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Pollen Micromorphological Studies of the Genus *Chenopodium* (Chenopodiaceae) in Iran

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Abstract: The main purpose of this study is to analyze palynomorphological characters among the member of this genus and evaluating the efficiency of these features in systematics of the genus. Pollen of 14 species belonging to genus *Chenopodium* was studied by Scanning Electron Microscope (SEM). All studied species have pollens with, spheroidal shape, radial symmetrical, isopolar and peripolporate. The exine surface ornamentation is scabrate. All studied species haven indicating that there are two pore types on pollen surface: foveat and perforate. The results of this studies show that pollen micromorphology can be considered used to superstition between species this genus.

Key words: *Chenopodium*, Chenopodiaceae, pollen, micromorphological

INTRODUCTION

According to Aellen (1960) there are over 120 species in the genus *chenopodium* in world wide that 15 species occur in Iran, 34 species Russia, 11 species Turkey, 18 species Afghanistan, 15 Pakistan and 8 species Iraq (Uotila, 1974). This the genus belong to Chenopodiaceae that 41 genera are distributed in Iran. Parsa (1949) recognized ten species genus *Chenopodium* in Iran and Uotila (1974) addad five more. Assadi (2001) recognized these fifteen species and 2 subspecies in his studies of the genus in Iran, that of these: *C. novopokrovskyanum* (Allen) Uotila, *C. rubrum* L., *C. urbicum* L., *C. murale* L., *C. glaucum* L., *C. opulifolium* Schrader ex Koch and Ziz., *C. sosnowskyi*, Kappeler, *C. ficifolium* Sm., *C. foliosum*, Aschers., *C. vulvaria* L., *C. botrys* L., *C. ambersoides* L. and *C. album* L. subsp. *album*, *C. album* L. subsp. *striatum* and *C. album* L. subsp. *iranicum*. (Chenopodiaceae). The species of the genus are currently identified on the basis of scanning electron microscopy studies of seed (Malekloo *et al.*, 2008). But difficulties are still encountered in the separation of some taxa. Pollen micromorphology can be considered used to separation between species this genus. The pollen morphology of *Chenopodium* has been considerable attention from several botanists (Erdtman, 1943, 1969; McAndrews and Swanson, 1967; Tsukada, 1967; Uotila, 1974).

MATERIALS AND METHODS

This study is mainly based on plant material deposited in the Iranian herbarium TARI (abbreviations

according to Holmgren *et al.* (1990), IAUGH (Islamic Azad University, Garmsar Herbarium) and collected from the localities from Iran during Jun-Oct. 2008-2009 (Table 1).

Pollen grains of 13 species of the genus *chenopodium* were studied by Scanning Electron Microscope (SEM). Samples were obtained mostly from fresh collected herbarium specimens. The voucher and the pollen specimens are deposited in TARI Herbarium are listed under Table 1 (acronyms according to Holmgren *et al.* (1990). For SEM, we used the protocol explained by Davies (1999) with some modifications. The 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. The specimens were examined and photographed by a LEO Scanning Electron Microscope (SEM) model 440 I, at an accelerating voltage of 10-15 kV. Cross-section of exine was also examined. The number of tecta perforations, according to Punt *et al.* (2007) per 25 µm² and length of larger perforations in proximal face and distal face were measured. The terminology used for describing the pollens features followed in general Moore *et al.* (1991).

RESULTS

The main features of the investigated pollen are shown in Table 1. Present studies show that the sculpturing of exine provides valuable characters for separating the species, sometimes even for closely related ones and delimitation of natural groups within the genus. Total pollen grains species are isopolar, radial

Table 1: Characteristic features of pollen grains in Iranian representatives of *Chenopodium*

