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Revision of Study of *Typha* Genus: Three New Records Species of the Genus *Typha* (Typhaceae) in Iran and Their Micromorphological Pollen and Capsule Studies

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Abstract: *Typha* genus belonging to Typhaceae family contained 12 species has been reported in Iran. This genus is one of the most systematically problematic identification. The main purpose of this study is to analyze palynomorphological characters among the member of *Typha* genus and evaluating the efficiency of these features in systematics. A total of 12 species, *Typha* species has been identified. Among them, *Typha angustifolia* L., *Typha caspica* Pobed. and *Typha shuttleworthii* W. Koch and Sonder in W. Koch. were recorded as three new species in the flora of Iran. These species were easily distinguished from its closest relative, *T. latifolia* L. In addition micromorphological characters i.e., capsule, pollen and leaf characters were examined under Scanning Electron Microscope (SEM). Also, *T. persica* was considered as a synonym of *T. shuttleworthii*. Relationships between closed species were discussed and geographical patterns were demonstrated. A diagnostic key to four species of *Typha* distribution in Iran was provided. The results of this study show that SEM studies can be considered as a tool to separate between species in the *Typha* genus.

Key words: New record, *Typha*, Typhaceae, micromorphology, macromorphology, *Typha persica*, Iran

INTRODUCTION

Typha is a genus of monocotyledons flowering plants in the monotypic family, Typhaceae. This genus has a largely Northern Hemisphere distribution, but is essentially cosmopolitan, being found in a variety of wetland habitats (Kronfeld, 1888), these plants known in Iran as Louee or Lookh (Mozaffarian, 2006) and in American English as Cattail (Jones *et al.*, 1998). Louee are wetlands plants, typically 1 to 4.5 m tall (*T. minima* and is smaller: 0.3-0.7 m), with spongy, strap-like leaves and starchy, creeping stems (rhizomes). The leaves are alternate and mostly basal to a simple, joint less stem that eventually bears the flowers. The rhizomes spread horizontally beneath the surface of muddy ground to start new upright growth and the spread of cattails is an important part of the process of open water bodies being converted to vegetated marshland and eventually dry land. *Typha* plants grow along lake margins, margin of river and in marshes, often in dense colonies and are sometimes considered a weed in managed wetlands. The larger *Typha* species can be serious weeds in managed aquatic systems worldwide, where they can invade canals, ditches, reservoirs, cultivated fields and farm ponds; they can be a nuisance in recreational lakes and they can

reduce biodiversity and displace species more desirable for certain kinds of wildlife (James and Robert 1982a,b, 1998; Thieret and Luken, 1996). *Typha* plants are monoecious, wind-pollinated and bear unisexual flowers developing in dense, complex spikes (male and female spike). The male (staminate) flowers are reduced to 1 to 4 of stamens, bare stem portion above the female inflorescence. The dense cluster of female flowers forms a cylindrical spike some 7 to as much as 40 cm long and 1 to 3.5 cm broad. Seeds are minute (about 0.2-0.5 mm long) and attached to a thin hair or stalk, which effects wind dispersal. *Typha* are often among the first wetland plants to colonize areas of newly exposed wet mud (Kronfeld, 1888; Jussieu, 2000; Hamdi and Assadi, 2003). Based on earlier studies, 24 *Typha* species have been recorded worldwide so far (Boissier, 1884; Kronfeld, 1888; Post, 1932; Komarov, 1934; Parsa, 1950; Townsend and Guest, 1968; Riedl, 1970; Hegi, 1981; Davis, 1982; Nassir, 1987; Takhtjan, 2001; Fedorov, 2001; Mavrodiev, 2002; Kim *et al.*, 2003; Hamdi and Assadi, 2003), of which 12 species occurs in Iran alone. The most widespread species is *Typha latifolia* and *T. domingensis*, extending across the entire temperate Northern Hemisphere (Jussieu, 2000). *Typha angustifolia* is nearly as widespread (Jussieu, 2000; Hegi, 1981), but does not

extend so far North. *Typha shuttleworthii* are largely restricted to Asia and parts of Southern Europe (Davis, 1982) and *Typha caspica* is restricted to Caucasian and North of Iran (Komarov, 1934). It must be remembered that the species of the genus are identified on the basis of the collected specimens from different locations in Iran; scanning electron microscopy studies of leaf, capsule and pollen can be considered useful in separating those species. As such, this study has used three new species records as well as *T. latifolia* L. Botanists have considerably been attentive so far as the pollen morphology of *Typha* is concerned hence; describing the pollen ornamentation, the present study has followed by Faegri and Iversen (1989), Kosenko (1999), Moore *et al.* (1991), Punt *et al.* (1999, 2007), Willis (1973) and Thanawan *et al.* (2005).

