

ARTICLE

Micromorphological studies on leaf, fruit and pollen of four species from *Typhaceae* (*Typha laxmannii*, *T. azerbaijanensis*, *T. minima* and *T. lugdunensis*) from Iran, and their thematic significance

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ABSTRACT Previous research has made it clear that the intrinsic taxonomy encountered difficulties in identifying species in the genus *Typha*. Therefore, in the present study we have tried to investigate the micromorphological characteristics of pollens, achenes and leaves as well as their systematic utility. Four *Typha* species identified from the Iranian flora *i.e.* *Typha minima* Funk in Hoppe, *Typha lugdunensis* Chab., *Typha azerbaijanensis* Hamdi & Assadi and *Typha laxmannii* Lepechin were taken into consideration. Three of them are easily distinguishable with their closest relative *i.e.* *T. laxmannii* Lepechin. Scanning electron microscopy was used to examine pollens, achenes and leaves of the four taxa of the proposed *Typha* from Iran. A part of these micromorphological studies attempted to investigate achenes, pollens and leaf characteristics of these species under SEM. Interestingly, *Typha martini* Jordan is also considered as a synonym of *T. lugdunensis*. The study does not only discuss relationships between close species but it also clarifies their geographical patterns. Finally, a diagnostic key is provided for the distribution of the four *Typha* species in Iran. The results show that the ornamentation characters of pollens, achenes and leaf cells prove to be very helpful. Ornamentation of pollens and achenes could be used to distinguish between the four morphological types. Micromorphological studies on achenes, pollens and leaves of *Typha* were found useful with respect to taxa differentiation, hence it provides a key to make distinctions between species or groups of species.

Acta Biol Szeged 54(2):117-125 (2010)

KEY WORDS

achene
Iran
leaf
pollen
SEM
Typha

Typha, a monotypic genus, is distributed widely throughout the world, the Mediterranean region is considered to be the most diversified center. *Typha* is a genus with approximately 24 species, 50% of which are present in Iran. Twelve *Typha* species were recorded by Hamdi and Assadi (2003), after the floristic contributions of others (Kronfeld 1888; Komarov 1934; Townsend and Guest 1968; Riedl 1970; Hegi 1981; Davis 1984; Nassir 1987; Fedorov 2001; Takhtajan 2001; Mavrodiev 2002; and Changkyun et al. 2003). The genus *Typha* is divided into *Typha* and *Bracteolata* sections. In the section *Typha*, there are no bracteolate female flowers like in *T. laxmannii* and *T. azerbaijanensis*. The section *Bracteolata* consists of bracteolate female flowers, such as in *T. minima* and *T. lugdunensis*. Some previous research has shown difficulties in identifying species belonging to the genus *Typhaceae*. Among these, we can name the palinology carried out by Moore et al. (1991); Moar (1993), and the biomolecular studies by Kuehn-Marcinko and White (1999). However, the

intrinsic taxonomic difficulties of the genus *Typhaceae* are increased further because important differential characters are observed only with difficulty and not at all on dried specimens. With regard to pollens and achenes, several studies have been carried out, each analyzing different aspect with scanning electron microscopy (SEM; e.g. Panich-pat et al. 2005). Interestingly, SEM allows observing structures that could be observed with difficult by other means. Some authors have highlighted the importance of this technique while studying achene coat, leaf surface and pollen ornamentation, especially for those families whose identification is complicated, particularly *Typhaceae* for hybridizing between species (Smith 1967; Kuehn-Marcinko et al. 1999), where morphological features of pollens, achenes and leaves were used widely in order to distinguish between different taxa or to find similarities between them. As mentioned earlier, the aim of the present study is to illustrate the role of pollens, achenes and leaf features to identify four species of *Typhaceae* in Iran as well as to help relate those characters to a systematic group. The present paper discusses four species *i.e.* *T. minima*, *T. lugdunensis*, *T. azerbaijanensis* and *T. laxmannii*. Here, *T. laxmannii* and

Accepted Aug 2, 2010

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Table 1. Macromorphological characteristic features of four species representing Iranian *Typha*.