Species	Vouchers	Pollen diameter (µm)	Pores diameter (µm)	No. of conical tuberculate (5 µm)	Pores high (µm)	No. of pore (5 µm)	No. of conical tuberculate on holes	Distance between of the centers of the adjacent pores (µm)	Pores number
<i>C. vulvaria</i>	Malekloo 2795 IAUGH	17.85	1.5-1.7	25-30	0.4-0.7	2	5-7	5	40
<i>C. urbicum</i>	Assadi and Nikchehreh 76207 TARI	17.30	1.52-1.82	50-55	0.3-0.4	3-4	4-5	3.75	50
<i>C. sosnowskyi</i>	Assadi and Akhani 61573 TARI	17.30	1.15-1.20	70-75	0.7-0.8	3	7-10	4.2-5	70
<i>C. rubrum</i>	Sharif 40284 TARI	13.70	0.66-0.93	35-40	0.10-0.13	3-4	4-5	3.10	36
<i>C. opulifolium</i>	Assadi 76802 TARI	20.20	0.9-1.0	45-50	0.5-0.9	3-4	5-7	3.12	88
<i>C. novopokrovskyanum</i>	Malekloo 2799 IAUGH	15.5	0.94-0.95	38-42	0.10-0.13	3-4	4-5	4.40	75
<i>C. murale</i>	Assadi 76805 TARI	18.60	1.44-1.55	45-48	0.07-0.08	2-3	5-7	4.2	48
<i>C. glaucum</i>	Assadi et al. 64449 TARI	12.50	1.0-1.47	35-40	0.10-0.13	2-3	4-5	2.4-2.7	52
<i>C. foliosum</i> subsp. <i>foliosum</i>	Mozaffarian and Norouzi 34481 TARI	21.08	0.85-1.76	25-30	0.6-0.8	1	8-9	5-5.2	34
<i>C. foliosum</i> subsp. <i>montanum</i>	Jouharchi 23602 FUMH	16.1	1.3-1.35	25-30	0.4-0.5	2-3	4-5	4.8	42
<i>C. ficifolium</i>	Mousavi and Pariab5211 MARI	19.30	0.905-1.18	150-155	0.25-0.30	2-3	8-12	2	65
<i>C. botrys</i>	Malekloo 2800 IAUGH	11.71	1.43-1.76	100-120	1.6-2.0	7-8	8-9	2.9	42
<i>C. ambersoides</i>	Gholipoor 83973 TARI	21.11	1-1.2	30-35	0.5-0.6	3	3-4	3	90
<i>C. album</i> subsp. <i>album</i>	Assadi 76804 TARI	23.36	0.94-0.95	90-95	0.10-0.15	4	4-6	4.8	92
<i>C. album</i> subsp. <i>iranicum</i>	Assadi 76806 TARI	19.30	0.96-0.97	25-30	0.08-0.10	5-6	5-6	3.20	100
<i>C. album</i> subsp. <i>straitum</i>	Boutler and Boutmer 22884 TARI	15.90	0.710-0.725	60-65	0.10-0.15	5-6	8-9	3.25	60

symmetrical, peripolyporate are spherical and 3-12 conical tuberculate on pore of pollen surface, holes of pollen surface 0.66-1.76 µm diameter, 2-6 pore number per 5 µm, 18-42 pore total of pollen, 21-76 conical tuberculate per 5 µm, tectal conical tuberculate 0.1-0.2 µm. The exine of the genus *chenopodium* is in the most cases perforate and faveat types ornamentation on tectum. That very important in recognizing natural groups within the genus. Pollen grains can be arranged in two groups which are accordance with the results from morphology and palynology resulted in the following grouping: Group 1 perforate type, that diameter of holes on the exine less than 1 µm². Group 1 includes the species such as: *C. album*, *C. novopokrovskyanum* and *C. rubrum*. Group 2 faveat type, that diameter of holes on the exine is more than 1 µm². Group 2 includes the species such as: *C. urbicum*, *C. murale*, *C. glaucum*, *C. opulifolium*, *C. sosnowskyi*, *C. ficifolium*, *C. foliosum*, *C. vulvaria*, *C. botrys* and *C. ambersoides*. On the basis of the exine sculpturing at proximal face, two main pollen types can be recognized which are described below:

Perforate: This type is homogeneous species examined and occurs in 3 species: *C. album*, *C. novopokrovskyanum* and *C. rubrum* (Fig. 1, 2, 5).

***C. album*:** *C. album* (Fig. 1) Pollen grains radial symmetrical, isopolar, peripolyporate, spherical, The largest pollen of all the species is classified under this type and

occurs in *C. album* subsp. *album* [23.01-23.36 µm in size], pollen diameter 23.36 µm in equatorial view, pores 0.94 to 0.95 µm in diameter; 0.1-0.15 µm high, 4-6 conical tuberculate on holes, distance between the centers of the adjacent pores 4.8 µm. Pore number 92. ornamentation scabrate; 9095- tuberculate conical per 5 µm²; 4 pore numbers per 5 µm², tectal tuberculate conical, 0.3 µm high, 0.2 µm wide. In *C. album* subsp. *straitum* [13.50-15.90 µm in size], pollen diameter 15.90 µm in equatorial view, pores 0.710-0.720 µm in diameter; 0.1-0.15 µm high, 8-9 conical tuberculate on holes, distance between the centers of the adjacent pores 3.25 µm. Pore number 60 ornamentation scabrate; 60-65 tuberculate conical per 5 µm²; 3 pore numbers per 5 µm², tectal tuberculate conical, 0.1 µm high, 0.15 µm wide in *C. album* subsp. *iranicum* [16.30-19.30 µm in size], pollen diameter 19.30 µm in equatorial view, Pores 0.96-0.97 µm in diameter; 0.08-0.10 µm high, 5-6 conical tuberculate on holes, distance between the centers of the adjacent pores 3.20 µm. Pore number 100, ornamentation scabrate; 25-30 tuberculate conical per 5 µm²; 5-6 pore numbers per 5 µm², tectal tuberculate conical, 0.3 µm high, 0.3 µm wide.

