphology of some Tamaricaceae species of *Tamarix*, *Reaumuria*, and *Myricaria.* Nair (1962) has studied the pollen characters of *Tamarix* species in India and provided an identification key base on features as the reticulum condition and the shape and pollen dimensions and separated five *Tamarix* species.

Pollen grains of Tamaricaceae in Pakistan by Qaiser and Perveen (2004) resulted in an identification key. They separated two groups as pollen grains with and without columellae. They also pointed out that there was no continuity between morphological and palynological features. Pollen grains in this genus are monad, isopolar, 3 (rarely 4) colpate (colporate) and with reticulate sculpture (Tsarenko and Tsymbalyuk 2016). Recently Elkordy and Faried (2017), studied the palynological features of six *Tamarix* species in Egypt. By the use of numerical methods, they found that pollen morphological characters are of diagnostic importance.

There is not a consensus opinion on the *Tamarix* species number in Iran, Assadi (1988) mentioned 22 species for Iran, while 39 were recorded by Mozaffarian 1994 for the country. Some authors consider invasive species as *T. pentandra*, *T. tetrandra*, *T. gallica*, *T. chinensis*, *T. ramosissima* and *T. parviflora* as separate species while others as Sudbrock considered them as a complex hybridising group of species under name of *T. pentandra*. So, there is always ambiguity and disagreement in species numbers in this genus.

There is also a problem with species separation in *T. ramosissima* and *T. chinensis* due to high morphological similarities to the extent that authors as Allred (2002) considered these two as synonyms. Finally, molecular studies by ITS sequences showed that these two species showed a lot of genetic differences far from America but hybrids were formed by the high rate of gene flow in America and made clear identification difficult (Gaskin and Schaal 2002). The same kind of hybrid formation, between *T. ramosissima* and *T. chinensis* and *T. aphylla*, was previously recorded (Gaskin and Shafroth 2005). This degree of gene flow in *Tamarix* species results in phenotypic variations, which makes the delimitation of the species more complex.

Its geographic distribution is from the southern parts of Europe from the Middle East to the northern parts of Africa. It stretches its distribution map also to Pakistan, India, and China (Zhang *et al.* 2002). Iran is considered to be one of its main speciation centres in the world besides Pakistan, Afghanistan, Turkmenia, east of China and eastern parts of the Mediterranean region. Due to De Loach *et al.* 1997, there are lots of taxonomic problems in this genus that the presence of hybrid taxa makes it more complex. *Tamarix* species are difficult to distinguish in the vegetative state (Crins 1989). As no palynological information is available on pollen features of *Tamarix* species of Iran, the purpose of the present investigation is to provide palynological data of some *Tamarix* species found in Iran to provide diagnostic features to separate *Tamarix* species studied.

METHODS AND MATERIALS

Palynological study

Pollen grains examined for *Tamarix* species in this study were collected from nature (Table 1). Selected species were representatives of three sections and some series of this genus in Iran. Ten individuals of each accession were studied. Vouchers are deposited at ALUH, acronyms of herbaria are according to Thiers (2018). Pollen grains were not acetolised and directly mounted on a metallic stub, by double-sided tape.

Light microscopy of pollen grains was carried and photographed by using an Olympus BX-51 microscope. The measurements were based on at least 50 pollen grains per specimen. Scanning electron micrographs were prepared by the use of a TESCAN scanning electron microscope. The specimens were coated with gold in an ion sputtering device. The means of Polar axis (P) and equatorial diameter (E) were measured and the P/E ratio was calculated over all specimens. To describe morphological characters, Punt *et al*. (2007) terminology was used.

Multivariate analysis

There were nonvariant features in pollen grains of species studied, by the omission of those, finally, 12 different quantitative and qualitative features were measured and evaluated for selected *Tamarix* species (Tables 2–3). Quantitative characters were averages of measurements of at least 10 specimens. Measurements were done by the use of Digimizer software. For multivariate statistical analysis PAST software (Paleontological Statistics Version 3.15) (Hammer *et al.* 2001) was used. Two types of analyses were performed with PAST as cluster analysis using average taxonomic distance and the WARD clustering.