	<i>T. azerbaijanensis</i>	<i>T. laxmannii</i>	<i>T. lugdunensis</i>	<i>T. minima</i>
<i>Vouchers</i>	Hamdi 81266 TARI	Mozaffarian 47032 TARI	Mozaffarian & al. 58975 TARI	Hamdi 80846 TARI
Rhizome (mm)	3	2-4	3-4	2-3
Stem (cm)	75-90	80-140	50-130	45-65
Largest of leaf long (cm)	75	80	110	25
Largest of leaf wide	8-9	6-9	2-4	2-3
Male inflorescence (cm)	9-15	10-32	4.5-7	1.5-5
Female inflorescence (cm)	7-10	4-11	2-4.5	2.5-4.5
Female inflorescence color	reddish- brown	brown	brown-light brown	brown
Rate of female inflorescence to male inflorescence	0.7	0.7	0.7	1
Gap of female inflorescence to male inflorescence (mm)	15-50	5-30	5-25	2-8
Scale of female flower (mm)	–	–	2.5-6	5-6
Number of hairs in female flower	35-40	25-35	10-15	12-25
Gynophores (mm)	3-7	4-5	1.5-4	3.5-4.5
Female flower (mm)	9-9.5	7-10	5-6	5.5-6
Stamen stalk (mm)	0.5-0.7	0.5-1	0.4-0.8	0.5
Stamen anther (mm)	1.5	1-2	1-1.5	1.2-1.7
Stamen number in male flowers	1-2	1-2	1	1-2

Table 2. Micromorphological features of pollen grains, achene and leaf in four species representing Iranian *Typha*.

	<i>T. azerbaijanensis</i>	<i>T. laxmannii</i>	<i>T. lugdunensis</i>	<i>T. minima</i>
<i>Vouchers</i>	Hamdi 81266 TARI	Mozaffarian 47032 TARI	Mozaffarian & al. 58975 TARI	Hamdi 80846 TARI
Shape of leaf testa cells	tetragonal	tetragonal & pentagonal	tetragonal & pentagonal	Pentagonal
Size of leaf testa cells	15-30×10-14	16-20×10-12	20-25×12-15	30-40×10-15
Shape of achene testa cell	pantagonal	pantagonal	Tetragonal	tetragonal & pentagona
Size of achene testa cells (μm)	100-125×12-15	80-110×12-15	10-12×6-8	35-55×10-15
Lumina (μm)	0.1-0.2	0.5-1.1	0.4-1.0	0.3-0.8
Length of large muri (μm)	1.9-2.1	3.5-4.0	1.0-1.8	0.8-1.0
Distal face				
Width of large muri (μm)	0.3-0.6	0.6-0.9	0.2-0.4	0.3-0.4
Distal face				
E (μm)	26-27	28-30	25-27	20-22
P(μm)	19-21	26-28	14-16	18-20
P/E ratio	0.73-0.77	0.92-0.93	0.56-0.59	0.90-0.91

T. minima are considered to be the most widespread species, extending throughout the temperate Northern Hemisphere (Smith 2000). *T. lugdunensis* is found in the Middle Eastern and Central Asian region (Riedl 1970) and *T. azerbaijanensis*, is restricted to northwestern Iran (Hamdi and Assadi 2003). At the same time, the scanning electron microscopic studies of leaves, achenes and pollens can be considered useful in separating those species. During studying the above-mentioned four species, botanists have been considerably attentive so far as the pollen morphology of *Typha* is concerned as well as the terminology for pollen ornamentation. The present study has followed Moore et al. (1991), Punt et al. (1999, 2007) and Panich-Pat et al. (2005) in order to test their results.

Materials and Methods

Pollen, capsule and leaf micromorphology was studied in four taxa of the *Typha* L. genus from Iran, using a scanning electron microscope. For every taxon, samples were collected from the populations within the study area. Mainly specimens from TARI (acronyms according to Holmgren et al. 1990) were used, although some of the samples acquired from a collection of Iranian fields that, however, proved problematic in finding pollens, achenes and leaves for the analysis. The collectors of samples and their localities are shown in the Appendix. The data represent the measurements done on 30-40 pollens, 10-15 achenes and selection of apex of middle

leaves per taxon, by means of direct visual observation under optical microscopy. For scanning electron microscopy, the pollen samples were examined and photographed using a LEO-440I scanning electron microscope. However, dry pollens, achenes and leaves were mounted directly on stubs using dual-sided adhesive tape and were coated with gold/palladium in a sputter coater. Morphological observations were made using a TESCAN microscope from the Electronic Microscopy Service of Razi Institute of Tehran. To describe the pollen ornamentation, the current study has followed the terminologies used by Moore et al. (1991) and Punt et al. (1999, 2007) and achene ornamentation used by Steam (1993) and acronyms of the herbariums follow Holmgren et al. (1990).

