

Palynological study of the *Geranium* (*Geraniaceae*) species from the Thrace region (Turkey-in-Europe)

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Abstract. This study presents a palynological analysis of 13 species of genus *Geranium* L. naturally distributed in Thrace. According to the analysis, the pollen grains of all species were large, exine ornamentation was reticulate-clavate, apertures were tricolporate, symmetries were radial, equatorial images were circular. *G. sanguineum* and *G. columbinum* species, with the largest pollen grains, were found with tectate tectum, while others had semitectate tectum. Although the shapes of pollen grains were generally spheroidal-subprolate, those of *G. asphodeloides*, *G. pusillum* and *G. rotundifolium* were only spheroidal.

Key words: *Geranium*, pollen, Thrace, Turkey

Introduction

Geranium is the most important genus of the *Geraniaceae* family. It consists of approximately 430 species in 24 sections, belonging to three subgenera (Yeo 1984, Aedo & al. 1998b, 2005, 2006). In Turkey, the genus is represented with 44 species and 46 taxa, 24% of which are endemic (Davis 1967; Davis & al. 1988; Aitchison 1995; Güner 2000; Conti & Uzunov 2006; Özhata & Kültür 2006; İlçim & Behçet 2006; Öner 2010).

Some morphological, palynological and karyological studies were carried out into the genus *Geranium*. Yeo analyzed the morphology and phylogeny of *Anemonifolia* R. Knuth section in 1973; *Lucida* R. Knuth and *Unguiculata* (Boiss.) Reiche sections in 2004; problems in the classification of genus *Geranium* in 1977; and fruit discharge types, their role in classification and effects on the evolutionary re-

lationships of genus *Geranium* in 1984. In 1992, he made revisions of *Geranium* plants in southwest China. Aedo & al. (1998a, 2002, 2003, 2005, 2006, 2007) have revised *Batrachioidea* W.D.J. Koch, *Divaricata* Rouy, *Brasiliensis* R.Knuth, *Trigonum* Aedo, *Gracile* Aedo, *Dissecta* Yeo, *Andina* R.Knuth, *Chilensis* R.Knuth, *Azoreloffia* Aedo, *Neoandina* Aedo, *Paramentia* R.Knuth sections, and *Tuberosa* (Boiss.) Reiche, and *Mediterranea* R. Knuth subsections, and have prepared a world checklist for *Geranium*. Aedo (2000, 2001a,b) also analyzed the annual and perennial *Geranium* species of North America and identified many new *Geranium* species. Perveen & Gaiser (1999) analyzed the pollen morphology of some *Geranium* species in Pakistan, Shehata (2008) analyzed it in Egypt. Deniz (1991) carried out morphological studies into *Geranium* species in Edirne. İlçim & al. (2008) analyzed the morphological and palynological properties of *G. tuberosum*.

As indicated in the present study, no comprehensive palynological study of genus *Geranium* exists in the botanical literature in Turkey. That is why, this study was aimed at conducting a palynological research into the *Geranium* species (*Geranium lucidum* L., *G. purpureum* Vill., *G. robertianum* L., *G. rotundifolium* L., *G. molle* L., *G. pusillum* L., *G. divaricatum* Ehrh., *G. columbinum* L., *G. dissectum* L., *G. tuberosum* L., *G. asphodeloides* Burm fil., *G. sanguineum* L., *G. pyrenaicum* Burm. fil. subsp. *pyrenaicum*) growing in the Thrace region.

Material and methods

The examined pollen grains of 13 *Geranium* species were taken from fresh specimens collected in their natural habitats (Table 1). All pollen grains for LM examination were prepared according to the standard procedure of Wodehouse (1935). They were observed in glycerin-water using a standard Olympus CX21FS1 microscope with D plan 1.00–1.25 160/0.17 oil immersion objective and NFK×3.3 LD 125 lens.

Pollen diameter, exine and intine thickness, and mesocolpium length were measured on the average on 30 different pollen grains, and were assessed by the biometric method. Arithmetic means and standard deviations were also calculated.

LEO 440 computer-controlled digital brand electron microscope was used to take SEM analyses of the pollen grains and microphotographs in the Dokuz Eylül University Characterization Laboratory of Metallurgy and Material Engineering. Mature pollen grains were secured on metal pollen-carrier stubs with two-side adhesive tape and binocular microscope was used. POLARON SC 7620 brand coating device was used to coat non-conductive seeds and mericarps by means of sputtering. Pollen grains were vacuumed; coating procedure lasted averagely for 1.5 minutes. The pollen grains were analyzed by SEM and LM. These analyses determined the form, aperture, tectum, and ornamentation types of the grains. On the basis of microscope images and analyses, the morphological characteristics of the pollen grains were evaluated, according to Punt & al. (2007), and the findings are presented in Tables 2 and 3.

Table 1. List of the studied species and specimens of *Geranium*.