***C. novopokrovskyanum*:** Pollen grains radial symmetrical, isopolar, peripolyporate, spherical (Fig. 5) in *C. novopokrovskyanum* [15.15-16.10 µm in size], pollen diameter 15.15 µm in equatorial view, pores 0.94-0.95 µm in diameter; 0.10-0.13 µm high, 4-5 conical tuberculate on holes, distance between the centers of the adjacent pores

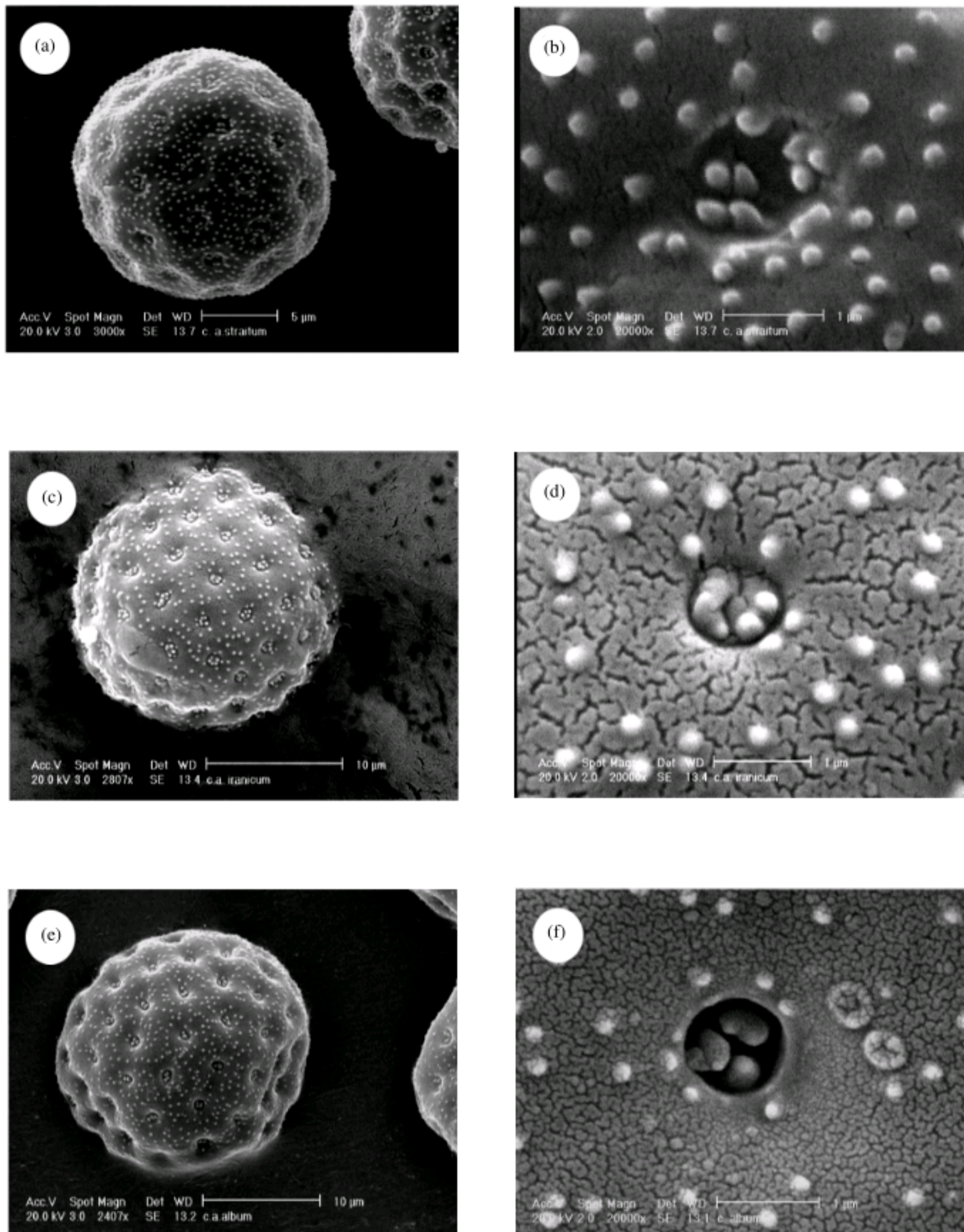


Fig. 1: Micrographs of pollen grains in *Chenopodium* (Chenopodiaceae). Perforate tectum at the proximal face in *C. album* subsp. *straitum* pollen with perfoarte ornamentation of exine (a, b), perforate tectum at the proximal face in *C. album* subsp. *iranicum* pollen with perfoarte ornamentation of exine (c, d) and perforate tectum at the proximal face in *C. album* subsp. *album* pollen with perfoarte ornamentation of exine (e, f). Scale bar = 10 µm (c, e), 1 µm (b, d, f) and 5 µm (a)