Results

Scanning electron microscopic observations indicated that two basic types of pollens could be found in Iranian *Typha* species. These are related to pollen dispersal in *Typha* which can be as tetragonal tetrads (Fig. 1) or monads (Fig. 2), (according to Punt et al. 1999, 2007). As a matter of fact, while tetrad pollens are found in two Iranian species: *T. lugdunensis* and *T. minima*, whereas monad pollens are found in two others: *T. azerbaijanensis* and *T. laxmannii* as listed in Table 2. The scanning electron microscopic observation could indicate three pollen types of *Typha*. Type 1: reticulate, Type 2: vermiculate, Type 3: perforate. Table 2 summarizes the main features of the investigated pollens. The study shows that the exine sculpturing provided valuable characteristics for separating species, sometimes even for closely related ones, and delimitation of natural groups within the genus. The exine of genus *Typha*, in most cases, is reticulated upon tectum. It seems that the structure of the muri is solid, which is very important in recognizing natural groups within the genus. With regard to the exine sculpture in proximal face, three basic types of pollen grains can be distinguished: perforate, reticulate, and vermiculate (Table 2). The pollen grains are partly medium in size. [Range: From 20-22_18-20 μm in *T. minima* to 28-30_26-28 μm in *T. laxmannii* P/E ratio ranges from 1-1.2. The aperture is simple, distal-polar (ana-sulcate), elongated, and reaches the proximal side of the pollen with rounded or acute ends (Figs. 1 and 2). The exine is perforated or rarely tectate-columellate upon tectum. The muri are solid or compound, simpli-, dupli- or pluricolumellate (according to Punt et al. 1999). Among the studied species, tectal perforations vary from 0.2 to 2.0 μm in diameter. Three main pollen types recognized on the basis of the exine sculpturing at proximal face are described below.

Type 1: Reticulate; this type occurs in *T. laxmannii* (Fig. 2). The largest pollen of all the species is classified under this type and occurs in *T. laxmannii* [26-28_28-30 μm in size].

Type 2: Vermiculate; occurs in two species: *T. minima* and *T. lugdunensis* (Fig. 1). The smallest pollen grains are found in *T. minima* [18-20_20-22 μm in size] and the largest in *T.*

lugdunensis [18-20_25-27 μm in size].

Type 3: Perforate; occurs in species *T. azerbaijanensis* (Fig. 1).

The basic palynomorphological characters of *Typha* are due to a shallow sulcus, a reticulate, microreticulate, vermiculate and perforate exine with solid or compound columellate muri. The taxonomy of the genus *Typha* is difficult due to emergence of several problems. Moreover, there are very controversial views with respect to subgeneric grouping within the genus. In the most recent published works, pollen morphological characters provide valuable characteristics for delimitation of the species in Typhaceae. This point of view has also been confirmed by the present study. Some of these groups are in congruence with the formerly supposed grouping within the genus. Of the micromorphological characteristics, the current paper examines achenes, pollens and leaf characters of species under SEM. Interestingly, here *Typha martini* Jordan is considered as a synonym of *T. lugdunensis*. The study discusses not only the relationship between close /nearest species rather gives their geographical patterns, too. Finally, a diagnostic key is provided to four *Typha* species distribution in Iran.

Table 1 indicates morphological differences between four taxa studied during the present research. Despite their overall close morphology, the four taxa could be distinguished from each other by a number of morphological traits. An identification key to these taxa has been provided. These species differ from *T. laxmannii* with respect to their female inflorescence long, gap between male and female inflorescence, male inflorescence long, female flower long, shape and size of leaf and achene (Figs. 3, 4, Table 1 and 2).

Discussion

Morphological differentiation between four taxa of *Typha*

Tables 1 and 2 present morphological differences among the four taxa studied above. Despite the overall close morphology, these taxa can be distinguished from each other by a number of morphological traits. These species differ from *others* in having long female and male inflorescences, a gap between the male and female inflorescences, female flowers are long, scale of female, number of hairs in female flower, shape and size of leaves, pollens and achenes (Figs. 1, 2, 3 and 4). Detailed pollen morphological characteristics are given for these species. Among the studied species, the newly described *T. lugdunensis*, and *T. minima* possess the tetrad pollen grains (Moar 1993) also, *T. laxmannii* and *T. azerbaijanensis* are monad pollen grain (Hamdi and Assadi 2003). The study shows that the sculpturing of pollen exine provides valuable characteristics for separating the species (Moore et al. 1991; Punt et al. 1999, 2007), sometimes even for closely related ones, and delimitation of natural groups within the genus. Re-