Table 1
Pollen grain specimens used in the studied taxa of *Tamarix* using light and scanning electron microscope (SEM)

Taxon	Locality	Longitude (E)	Latitude (N)
T. androssowii	Semnan, 20 km to Garmsar, 995 m, Shagholi, 2794 ALUH	52° 07.33	35° 18.98
	Semnan, Damghan, Begh Village, 1,109 m, Shagholi, 2894 ALUH	54° 26.74	36° 12.51
	Semnan, 5 km to Damghan, 1,146 m, Shagholi 2994 ALUH	54° 15.93	36° 07.15
	Semnan, Amiriyeh Village, 1,144 m, Shagholi 3294 ALUH	54° 14.47	36° 06.48
	Semnan, Khar Turan National Park, 1,007 m, Shagholi 3394 ALUH	55° 42.99	36° 06.48
T. androssowii var. *transcaucasica*	Semnan, 47 km to Shahroud, 1,114 m, Shagholi 3694 ALUH	54° 29.21	36° 12.81
T. arceuthoides	Semnan, 25 km to Garmsar, 1,033 m, Shagholi 2394 ALUH	52° 06.08	35° 19.76
T. aucheriana	Semnan, Damghan, Begh Village, 1,109 m, Shagholi, 1294 ALUH	54° 26.74	36° 12.51
	Tehran, 25 km to Garmsar, 1,033 m, Shagholi, 1394 ALUH	52° 60.08	35° 19.76
	Semnan, 5 km to Damghan, 1,146 m, Shagholi 1494 ALUH	54° 15.93	36° 07.15
T. karakalensis	Semnan, Haddadeh, 1,124 m, Shagholi 3494 ALUH	54° 44.51	36° 16.66
T. meyeri	Semnan, Sorkhe, 1,149 m, Shagholi 1594 ALUH	53° 10.93	35° 27.21
	Semnan, 10 km to Semnan, 1,165 m, Shagholi 1694 ALUH	53° 17.24	35° 30.49
	Semnan, Damghan, Begh Village, 1,109 m, Shagholi, 1794 ALUH	54° 26.74	36° 12.51
	Semnan, 5 km to Damghan, 1,146 m, Shagholi 1894 ALUH	54° 15.93	36° 07.15
T. ramosissima	Semnan, Cheshmeh Ali Basin, 1,395 m, Shagholi 1994 ALUH	54° 09.54	36° 15.21
	Semnan, Cheshmeh Ali Basin, 1,400 m, Shagholi 2194 ALUH	54° 09.29	36° 15.17
	Semnan, Cheshmeh Ali Basin, 1,376 m, Shagholi 2294 ALUH	54° 10.54	36° 14.67
T. szowitsiana	Semnan, 20 km to Garmsar, 995 m, Shagholi, 2594 ALUH	52° 07.33	35° 18.98
	Semnan, 10 km to Garmsar, 844 m, Shagholi, 2694 ALUH	52° 17.77	35° 14.41

RESULTS

Pollen grains were prolate (*T. androssowii*, *T. arceuthoides*, *T. aucheriana*, *T. szowitsiana*) to sub-prolate (*T. ramosissima*) or pre-prolate (*T. karakalensis*, *T. androssowii* var. *transcaucasica*, *T. meyeri*). Pollen ornamentations were of reticulate cristatum (*T. szowitsii* and *T. karakalensis*) or micro-reticulate (in other species studied as in *T. ramosissima*). All species studied are tricolpate and only some individuals of *T. androssowii* showed both 3- and 4-colpate. The largest pollen grain was observed in *T. androssowii* var. *transcaucasica* and the

Table 2
Average values of quantitative pollen features used in the present study. All measures in μm.