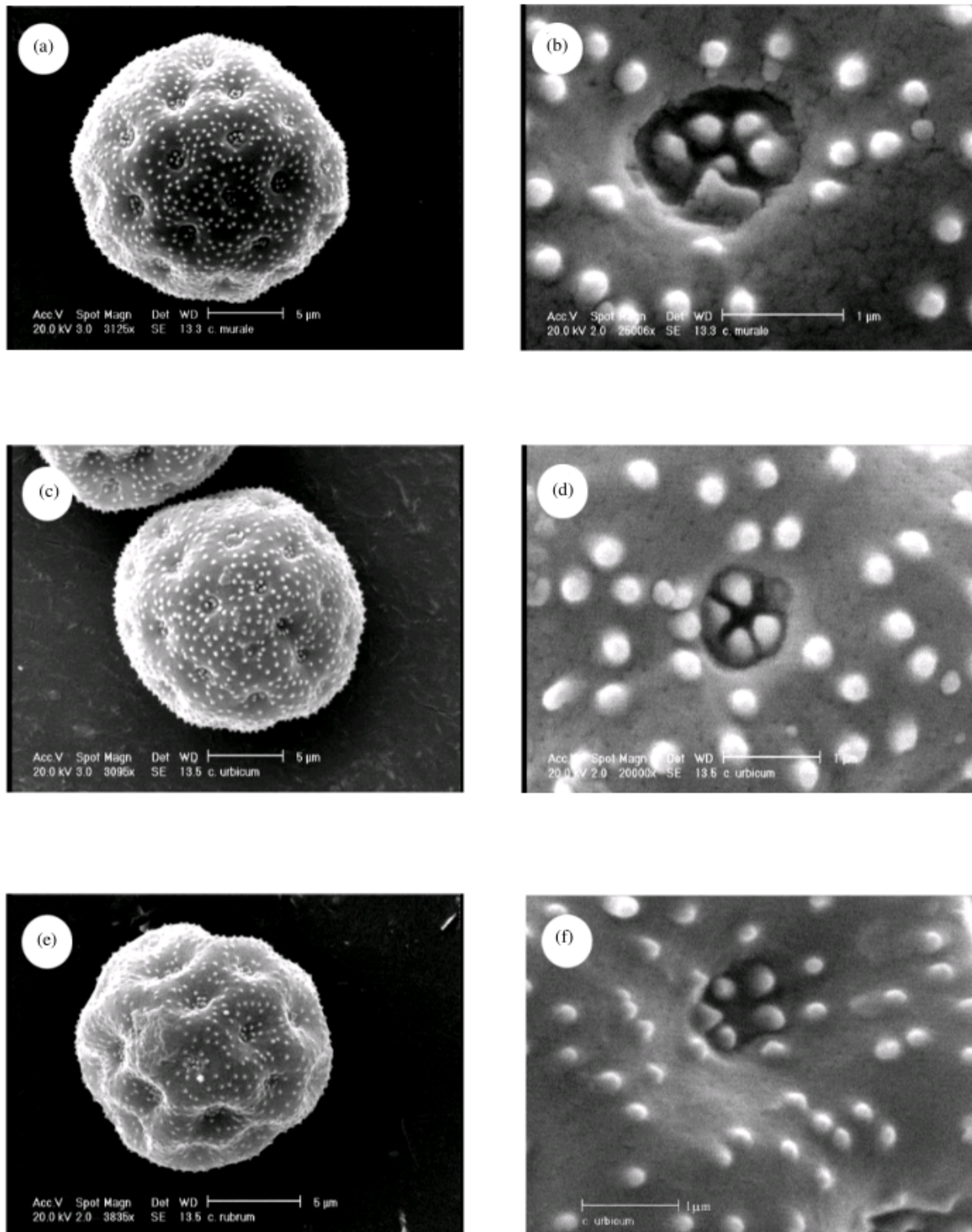


Fig. 2: Micrographs of pollen grains in *Chenopodium* (Chenopodiaceae). Faveate tectum at the proximal face in *C. murale* pollen with faveate ornamentation of exine (a, b), faveate tectum at the proximal face in *C. urbicum* pollen with faveate ornamentation of exine (c, d) and perforate tectum at the proximal face in *C. rubrum* pollen with faveate ornamentation of exine (e, f). Scale bar = 5 µm (a, c, e) and 1 µm (b, d, f)

4.40 μm , pore number 75, ornamentation scabrate, 38 to 42 tuberculate conical per 5 μm^2 ; 3-4 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.06-0.08 μm high, 0.18-0.20 μm wide.

C. rubrum: The smallest one is observed in *C. rubrum* [11.80-13.70 μm in size] which also belongs to this type (Fig. 2). Pollen grains radial symmetrical, isopolar, peripolporate, spherical, pollen diameter 13.70 μm in equatorial view, pores 0.66-0.93 μm in diameter; 0.10-0.13 μm high, 4-5 conical tuberculate on holes, distance between the centers of the adjacent pores 3.10 μm , pore number 36, ornamentation scabrate, 35 to 40 tuberculate conical per 5 μm^2 ; 3-4 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.02-0.03 μm high, 0.04-0.06 μm wide.

Faveat: This type is heterogeneous and more frequent between species examined and occurs in 10 species: *C. urbicum*, *C. murale*, *C. glaucum*, *C. opulifolium*, *C. sosnowskyi*, *C. ficifolium*, *C. foliosum*, *C. vulvaria*, *C. botrys* and *C. ambersoides*.