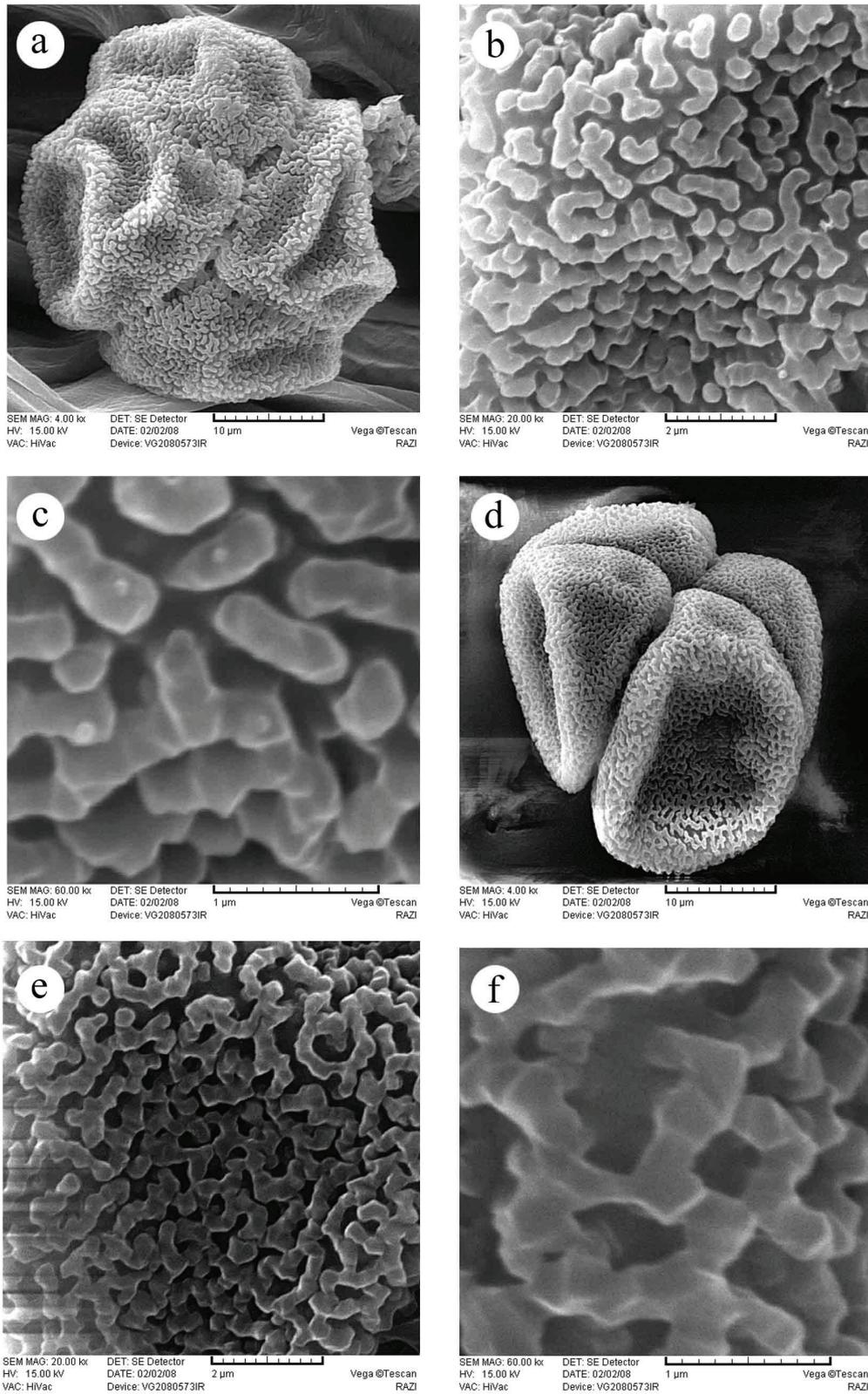


Figure 1. Micrographs of pollen grains in *Typha* (Typhaceae). Fig. a-c, vermiculate tectum at the proximal face in *T. minima* pollen with vermiculate muri. Fig. e-f, vermiculate tectum at proximal face of pollen of *T. lugdunensis* with vermiculate muri. Scale bar = 1 µm; Figs c and f, scale bar = 2 µm; Figs b and e, scale bar = 10 µm; Fig. a and d.