Species	Apocolpium length	Mesocolpium	Polar axis length	Equator axis length	P/E ratio	Lumen diameter	Muri diameter	Length of colpus
T. androssowii	4.91±0.35	8.96±0.10	23.45±0.05	14.9±0.83	1.57	0.678±0.10	0.300±0.03	22.32±1.12
T. androssowii var. *transcaucasica*	2.87±0.45	7.61±0.06	26.55±0.02	13.13±0.02	2.02	0.374±0.08	0.237±0.04	24.78±0.68
T. arceuthoides	4.98±0.41	7.75±0.07	19.15±0.02	13.48±0.33	1.42	0.367±0.05	0.362±0.06	15.82±0.09
T. aucheriana	5.72±0.23	9.07±0.13	22.53±0.09	13.03±0.20	1.73	0.280±0.07	0.287±0.03	21.54±0.64
T. karakalensis	3.72±0.30	9.08±0.09	25.05±0.03	10.95±0.09	2.28	0.656±0.14	0.326±0.04	24.41±1.81
T. meyeri	3.84±0.24	7.21±0.10	25.82±0.05	11.02±0.86	2.34	0.433±0.08	0.288±0.03	23.11±0.26
T. ramosissima	3.22±0.42	6.22±0.03	17.08±0.05	14.13±0.28	1.21	0.254±0.06	0.349±0.06	14.97±0.71
T. szowitsiana	3.68±0.14	7.62±0.04	21.51±0.06	13.13±0.26	1.64	0.259±0.05	0.355±0.03	21.11±0.36

Table 3
Qualitative pollen features used in the present study

Species	Lumen shape	Reticulum type	Number of colpi	Exine sculpture
T. androssowii	circular	heterobrochate	3	microreticulate
T. androssowii var. *transcaucasica*	circular	heterobrochate	3	microreticulate
T. arceuthoides	circular	heterobrochate	3	microreticulate
T. aucheriana	angled	heterobrochate	3	microreticulate
T. karakalensis	circular	heterobrochate	3	microreticulate cristatum
T. meyeri	circular	heterobrochate	3	microreticulate
T. ramosissima	angled	heterobrochate	3	microreticulate
T. szowitsiana	circular	heterobrochate	3	microreticulate cristatum

smallest in *T. ramosissima*. The largest P/E ratio was observed in *T. meyeri* and the lowest in *T. ramosissima* (Figs 1–2).

To demonstrate the species relationship between species studied, results were analysed by the use of the WARD method (Fig. 3). In the WARD diagram, two main clusters were formed. First main cluster composed of *T. ramosissima and T. aucheriana.* These two species are representative of different sections as *Tamarix* and *Polyadenia*, respectively. In the second cluster, there are two subsets. First subset composed of *T. androssowii* and *T. karakalensis*.