MATERIALS AND METHODS

Pollen grains, leaf and capsules of the aforementioned four species of the genus *Typha* were studied through Scanning Electron Microscope (SEM). Samples were obtained mostly from fresh collected herbarium specimens namely: FUMH, IRAN, TARI, TUH (abbreviations forwarded by Holmgren and Holmgren, 1998) as well as author's own collection from

wild population. Measurements of vegetative and floral parts as well as capsules, pollen and leave were carried out under a stereomicroscope (Olympus SZH). Table 1 shows voucher and pollen specimens deposited in TARI Herbarium, which are listed under (acronyms according to Holmgren and Holmgren, 1998). The SEM, procedure was done following Davies (1999) although with minor modifications. The collected specimens were mounted on 12.5 mm diameter stubs and attached with sticky tabs and then coated in a sputter coater with approximately 25 µm of Gold-Paladium. Thereafter, a TESCAN Scanning Electron Microscope (SEM) examined and photographed the specimens, at an accelerating voltage of 10-15 kv. A cross-section of exine was also examined. We also measured the number of tecta perforations (according to Punt *et al.*, 2007) per 25 µm² as well as the length of larger perforations in proximal face and distal face. To describe the pollen ornamentation, the current study followed the terminology used by Moore *et al.* (1991) and Punt *et al.* (1999, 2007). The study on the family Typhaceae in Iran has partially been supported by a project (No. 108) conducted during 1, June, 2008 and 19, April, 2009 that was awarded to Seyed Mohammad Mahdi Hamdi by Research Council of Islamic Azad University, Garmsar Branch.

Table 1: Comparison of morphological and micromorphological characters of four *Typha* species

	<i>T. angustifolia</i>	<i>T. shuttleworthii</i>	<i>T. caspica</i>	<i>T. latifolia</i>
Rhizome (mm)	15-20	5-10	5	20-30
Stem (m)	1.3-1.8	1.5-2.5	1.5-1.8	1.5-2.8 (3.5)
Largest Leaf long (cm)	75-90	70-75	70-85	75-120
Largest Leaf wide (mm)	5-15	10-17	6-10	7-16
Male inflorescence (cm)	12-32	7-15	13-22	10-20
Female inflorescence (cm)	10-28	8-30	8-15	10-23
Female inflorescence color	Reddish brown	Reddish brown	Dark brown	Dark brown
Rate of female inflorescence to male inflorescence	A little shorter	Longer	Shorter	Equal- a little shorter
Gap of female inflorescence to male inflorescence (mm)	10-50	Without	5-15	5-10
Scale of female flower (mm)	2.5-5	Without	Without	6.5-11
Number of hairs in female flower	25-30	25-40	30	20-40
Shape of leaf testa cells	Tetragonal	Tetra/pentagonal	Polygonal	Polygonal
Size of leaf testa cells (µm)	15-30×7-15	25-40×10-20	12-18×7-10	23-30×10-15
Gynophores (mm)	1	0.5	0.5-1	1.5-3.5
Female flower (mm)	2.5-6	8-11.5	6.5-9	8-13
Sterile female flowers (mm)	1.5-4.5	6.5-9	7.5-8	3-8.5
stamen stalk(mm)	1.5-2.5	1.5-3	1.5-2.5	1.5-2
Stamen anther (mm)	1.5-2.5		2-2.2	2-3
Stamen number in male flowers	1-4	1-3	1-3	2-3
Capsule (mm)	0.4-1	1.2-2	0.5-1	0.8-1.5
Shape of capsule testa cells	Pentagonal	Tetragonal	Pentagonal	Pentagonal
Size of capsule testa cells (µm)	75-100×10-12	160-180×14-16	80-100×8-12	125-140×12-20
Exine type	Perforate	Vermiculate	Perforate	Reticulate-Microreticulate/ Rugulate
Lumina (µm)	0.7-1.9	0.3-0.6	0.1-0.3	0.6-1.2
Length of large muri (µm) distal face	2.0-2.2	2.2-2.7	2.5-3.0	2.0-3.0
Width of large muri (µm) distal face	0.5-0.7	0.3-1.1	0.6-1.0	0.3-1.0
E (µm)	20-22	25-26	25-26	30-31
P (µm)	12-14	16-18	18-19	25-26
P/E ratio	0.60-0.63	0.64-0.69	0.72-0.73	0.83-0.84

RESULTS

The present study discusses three new species records i.e., *Typha angustifolia* L., *Typha caspica* Pobed. and *Typha shuttleworthii* W. Koch and Sonder in W. Koch, which have been reported for the first time for the flora of Iran. The species of the genus are currently identified on the basis of collected field from some location of Iran and because scanning electron microscopy studies of leaf, capsule and pollen can be considered used to separation between species this genus. Therefore, these studies used on the of three new records and *T. latifolia* L.

The most reliable identification characters for distinguishing four *Typha* species i.e., *T. shuttleworthii*, *T. caspica*, *T. latifolia* and *T. angustifolia*:

- Female inflorescence reddish brown
- Female inflorescence dark brown
- Male inflorescence 1/2 to 1/3 female inflorescence (*T. shuttleworthii*)
- Male inflorescence 5/4 female inflorescence (*T. angustifolia*)
- Male inflorescence equal to female inflorescence or a little shorter than that (*T. latifolia*)
- Male inflorescence 5/4 female inflorescence (*T. caspica*)

Typha shuttleworthii W. Koch and Sonder in W. Koch, Fl. Germ. ed. 2: 786 (1844). Syn.: *T. persica* Ghahreman A. and Sanei H., Bull. Soc. Bot. Fr., 126(3): 373 (1979).