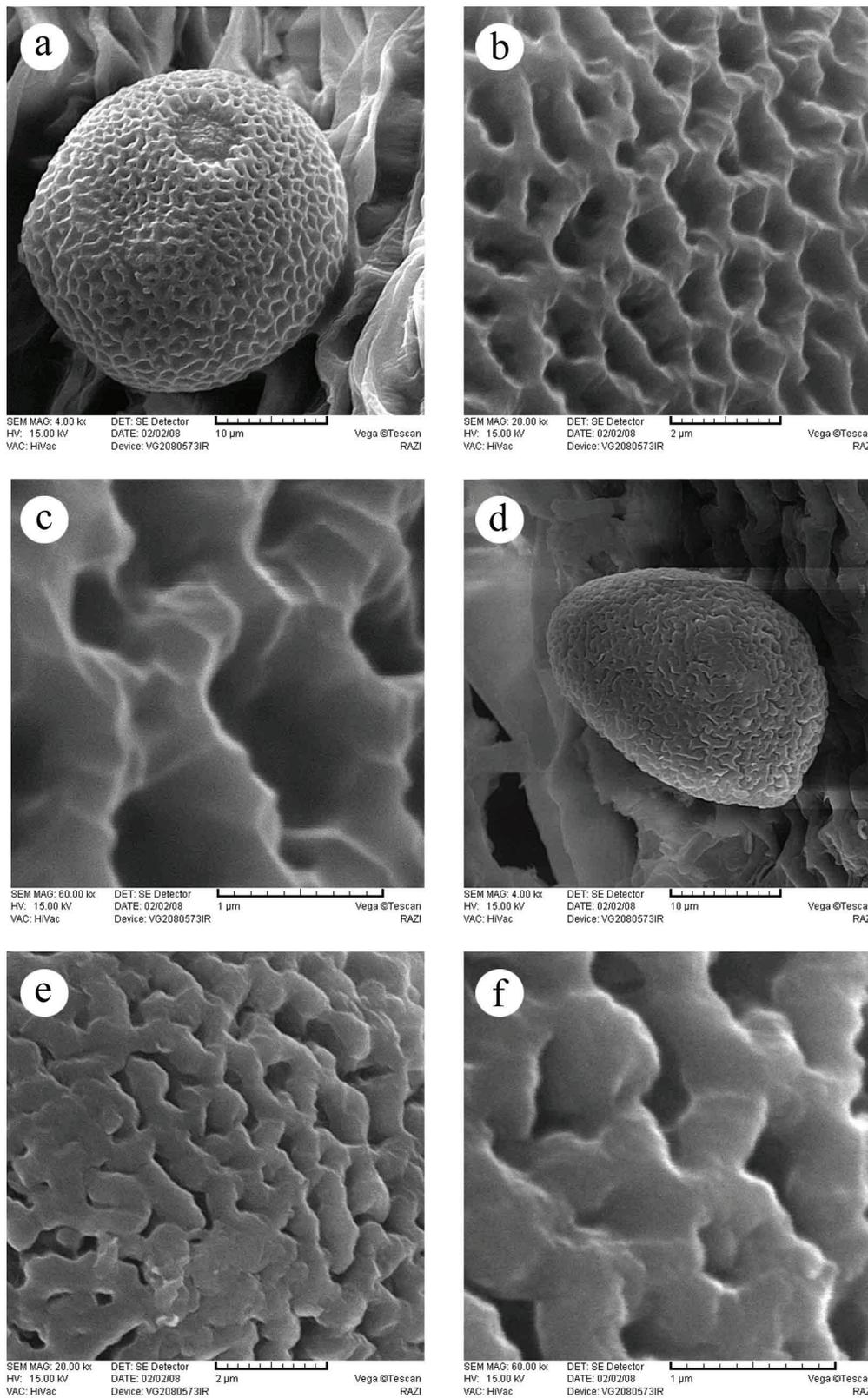


Figure 2. Micrographs of pollen grains in *Typha* (Typhaceae). Fig. a-c, Reticulate tectum at the proximal face in *T. laxmanii* pollen with perforate muri. Fig e-f, perforate tectum at proximal face of pollen of *T. azerbaijanensis* with perforate muri. Scale bar = 1 μ m; Figs c and f, scale bar = 2 μ m; Figs b and e, scale bar = 10 μ m; Fig. a and d.