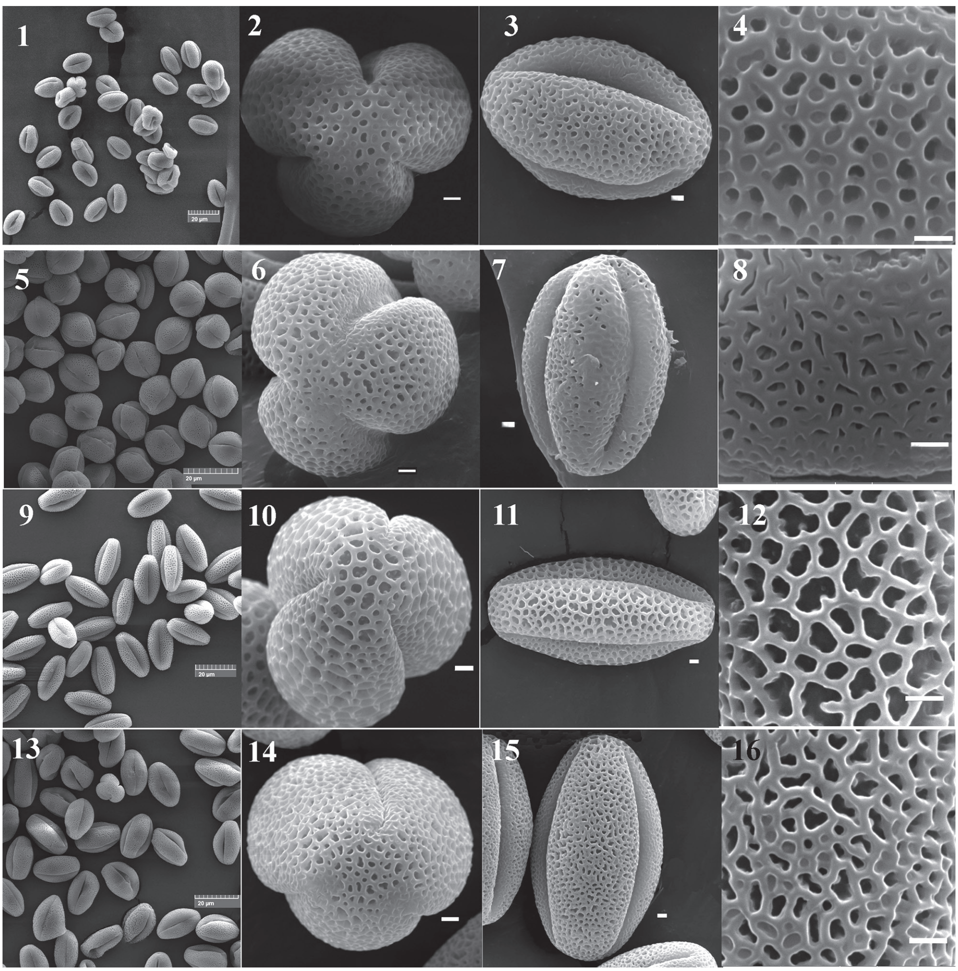

Fig. 1. Scanning micrograph of pollen grains, leaf to right; columns show the general shape, polar view, equatorial view, and exine sculpture, respectively. 1–4 = *Tamarix arceuthoides* from 25 km Garmsar population; 5–8 = *T. ramosissima* from Cheshme Ali Basin population; 9–12 = *T. meyeri* from Sorkhe population; 13–16 = *T. aucheriana*, from Begh village population (white line equals 1 μm)

The second subset composed of *T. meyeri*, *T. androssowii* var. *T. arceuthoides* and *T. szowitsiana*.

DISCUSSION

Recent molecular and morphological study of Sheidai *et al.* (2019) on the same *Tamarix* species revealed that *T. arceuthoides*, *T. ramosissima*, *T. karakalensis* (members of section *Tamarix*) are closely related morphologically. *T. aucheri-*