Selected specimens

Azerbaijan: Uromieh, 3 km to Sero (near Turkey), Hingervan village, 1000 m, Hamdi 81261 TARI; Uromieh, 70 km to Ziveh village, Hashemabad village, 1100 m, Hamdi 81257 TARI; Uromieh, Road of Tallatapeh, between Uromieh and Yoghoonabad, 1190 m, Izadpanah and Taheri 68332 TARI; Oshnavieh, 50 km to Uromieh, margin of road, 1750 m, Assadi 78892 TARI. This species is easily distinguishable from its closest relative, *T. latifolia* L. which is a new record for flora of Iran. Previously, Davis (1982) identified it from Turkey until authors collected specimens of this species from flowering culture of Northwestern Iran. The authors also studied *Typha persica* Ghahreman and Sanei (1978) and other specimens. The material types are not been seen but the specimens collected from the localities were studied. It is seen that *T. persica* is closely related to *T. shuttleworthii* which has shorter male inflorescence, female flowers scale, flower hairs. Some specimens of the group were also studied in

Western Iran (Boroujerd). Actually, these specimens are *T. shuttleworthii* distributed between Northwestern Iran to Eastern Turkey. Characters of these specimens are similar to of *T. persica* from West of Iran. As such, the characters mentioned for *T. persica* are observable on *T. shuttleworthii* hence; *T. persica* is considered as a synonym for *T. shuttleworthii*.

Typha caspica Pobed., Not. Syst. Leningrad 12: 21 (1949). Guilan; Astara, margin of sea port, Hamdi 80869 TARI. Riedl (1970) identified *T. caspica* Pobed. with Caucasian and distributed it to Republic of Azerbaijan. This is similar to *T. latifolia* L. but differs in inflorescence, scale of female flowers, gap between female and male inflorescence and size of capsule testa cells. The type materials have not been seen but the specimens collected from near the type localities were studied.

T. angustifolia L., sp. Pl. 971 (1753)

Mazandaran: Noushahr, 20 m, Sabeti 1715 TARI; Chalous, Chalous to Tonekabon, 35 km, margin of road, Hamdi 80850 TARI; Noushahr, Sabeti 4466 TARI. Azerbaijan: Parsabad, 3 km to Germe, Hamdi 80874 TARI; Parsabad, 5 km towards Aslandouz, Hamdi 80876; Khoy, to Marand, 10 km, margin of road, Hamdi 81265. Kourdestan: Paveh, 48 km to Kermanshah, Ronehmark and Mozaffarian 27452 TARI. Kermanshah: Ghasreshirin, Hamdi 81255 TARI; Sarepolezahab, Rigab village, Hamdi 81255 TARI; Kermanshah, 15 km to Islamabad, margin of road near Mahidasht, Hamdi 81256 TARI. Fars: Sivand, Sivand River, Zehzad and Taheri 66915 TARI. Khorasan: Mashad, 110 km to Sarakhs, Bazangan village, Hamdi 80890 TARI; Kalat, 65 km to Mashad, margin of road, Hamdi 80891 TARI; Kalat, 35 km to Mashad, margin of road, Hamdi 80892 TARI; Nishabour, 30 km to Ghouchan, Bar village, Hamdi 80901 TARI, Bojnourd, 15 km to Shirvan, Babaaman village, Hamdi 80888 TARI. Tehran, margin Hemat Exempt. Way, Hamdi 80866 TARI; Tehran, Ivankey, Akhani 65340 TARI.

DISCUSSION

Morphological differentiations of four taxa of *Typha*:

Table 1 shows morphological differences among the four taxa studied during the present research. Despite their overall close morphology, the four taxa can be distinguished from each other by a number of morphological traits. An identification key to the four taxa has been provided.

These species differ from *T. latifolia* with respect to their longer female inflorescence, gap between male and female inflorescence, shorter male inflorescence, female flower long, shape and size of leaf and capsule (Fig. 1-4).