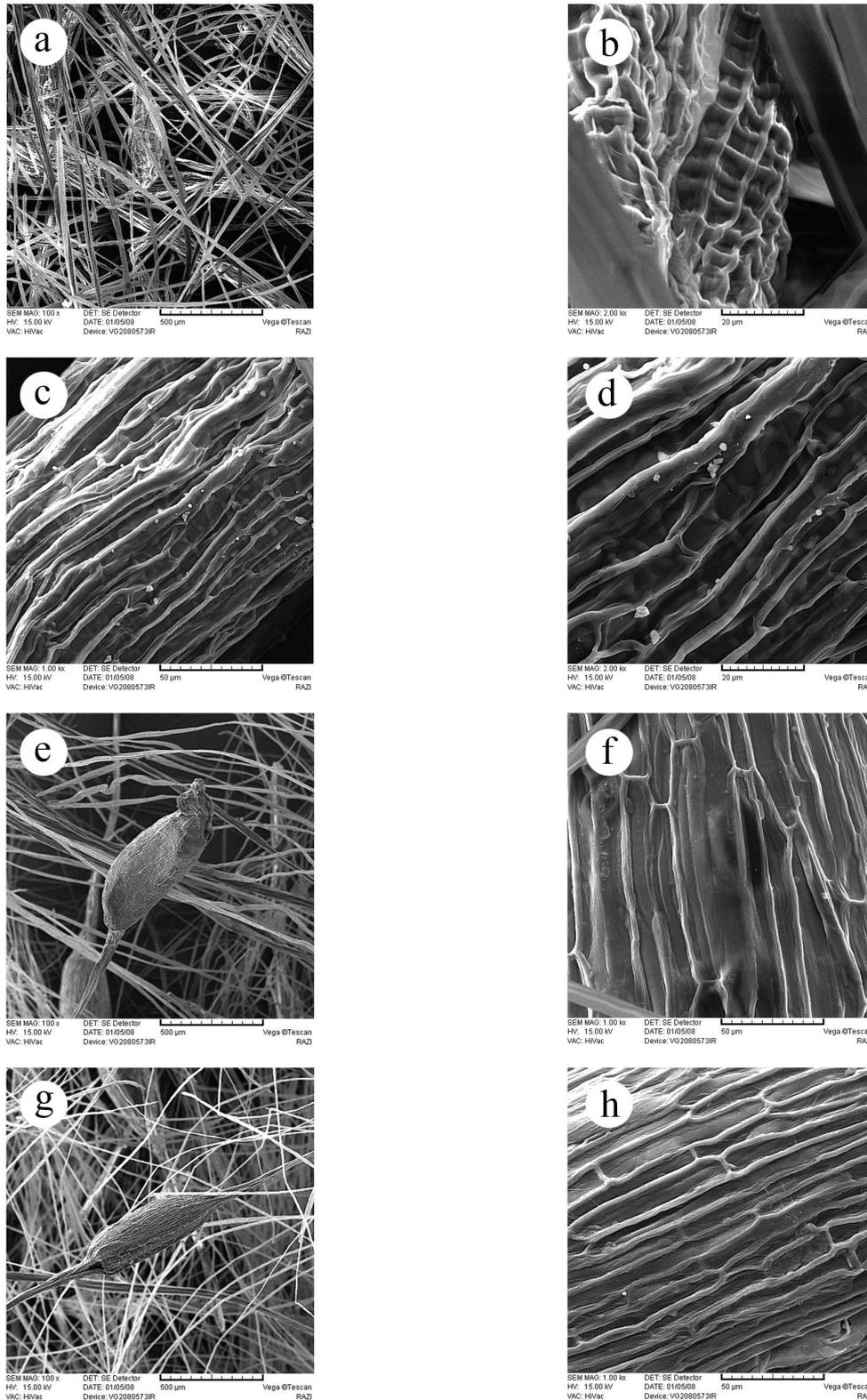


Figure 3. Scanning electron micrographs of *Typha* (Typhaceae) - a-b: *T. lugdunensis* from 58975 Mozaffarian and Abouhamze (TARI), overview of capsule (a) testa cells of capsule (b); -c-d: *T. minima* from 80846 Hamdi (TARI), testa cells of capsule (c, d); e-f: *T. azerbaijanensis* from 81266 Hamdi (TARI), overview of capsule (e) testa cells of capsule (f); -g-h: *T. laxmannii* from 61619 Assadi and Akhani (TARI), overview of capsule (g) testa cells of capsule (h); - Scale bars: a = 500 µm, b = 20 µm, c = 50 µm, d = 20 µm, e = 500 µm, f = 50 µm, g = 500 µm, h = 50 µm.