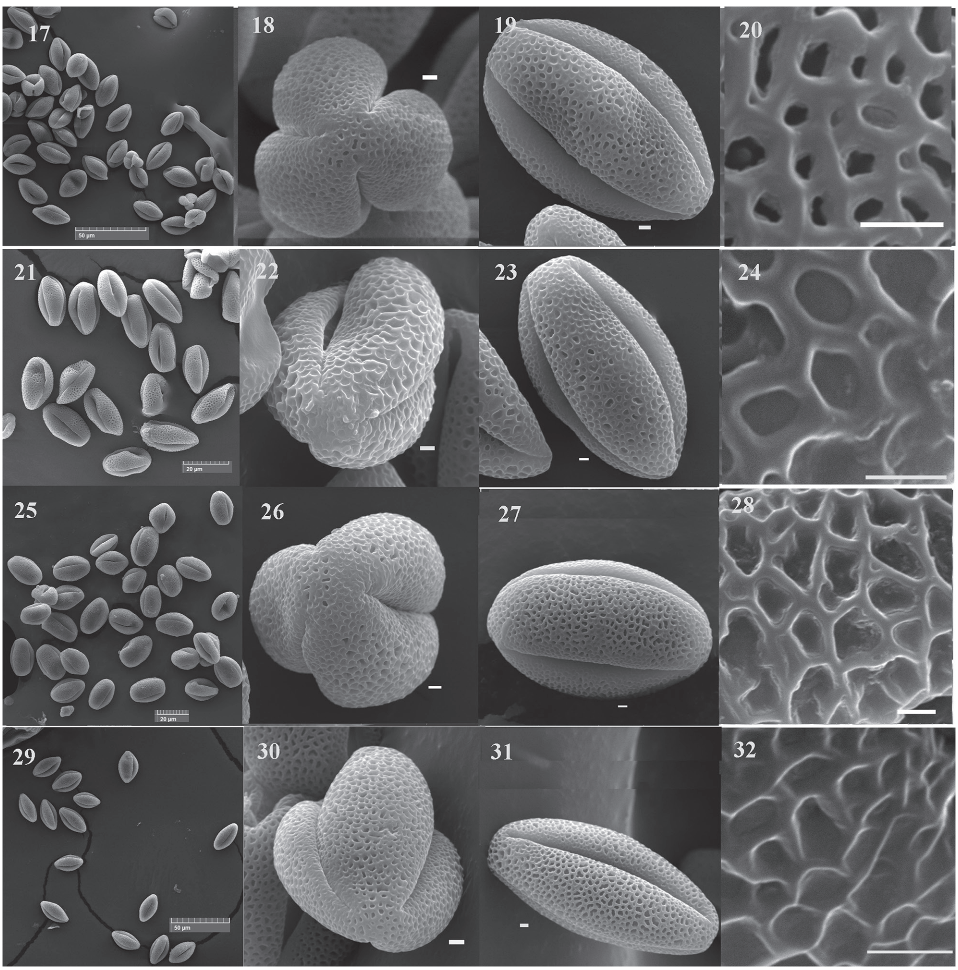

Fig. 2. Scanning micrograph of pollen grains. leaf to right; columns show the general shape, polar view, equatorial view, and exine sculpture, respectively. 17–20 = *Tamarix szowitsiana* from 20 km to Garmsar population; 21–24 = *T. karakalensis* from Haddadeh population; 25–28 = *T. androssowii* from Begh Village population; 29–32 = *T. androssowii* var. *transcaucasica* from 47 km to Shahroud population (white line equals 1 µm)

ana is distinct from its morphological features. The tree base on ITS sequences for these species illustrated somehow different topology but still, *T. androssowii* and *T. szowitsiana* were grouped near each other and *T. aucheraiana* show a separate position.

Identification and determination of the boundaries of species are of great importance in many biological sciences such as biogeography, ecology, population genetics, systematic phylogenetics, conservation biology and biodiversity (Duminil and Di Michele 2009). This is very difficult in the species with vast geographic distribution in different ecological conditions as these are capable of environmental adaptations by their phenotypic plasticity. The latter capacity caused morphological variation in plants and as a result, there are many identification and taxonomic problems. Identification of species and their boundary determination are observed in complex species groups with a high degree of hybrid development (Sudbrock 1993).

The existence of genetic flow between similar and closely related species produces plants that exhibit a wide variety of morphological traits in parental species. As a result, the identification and taxonomy of these plants are difficult and may lead to errors. *Tamarix* species are encountered with such prob-