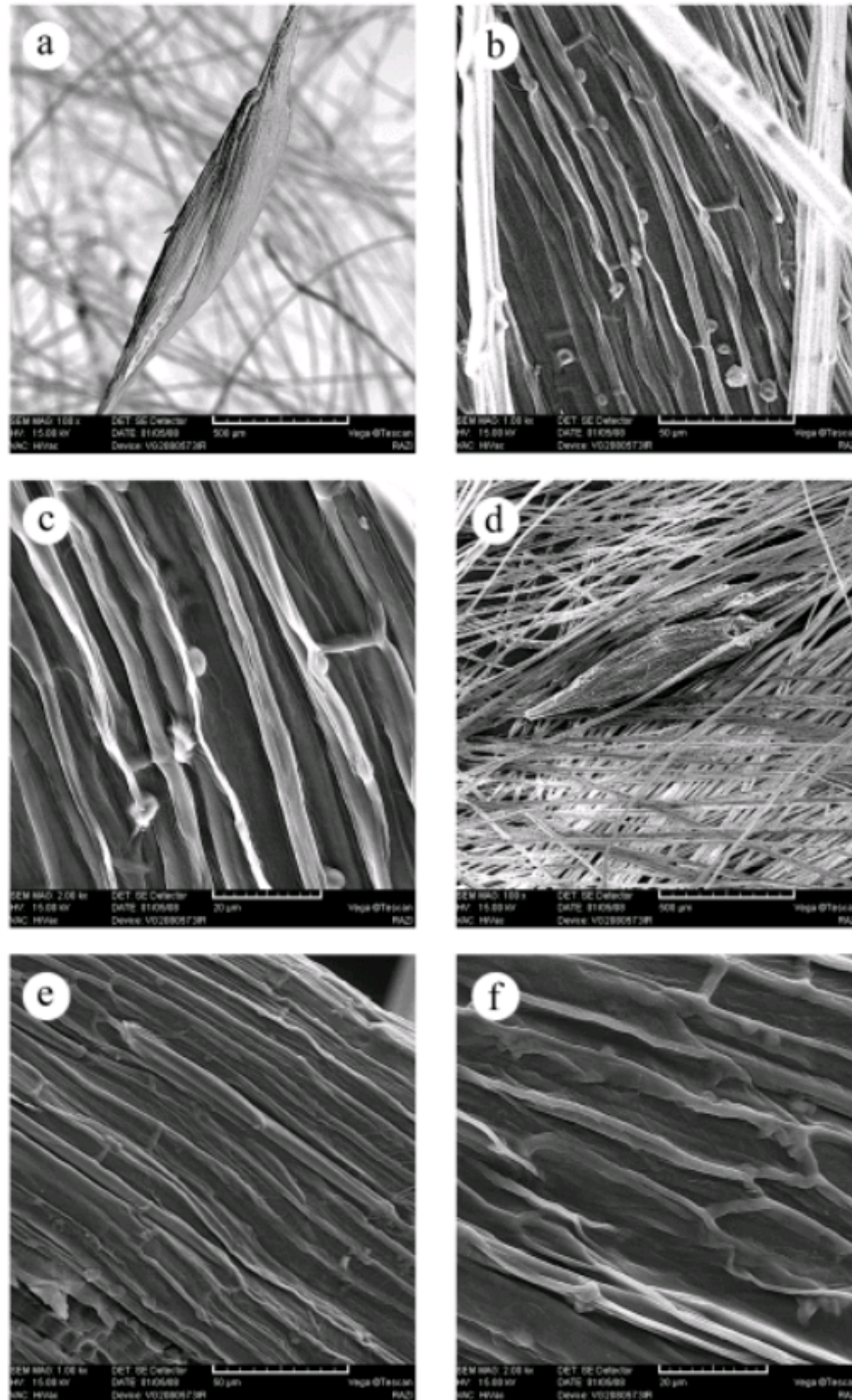


Fig. 1: Scanning electron micrographs of *Typhs* (Typhaceae). (a-c): *T. shuttleworthii* from TARI 81261 (Azerbaijan, Uroumieh, 3 km to Sero, near of Turkey border, 1000 m, 15.8.2001, Hamdi), (a) overview of capsule, (b, c) testa cells of capsule, (d, f): *T. caspica* from TARI 80869 (Guilan, Astara, seaport, 7.27.2001, Hamdi), (d) overview of capsule, (e, f) testa cells of capsule. Scale bars: a = 500 μ m, b = 50 μ m, c = 20 μ m, d = 500 μ m, e = 50 μ m and f = 20 μ m

For these species, detailed pollen morphological characteristics are provided. Among them, the newly

described *T. latifolia* together with three recorded species i.e., *T. shuttleworthii*, *T. caspica* and *T. angustifolia*