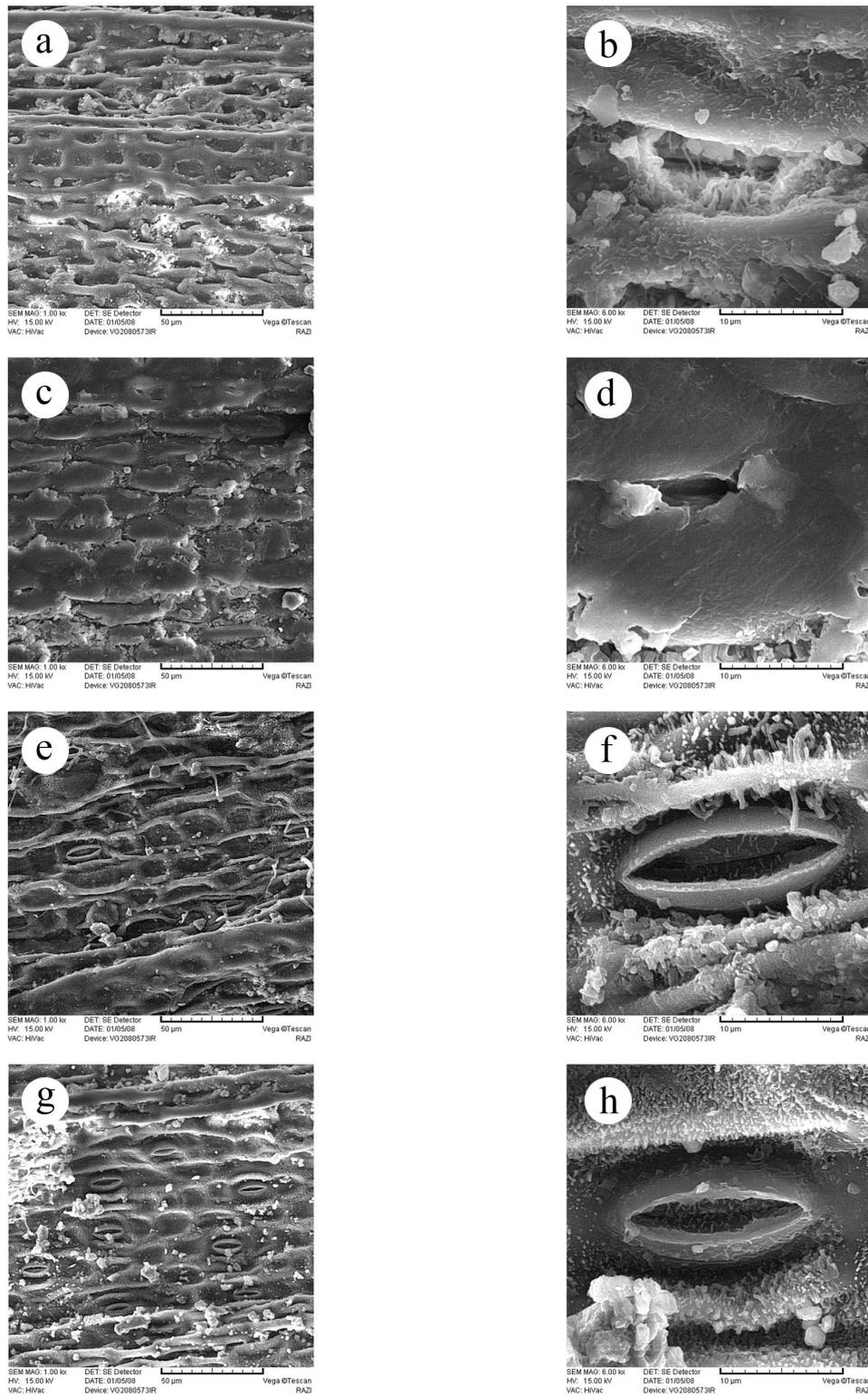


Figure 4. Scanning electron micrographs of *Typha* (Typhaceae) - a-b: *T. lugdunensis* from 58975 Mozaffarian and Abouhamze (TARI), testa cells of leaf (a), stomata ornamentation surface (b); -c-d: *T. minima* from 80846 Hamdi (TARI), testa cells of leaf (c) stomata ornamentation surface (d); e-f: *T. azerbaijanensis* from 81266 Hamdi (TARI), testa cells of leaf (e) stomata ornamentation surface (f); - g-h: *T. laxmannii* from 18559 Wendelboo and Assadi (TARI), testa cells of leaf (g) stomata ornamentation surface (h); - Scale bars: a = 50 µm, b = 10 µm, c = 50 µm, d = 10 µm, e = 50 µm, f = 10 µm, g = 50 µm, h = 50 µm.