C. ficifolium: The largest pollen of all the species is classified under this type and occurs in *C. ficifolium* [19.30-22.40 μm in size] (Fig. 3). Pollen grains radial symmetrical, isopolar, peripolporate, spherical, pollen diameter 19.30 μm in equatorial view, pores 0.905-1.18 μm in diameter; 0.25-0.3 μm high, 8-12 conical tuberculate on holes, distance between the centers of the adjacent pores 2 μm , pore number 65, ornamentation scabrate, 150 to 155 tuberculate conical per 5 μm^2 ; 2-3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.30-0.34 μm high, 0.2-0.3 μm wide.

C. botrys: The smallest one is observed in *C. botrys* [9.07-11.71 μm in size] which also belongs to this type (Fig. 3). Pollen grains radial symmetrical, isopolar, peripolporate, spherical, pollen diameter 11.71 μm in equatorial view, pores 1.43-1.76 μm in diameter; 1.6-2.0 μm high, 8-9 conical tuberculate on holes, distance between the centers of the adjacent pores 2.9 μm , pore number 42, ornamentation scabrate, 100-120 tuberculate conical per 5 μm^2 ; 7-8 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.1-0.2 μm high, 0.3-0.4 μm wide.

C. urbicum: This species (Fig. 2) with, Pollen grains radial symmetrical, isopolar, peripolporate, spherical, pollen diameter 17.30 μm in equatorial view, pores 1.52-1.82 μm in diameter; 0.3-0.4 μm high, 4-5 conical tuberculate on holes, distance between the centers of the adjacent pores 3.75 μm , pore number 50, ornamentation scabrate,

50-55 tuberculate conical per 5 μm^2 ; 3-4 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.1-0.2 μm high, 0.3-0.4 μm wide.

C. murale: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig. 2), pollen diameter 18.61 μm in equatorial view, pores 1.44-1.55 μm in diameter; 0.4-0.5 μm high, 5-7 conical tuberculate on holes, distance between the centers of the adjacent pores 4.2 μm , pore number 48, ornamentation scabrate, 45-48 tuberculate conical per 5 μm^2 ; 2-3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.07-0.08 μm high, 0.3-0.35 μm wide.

C. glaucum: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig. 3), pollen diameter 12.50 μm in equatorial view, pores 1.0-1.47 μm in diameter; 0.10-0.13 μm high, 4-5 conical tuberculate on holes, distance between the centers of the adjacent pores 2.4-2.7 μm , pore number 52, ornamentation scabrate, 35-40 tuberculate conical per 5 μm^2 ; 2-3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.4-0.5 μm high, 0.3-0.4 μm wide.

C. opulifolium: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig 4), pollen diameter 20.20 μm in equatorial view, pores 0.9-1.0 μm in diameter; 0.5-0.9 μm high, 5-7 conical tuberculate on holes, distance between the centers of the adjacent pores 3.12 μm , pore number 88, ornamentation scabrate, 45-50 tuberculate conical per 5 μm^2 ; 3-4 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.2-0.5 μm high, 0.2-0.9 μm wide.

C. sosnowskyi: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig. 4), pollen diameter 17.30 μm in equatorial view, pores 1.15-1.20 μm in diameter; 0.7-0.8 μm high, 7-10 conical tuberculate on holes, distance between the centers of the adjacent pores 4.2-5 μm , pore number 70, ornamentation scabrate, 70-75 tuberculate conical per 5 μm^2 ; 3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.1-0.2 μm high, 0.2-0.3 μm wide.

C. foliosum: Pollen grains radial symmetrical, isopolar, peripolporate, spherical and occurs in *C. foliosum* subsp. *foliosum* (Fig. 5), pollen diameter 21.08 μm in equatorial view, pores 0.85-1.76 μm in diameter; 0.6-0.8 μm high, 8-9 conical tuberculate on holes, distance between the centers of the adjacent pores 5-5.2 μm , pore number 34, ornamentation scabrate, 25-30 tuberculate conical per 5 μm^2 ; 1 pore numbers per 5 μm , tectal tuberculate conical, 0.1-0.2 μm high, 0.3-0.4 μm wide. In *foliosum* subsp. *montanum* (Fig 5), pollen diameter 16.1 μm in equatorial view, pores 1.3-1.35 μm in diameter; 0.5-0.6 μm