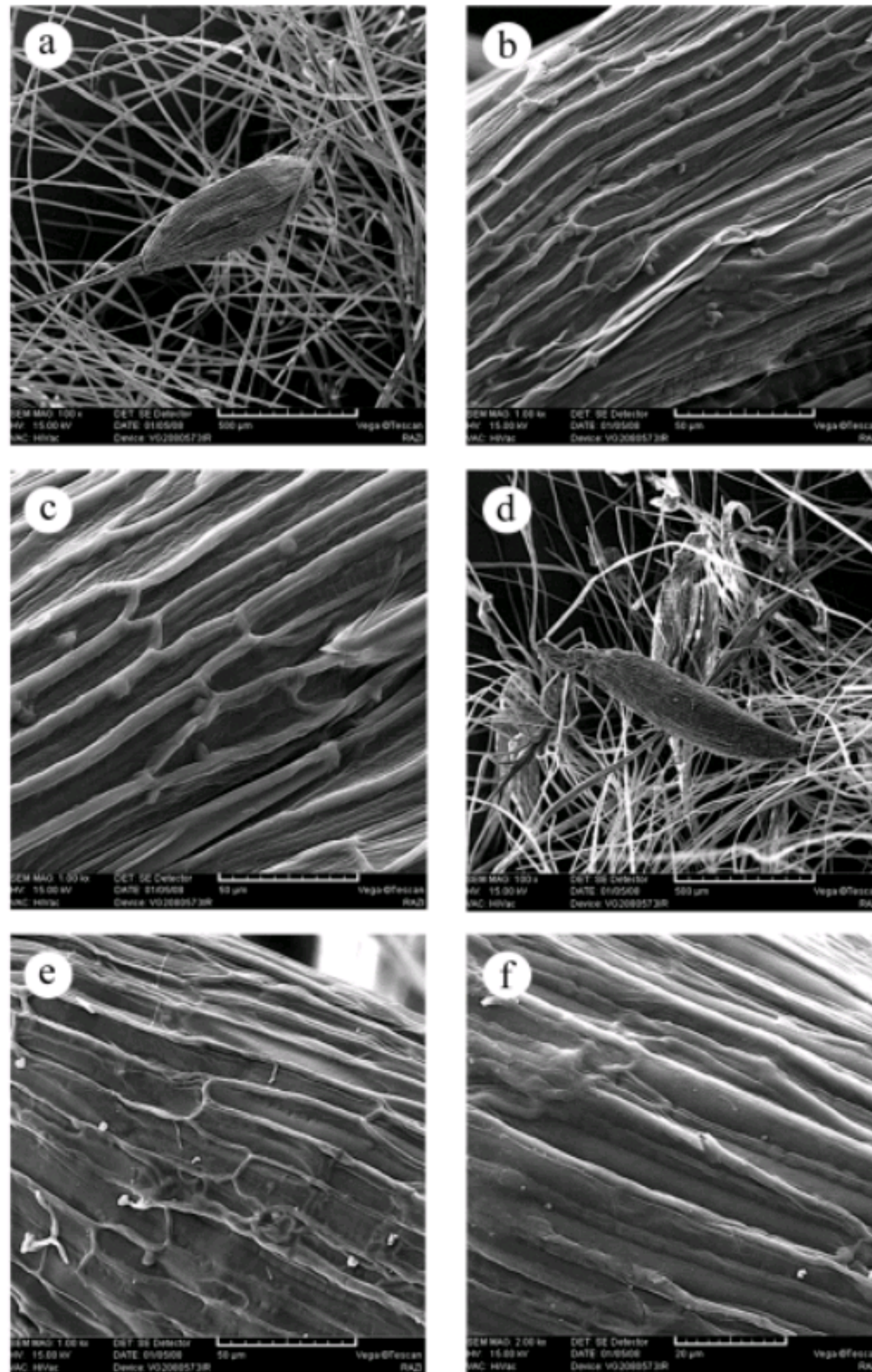


Fig. 2: Scanning electron micrographs of *Typha* (Typhaceae). (a-c): *T. latifolia* from TARI 80858 (Mazandaran, Baboulsar, 0 m, 5.6.2000, Hamdi), (a) overview of capsule, (b, c) testa cells of capsule, (d, f): *T. angustifolia* from TARI 80890 (Khorasan, Mashhad, 110 km to Sarakhs, Bazangan village, 6.15.2000, Hamdi), (d) overview of capsule, (e, f) testa cells of capsule. Scale bars: a = 500 μ m, b = 50 μ m, c = 20 μ m, d = 500 μ m, e = 50 μ m and f = 20 μ m

possess the tetrad pollen grains (Moar, 1993). Present studies show that the sculpturing of pollen exine provides

valuable characters for separating the species (Punt *et al.*, 1999, 2007; Moore *et al.*, 1991), sometimes even for