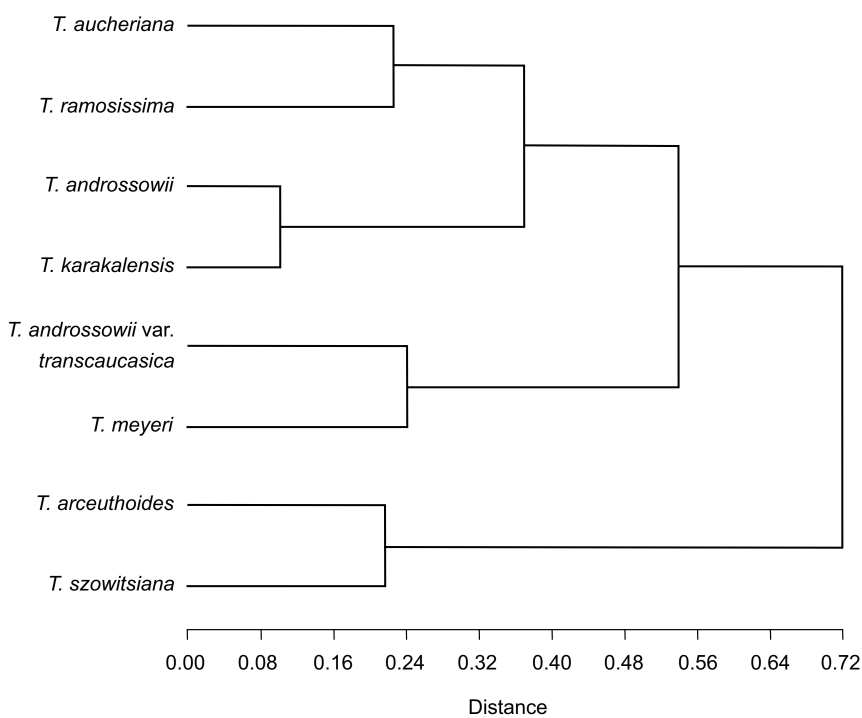

Fig. 3. WARD diagram of pollen grains feature in studied taxa

lems and their identification is very difficult (De Loach *et al.* 1997). Some *Tamarix* species show a high level of variability, in response to ecological niches and phenotypic plasticity (Van den Berg and Groendijk Wilders 1999), which make *Tamarix* species separation much more difficult.

Tamarix is known as a stenopalynous genus. The general morphology of pollen grains is similar, all are tricolpate and reticulate. These results show congruence with those of Elkordy and Faried (2017) who had studied *Tamarix* species of Egypt. There is no difference in general shape, condition of the aperture the general form of sculpture. Qualitative features showed variations with diagnostic value.

The presence of individuals with intermediate features and a high rate of morphological variation showed the important role of hybridisation in observed variation. Hybrid individuals go to a repeated crossing with each other and parental species so makes hybrid swarm, which is only distinguished by careful molecular methods.

Based on the results of the present study, the pattern of species grouping based on the pollen grains features, are not in agreement with previous morphological and molecular genetics. Qaiser and Perveen (2004) pointed to this disagreement, too. For example, *T. arceuthoides* and *T. karakalensis* showed gene flow in the previous study of Shagholi (2016) but pollen grains showed differences. *T. arceuthoides*, *T. szowitsiana*, and *T. aucheriana* are grouped near each other despite their morphological differences. Arianmanesh *et al.* (2015, 2016) studied 15 *Tamarix* species, through phylogenetic analysis based on internal transcribed spacer (ITS). Our results are in disagreement with those of Arianmanesh *et al*. (2016).

The present study is in agreement with Baum *et al.* (1970). They studied pollen grains of some *Tamarix* species and found that despite high morphological similarities between *T. kermanensis* and *T. aphylla* their pollen grains are different. Micro-morphological features of pollen grains were not in agreement with morphological findings. The results of the present study showed a grouping pattern, which is not obeyed the traditional sectional classification. *Tamarix* is still a complex genus with many taxonomic problems.

CONCLUSIONS

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 differentiating morphological features. The present study showed that, in the identification of *Tamarix* species in Iran, the pollen features are less important than morphological characters and should be used as an additional one with molecular data support. A combination of both morphological and molecular results provides a more reliable and consistent method of identifying *Tamarix* species.

Further studies using various approaches with a complete set of species native to Iran, is recommended.

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