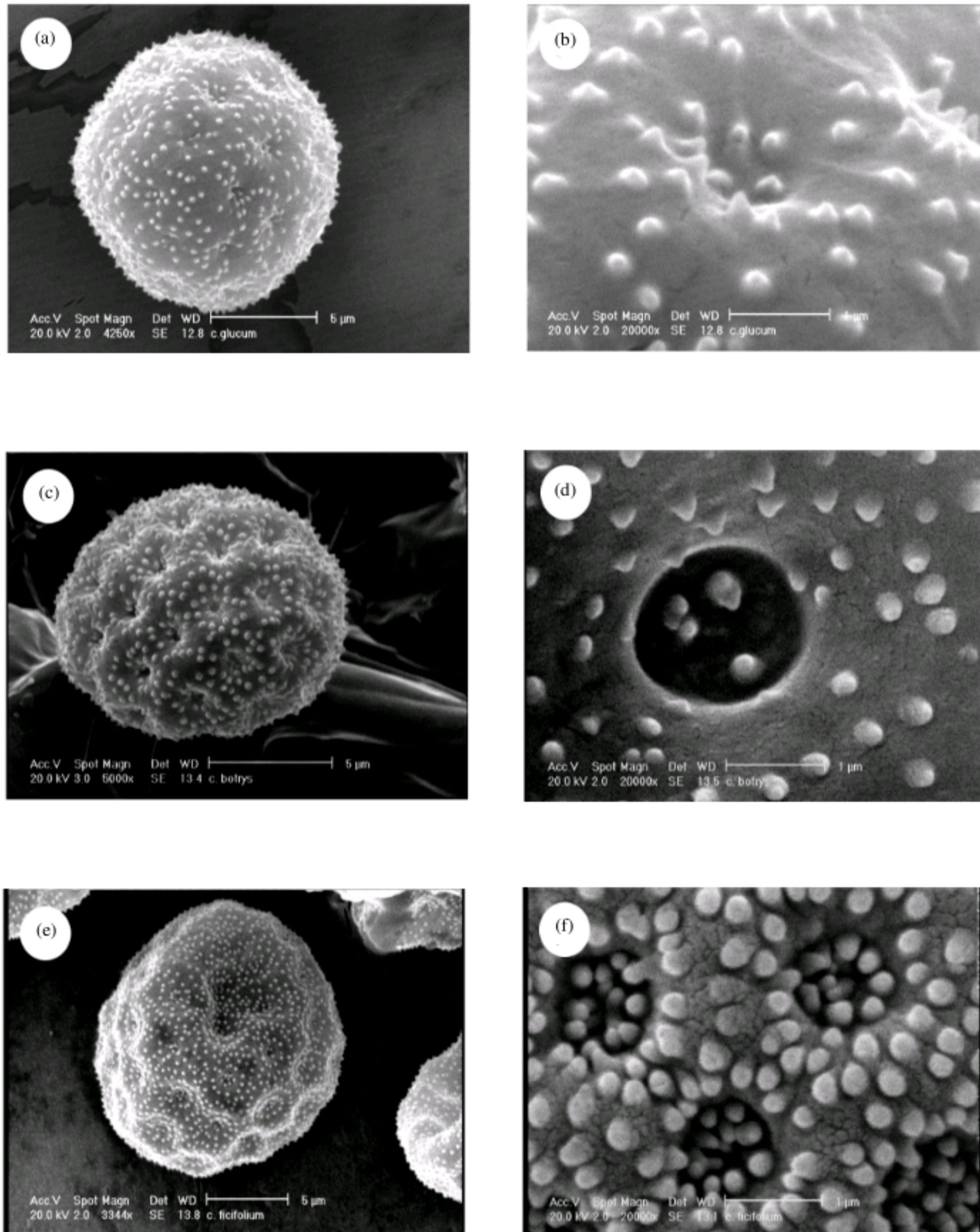


Fig. 3: Micrographs of pollen grains in *Chenopodium* (Chenopodiaceae). Faveat tectum at the proximal face in *C. glaucum* pollen with faveat ornamentation of exine (a, b), Faveat tectum at the proximal face in *C. botrys* pollen with faveat ornamentation of exine (c, d) and Faveat tectum at the proximal face in *C. ficifolium* pollen with faveat ornamentation of exine (e, f). Scale bar = 5 µm (a, c, e) and 1 µm (b, d, f)