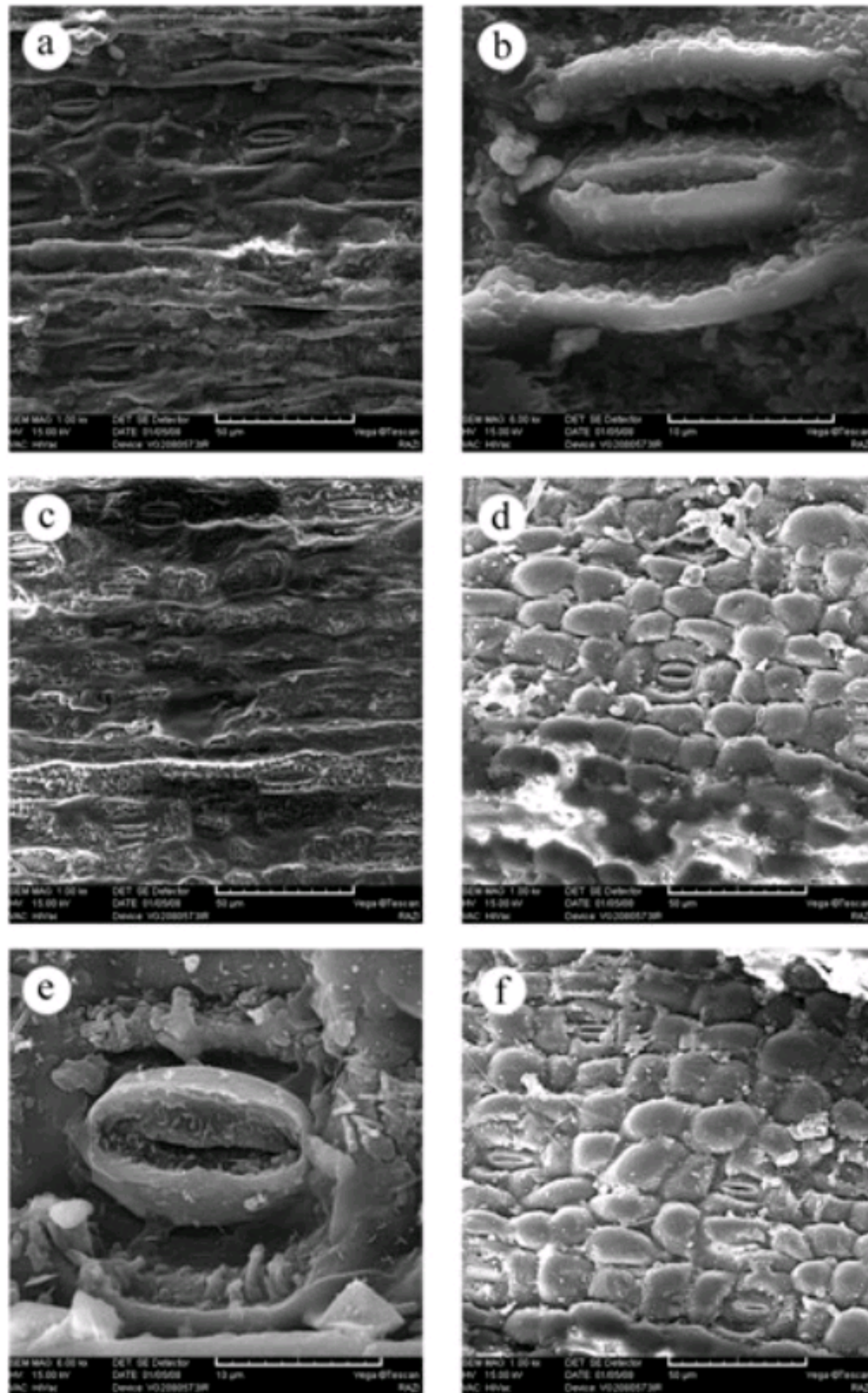


Fig. 3: Scanning electron micrographs of *Typha* (Typhaceae). (a-c): *T. shuttleworthii* from TARI 68332 (Azerbaijan, Uroumieh, Talataphe road, between Uroumieh and Youghenabad. 1190 0 m, 6.16.1998, Izadpanah and Tahreri), (a, c) testa cells of leaf, (b) stoma ornamentation surface; (d, f): *T. latifolia* from TARI 18331 (Guilan, Bandaranzali, Siyahdarvishan march, 7.11.1985, Wendelbo and Assadi), (d, f) testa cells of leaf, (e) stoma ornamentation surface. Scale bars: a = 50 μm, b = 10 μm, c = 50 μm, d = 50 μm, e = 10 μm and f = 50 μm

closely related ones and delimitation of natural groups within the genus. With regard to sculpturing of exine in

proximal face, three basic types of pollen grains can be distinguished: Perforate, Vermiculate and Reticulate-

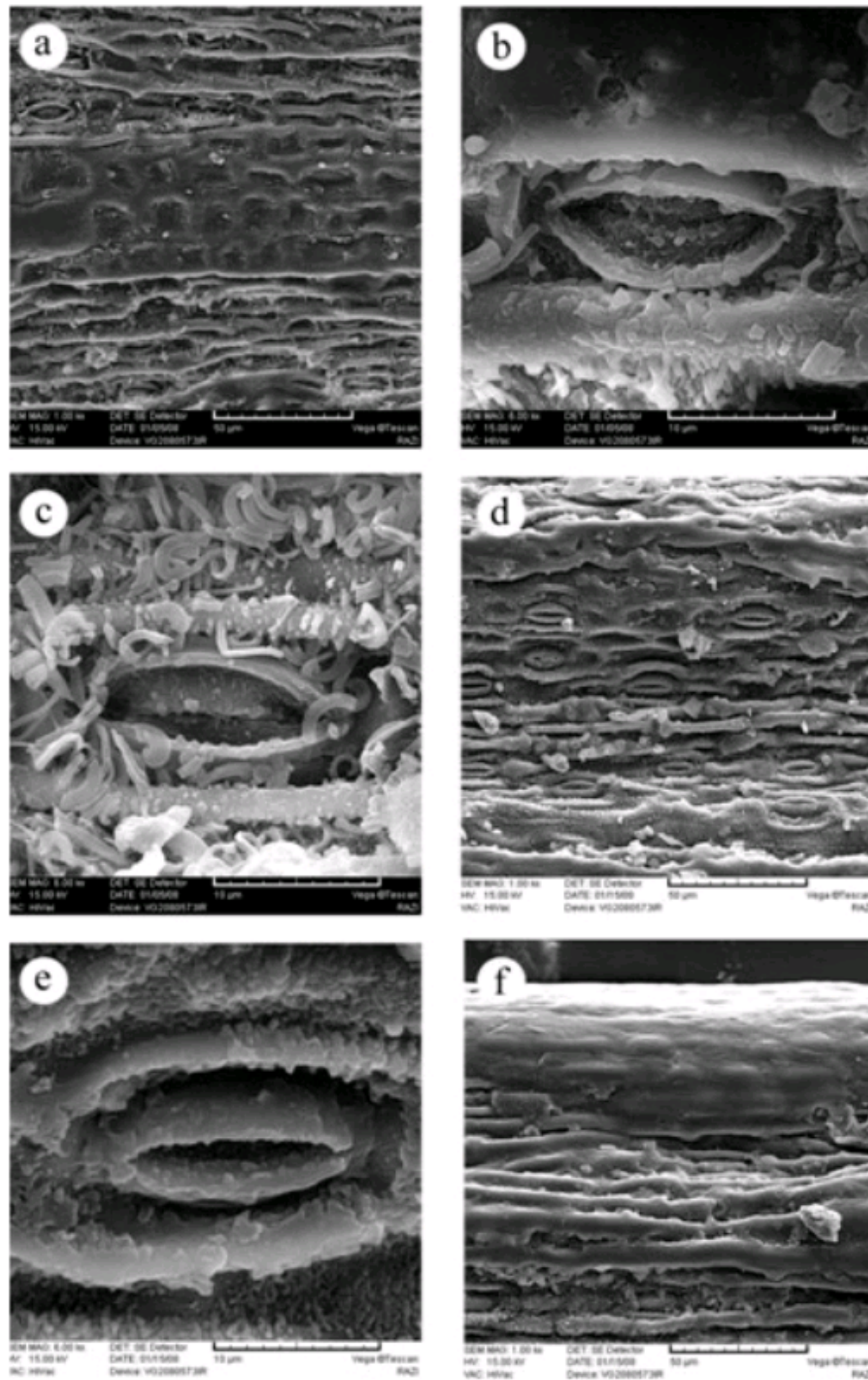


Fig. 4: Scanning electron micrographs of *Typha* (Typhaceae). (a-c): *T. angustifolia* from TARI 80890 (Khorasan, Mashhad, 110 km to Sarakhs, Bazangan village, 6.15.2000, Hamdi), (a) testa cells of leaf, (b) stoma ornamentation surface; (b, c): *T. caspica* from TARI 80890 (Guilan, Astrara, seaport, 7.8.1985, Hamdi), (d, f) testa cells of leaf stoma, (e) ornamentation surface. Scale bars: a = 50 μm , b = 10 μm , c = 10 μm , d = 50 μm , e = 10 μm and f = 50 μm