garding exine sculpturing in proximal face, three basic types of pollen grains can be distinguished: perforate, vermiculate and reticulate. Perforate type occurs in: *T. azerbaijanensis* (Figs. 2, 3 and 4) *T. azerbaijanensis* with lumina 0.1-0.2 µm, length and width of large muri distal face 1.9-2.1×0.6-0.9 µm, E 26-27 µm, P 19-21 µm, P/E ratio 0.73-0.77. Reticulate type occurs in *T. laxmannii*, with lumina 0.5-1.1 µm length and width of large muri distal face 3.5-4.0×0.6-0.9 µm, E 28-30 µm, P 26-28 µm, P/E ratio 0.92-0.93. (Fig. 2). Vermiculate type occurs in *T. minima* and *T. lugdunensis* (Fig. 1). *T. minima*, with lumina 0.3-0.8 µm length and width of large muri distal face 0.8-1×0.3-0.4 µm, E 20-22 µm, P 18-20 µm, P/E ratio 0.90-0.91 and *T. lugdunensis* with lumina 0.4-1 µm, length and width of large muri distal face 1.0-1.8-0.2-0.4 µm, E 25-27 µm, P 14-16 µm, P/E ratio 0.56-0.59 (Table 2). Table 2 presents micromorphological achene character differences between the four taxa. These differ in size, shape of testa cells and size of testa cells. The acquired results confirm the usefulness of pollen, achene and leaf surface characters for identifying most of the studied species, and highlight their division into three (*i.e.* reticulate, vermiculate and perforate), as has been traditionally recognized (Komarov 1934; Davis 1984; Hamdi and Assadi 2003). The results of this studies show that SEM can be considered useful in making separation between the aforementioned species of this genus.

Acknowledgements

We express our gratitude for the proposed research at by Research Council of Islamic Azad University, Garmsar branch, as well as Dr. Shahrookh Ranjbar Bahadori for his partial support for the project (No. 108). We would also like to thank the Electron Microscopy Unit of Razi Institute in Tehran especially Mr. Rahmani for his valuable contribution. Last but not the least is Dr. Massoumi from National Botanic Garden of Iran.

Appendix

Localities of collections studied

Typha lugdunensis CHAB. IRAN. Azerbaijan province; Aras Lake, between Pol-Dasht, 730 m, IZADPANAHA and TAHERI (TARI 68417). Khorasan province; Kalat, Archangan, 950 m, HAMDI (TARI 80898); Kalat, 25 km to Kalat, near Soltan Abad village, ASSADI and MASSOUMI (TARI 55806). Tehran province; Taleghan, Karaj, Sikaroud, 185 m, AMIN and BAZARGAN (TARI 18580); Firouzkouh, margin of Nemroud, MOZAFFARIAN and ABOUHAMZEH (TARI 58975).

Typha minima FUNK in HOPPE. IRAN. Gorgan province; Kourdkoy, protected area Jahan nema, 1800-2000 m, MASSOUMI (TARI 55059). Azerbaijan province, Parsabad, 5 km to Aslandouz, HAMDI (TARI 80879). Tehran province, Tehran toward Firouzkouh, Harandeh village, margin of river of Nomroud, HAMDI (TARI 80846).

Typha azerbaijanensis HAMDI and ASSADI. IRAN. Azerbaijan province; Khoy, 5 km to Marand, margin of road, 1100 m, HAMDI (TARI 81266).

Typha laxmannii LEPECHIN. IRAN. Gorgan; 15 km west of Gorgan, ILKA COCONEN (TARI 7790); Kourdkoy, 5 km to Bandare-Tourkemn, margin of road, HAMDI (TARI 80854). Mazandaran province; Pole-Sefid; 150 m, AKHANI (TARI 65315). Guilan province; Bandare-Anzali, 25 m, MOZAFFARIAN (TARI 66213); Rasht, 3 km to Bandare-Anzali, margin of road, HAMDI (TARI 80867). Azerbaijan province; Ardebil, 30 km to Naier, Yamchi Sofla village, HAMDI (TARI 80881); Parsabad, toward Aslandouz village, 5 km of Pirevatloo village, HAMDI (TARI 80875). Fars province; Stahbanat, south of Bash moountain, 1700- 2200 m, MOZAFFARIAN (TARI 47032).

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