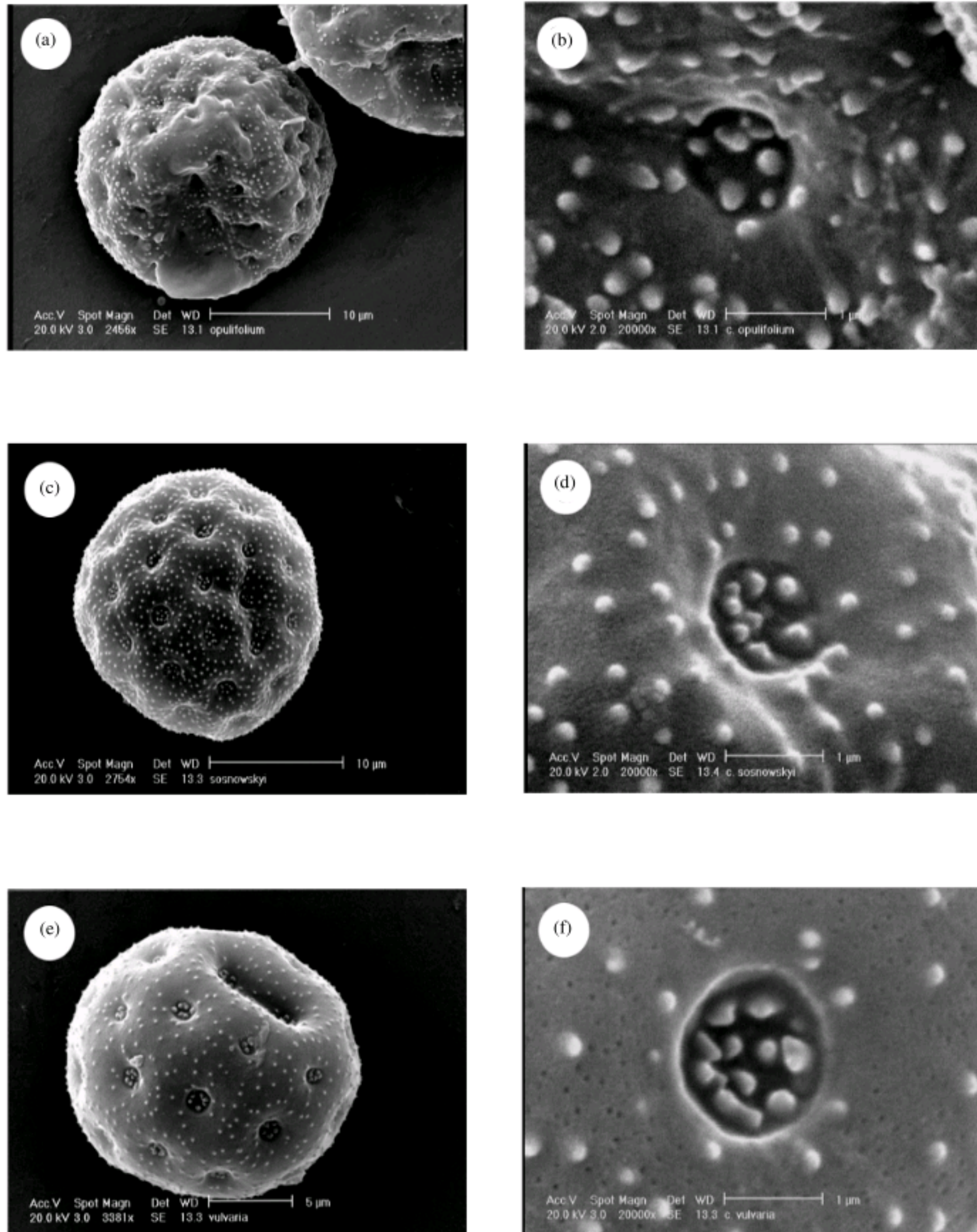


Fig. 4: Micrographs of pollen grains in *Chenopodium* (Chenopodiaceae). Faveate tectum at the proximal face in *C. opulifolium* pollen with faveate ornamentation of exine (a, b), Faveate tectum at the proximal face in *C. sosnowskyi* pollen with faveate ornamentation of exine (c, d) and Faveate tectum at the proximal face in *C. vulvaria* pollen with faveate ornamentation of exine (e, f). Scale bar = 10 µm (a, c), 1 µm (b, d, f) and 5 µm (e)