Microreticulate/Rugulate type. Porforate type is heterogeneous and more frequent between species and

occurs in *T. angustifolia* and *T. caspica* (Fig. 5, 6). *T. angustifolia* with Lumina 0.7-1.9 μm , length and width

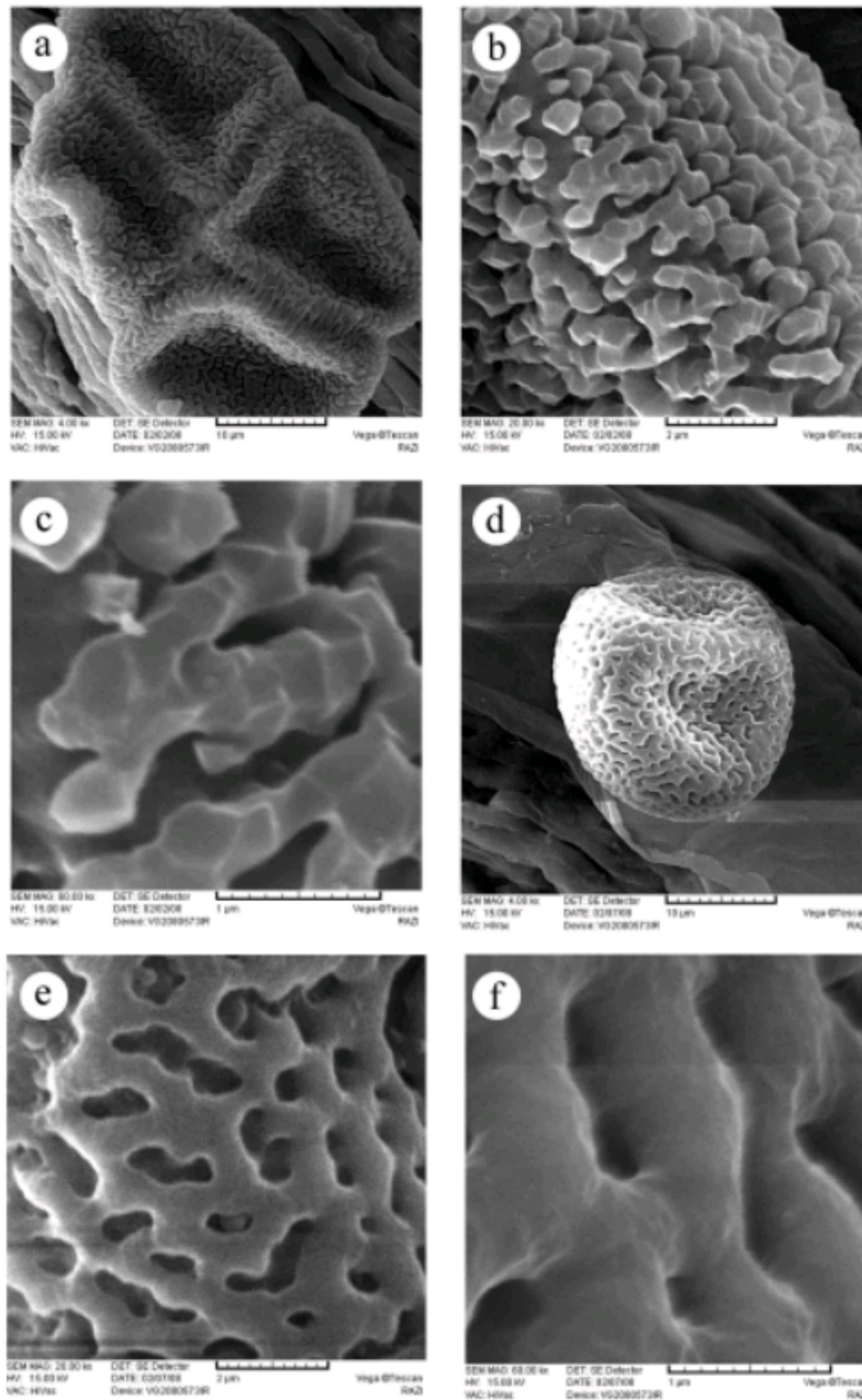


Fig. 5: Micrographs of pollen grains in *Typha* (Typhaceae). (a-c): Vermiculate tectum at the peroximal face in *T. shuttleworthii* pollen with vermiculate muri, (e, f) Perforate tectum at peroximal face of pollen of *T. angustifolia* with perforate muri. (c, f) Scale bar = 1 μ m, (b, e) Scale bar = 2 μ m, (a, d) Scale bar = 10 μ m

of large muri distal face 2.0-2.2 \times 0.5-0.7 μ m, E 20-22 μ m, P 12-14 μ m, P/E ratio 0.60-0.63. *T. caspica* with Lumina 0.1-0.3 μ m, length and width of large muri distal face 2.5-3.0 \times 0.6-1.0 μ m, E 25-26 μ m, P 18-19 μ m, P/E ratio 0.72-0.73. Reticulate-Microreticulate/Rugulate type

occurs in *T. latifolia*, with Lumina 0.6-1.2 μ m, length and width of large muri distal face 2.0-3.0 \times 0.1-0.3 μ m, E 30-31 μ m, P 25-26 μ m, P/E ratio 0.83-0.84 (Fig. 5). Vermiculate type occurs in *T. shuttleworthii* (Fig. 6), with Lumina 0.3-0.6 μ m, length and width of

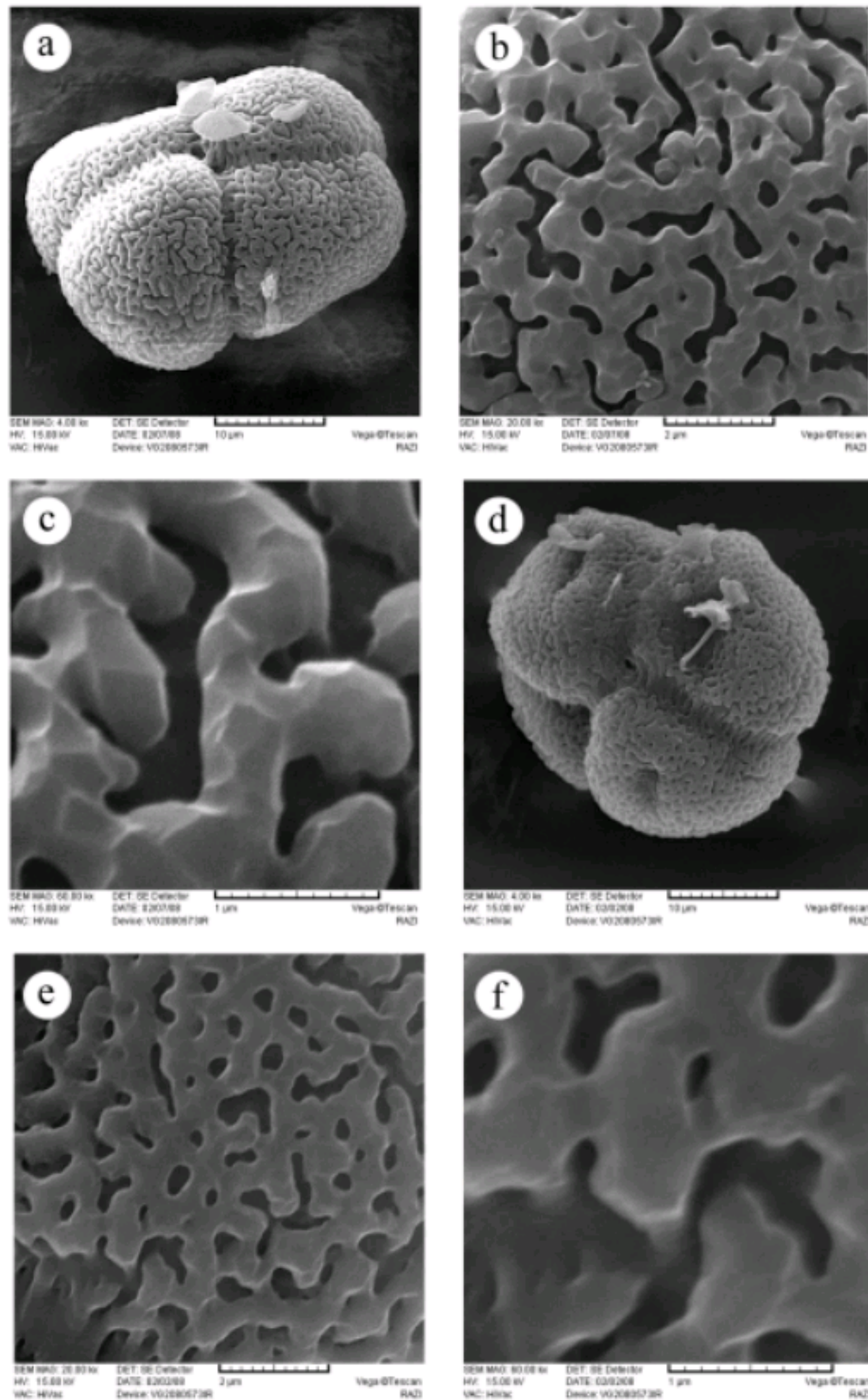


Fig. 6: Micrographs of pollen grains in *Typha* (Typhaceae). (a, c): Perforate tectum at the proximal face in *T. caspica* pollen with perforate muri. (e, f): Perforate tectum at proximal face of pollen of *T. latifolia* with perforate muri. (c, f) Scale bar = 1 µm, (b, e) Scale bar = 2 µm, (a, d) Scale bar = 10 µm

large muri distal face 2.2-2.7×0.3-1.1 µm, E 25-26 µm, P 16-18 µm, P/E ratio 0.64-0.69 (Table 1). Table 1 shows differences of micromorphological capsule characters among the four taxa. These differ in capsule size as well

as shape and size of capsule testa cells. The results of this studies show that SEM can be considered in separating species of the aforementioned newly recorded genus.

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