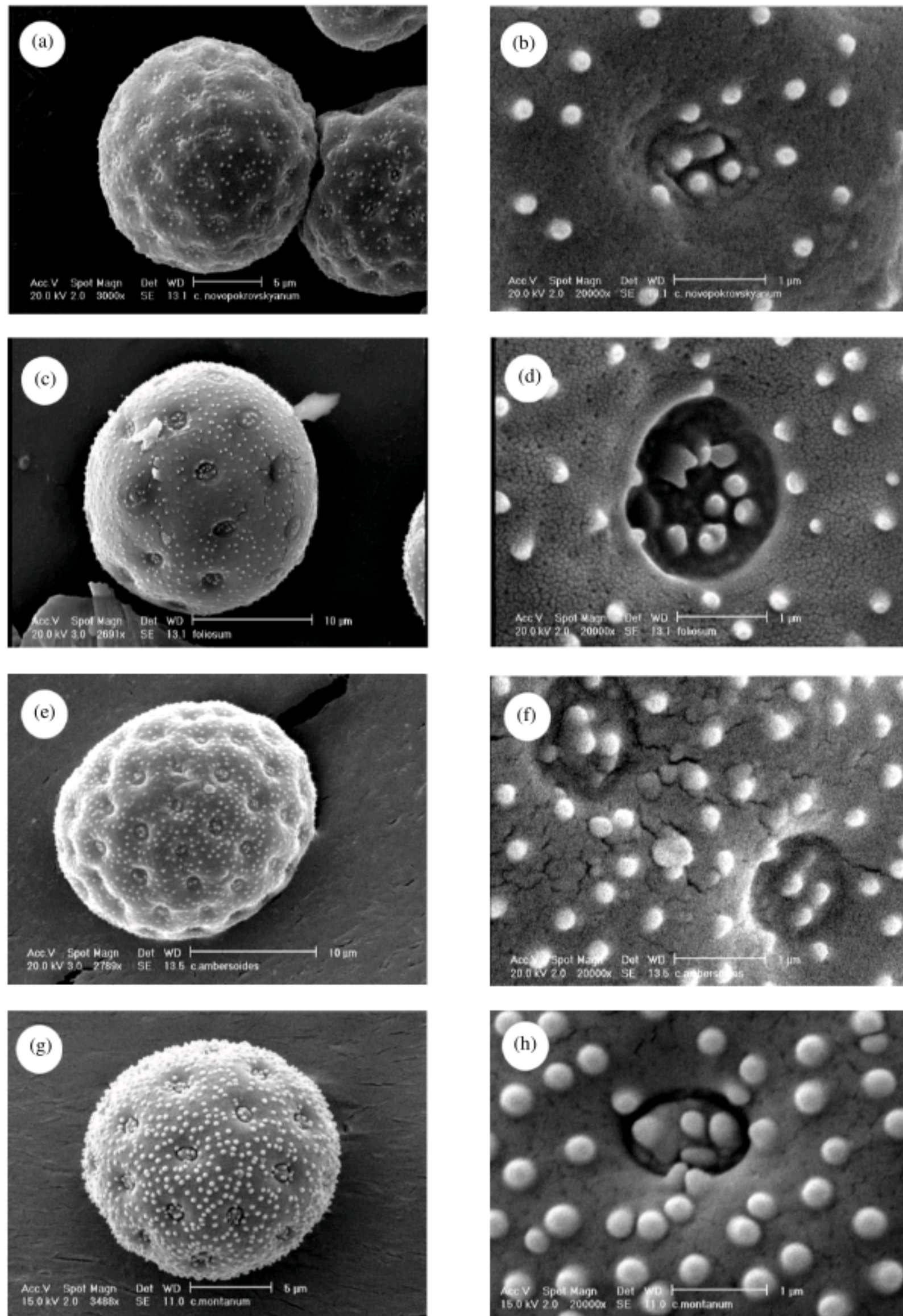


Fig. 5: Micrographs of pollen grains in *Chenopodium* (Chenopodiaceae). Perforate tectum at the proximal face in *C. novopokrovskyanum* pollen with perforate ornamentation of exine (a, b), faveate tectum at the proximal face in *C. foliosum* subsp. *foliosum* pollen with faveate ornamentation of exine (c, d), faveate tectum at the proximal face in *C. ambersoides* pollen with faveate ornamentation of exine (e, f) and faveate tectum at the proximal face in *C. foliosum* subsp. *montanum* pollen with faveate ornamentation of exine (g, h). Scale bar = 5 µm (a, g), 10 µm (c, e) and 1 µm (b, d, f, h)

high, 4-5 conical tuberculate on holes, distance between the centers of the adjacent pores 4.8 μm , pore number 42, ornamentation scabrate, 85-90 tuberculate conical per 5 μm^2 ; 2-3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.4-0.5 μm high, 0.3-0.4 μm wide.

C. vulvaria: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig. 4), pollen diameter 17.85 μm in equatorial view, pores 1.5-1.7 μm in diameter; 0.4-0.7 μm high, 5-7 conical tuberculate on holes, distance between the centers of the adjacent pores 5 μm , Pore number 40, ornamentation scabrate, 30-35 tuberculate conical per 5 μm^2 ; 2 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.1-0.2 μm high, 0.2-0.3 μm wide.

C. ambersoides: Pollen grains radial symmetrical, isopolar, peripolporate, spherical (Fig. 5), pollen diameter 21.11 μm in equatorial view, pores 1-1.2 μm in diameter; 0.5-0.6 μm high, 3-4 conical tuberculate on holes, distance between the centers of the adjacent pores 3 μm , pore number 90, ornamentation scabrate, 30-35 tuberculate conical per 5 μm^2 ; 3 pore numbers per 5 μm^2 , tectal tuberculate conical, 0.1-0.12 μm high, 0.2-0.3 μm wide.

DISCUSSION

The basic palynomorphological characters of *Chenopodium* are the occurrence of an all studied species has pollens with, spherical shape, radial symmetrical, isopolar and peripolporate. The exine surface ornamentation is scabrate. Uotila (1997) pointed out the importance of the pore number and C/D ratio in the *Chenopodium* species, also, the C/D ratio is used by McAndrews and Swanson (1967). The figure shows no correlation between pore number and pollen size of the species examined for the present study. Therefore, this present study, All studied species haven indicating that there are two holes types on pollen surface: foveat and perforate. The present study confirms this point of view the majority of the species have pollen 11-23 μm in diameter, there is a tendency of *C. ficifolium* to have larger pollen grains. We also gave variation in Distance between the centers of the adjacent pores, corresponding pore number according to McAndrews and Swanson (1967) and pollen size in Fig. 1-5. This studies suggests that there are significant differences in pollen size, tuberculate on surface exine, distance between the centers of the adjacent pores and pore number on surface exine within *Chenopodium* species. The results of this studies show that pollen micromorphology can be considered

used to superstition between species this genus. Pollen morphological characters provide valuable characters for a delimitation of the species in *Chenopodium*.

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