1. <i>Geranium lucidum</i> L.	A1(E) Kırklareli: Kömürköy-Vize 3. km, in forest, 260 m, 20.05.2009, İ.Deniz 423, EDTU 11027. Pınarhisar-İğneada 35. km, Kadıkule region, in forest, 650 m, 11.06.2009, İ.Deniz 452, EDTU 11046.
2. <i>G. purpureum</i> Vill.	A1(E) Edirne: Seydiköy-Malkara 1. km, near watercourse, under trees, 60 m, 17.05.2009, İ.Deniz 385, EDTU 11069. A2(E) İstanbul: Hacıosman slopes, meadow, in forest, 80 m, 24.04.2009, İ.Deniz 314, EDTU 11051.
3. <i>G. robertianum</i> L.	A1(E) Kırklareli: Pınarhisar-İğneada 35. km, Kadıkule region, in forest, 650 m, 11.06.2009, İ.Deniz 452, EDTU 11100.
4. <i>G. rotundifolium</i> L.	A1(E) Çanakkale: Kavaklı village, Demirci lake, in bushes, 40 m, 18.05.2009, İ.Deniz 401, EDTU 11118. A2(E) Tekirdağ: Sultanköy-İstanbul 1. km, near road, hard pan, 30 m, 20.05.2009, İ.Deniz 415, EDTU 11121.
5. <i>G. molle</i> L.	A1(E) Edirne: Seydiköy-Malkara 1. km, near watercourse, under trees, 60 m, 17.05.2009, İ.Deniz 385, EDTU 11200. A1(E) Kırklareli: Between Kızılağaç-sivriler, near road, Quercus forest, 400 m, 21.05.2010, İ.Deniz 457, EDTU 11238.
6. <i>G. pusillum</i> L.	A1(E) Edirne: Üyüklüttatar-Edirne, near road, meadow, wetland, 40 m, 02.05.2009, İ.Deniz 333, EDTU 11240. Küçünlü-Hacıdanışment 4. km, near watercourse, in bushes, 280 m, 24.05.2009, İ.Deniz 431, EDTU 11257.
7. <i>G. divaricatum</i> Ehrh.	A1(E) Edirne: Tatarlar-Süleymandanışment 1. km, under <i>Genista</i> sp., 250 m, 24.05.2009, İ.Deniz 428, EDTU 11447. Between Hamzabeyli-otoyol, near trees, wetland, 370 m, 28.05.2009, İ.Deniz 436, EDTU 11450.
8. <i>G. columbinum</i> L.	A1(E) Kırklareli: Topçular-Tatlıpınar 1. km, in forest, near watercourse, 470 m, 28.05.2009, İ.Deniz 439, EDTU 11299. A1(E) Tekirdağ: Dereköy-Yuva 2. km, in forest, in bushes, 160 m, 19.05.2009, İ.Deniz 408, EDTU 11280.
9. <i>G. dissectum</i> L.	A1(E) Edirne: Karpuzlu-İpsala 1. km, near road, 10 m, 02.05.2009, İ.Deniz 343, EDTU 11352. Kayapa village, near watercourse, 100 m, 24.05.2009, İ.Deniz 425, EDTU 11426.
10. <i>G. tuberosum</i> L.	A1(E) Kırklareli: Edirne-İstanbul road, Sarıcaali junction, meadow, 50 m, 23.04.2009, İ.Deniz 308, EDTU 11451.
11. <i>G. asphodeloides</i> Burm fil.	A1(E) Edirne: Barağı village-Enez 3. km, under <i>Quercus</i> , 30 m, 03.05.2009, İ.Deniz 351, EDTU 11461. A1(E) Kırklareli: Kıyıköy-Saray, Hamidiye junction, in forest, 150 m, 20.05.2009, İ.Deniz 422, EDTU 11474.
12. <i>G. sanguineum</i> L.	A1(E) Edirne: Hamzabeyli-Uzunbayır 1. km, <i>Ouerqus</i> forest, 260 m, 28.05.2009, İ.Deniz 435, EDTU 11485. Kalkansöğüt-Vaysal 1. km, <i>Ouerqus</i> forest, 440 m, 28.05.2009, İ.Deniz 437, EDTU 11486.
13. <i>G. pyrenaicum</i> Burm. fil. subsp. <i>pyrenaicum</i>	A1(E) Kırklareli: Kadıköy-Kuzulu 2. km, in bushes, near road, 360 m, 10.06.2009, İ.Deniz 443, EDTU 11505. A2(E) İstanbul: Celepköy-Erencik 2. km, in forest, 90 m, 20.05.2010, İ.Deniz 455, EDTU 11512.

Table 2. Morphological pollen data of the *Geranium*-I species (values in μm).
 (Abbreviations: M – mean value, V – variation, minimum – maximum values)

	Polar length (P)		Equatorial width (E)		P/E ratio
	V	M	V	M	M
<i>G. asphodeloides</i> EDTU 11474	67.27–79.12	73.84 \pm 3.37	65.10–78.12	71.06 \pm 3.57	1.04 \pm 0.03
<i>G. columbinum</i> EDTU 11299	65.10–82.46	74.16 \pm 4.57	60.76–75.95	70.58 \pm 3.93	1.05 \pm 0.05
<i>G. dissectum</i> EDTU 11426	47.74–75.95	59.97 \pm 4.76	43.40–69.44	56.14 \pm 4.73	1.06 \pm 0.04
<i>G. divaricatum</i> EDTU 11450	56.42–67.27	62.31 \pm 2.69	47.74–62.93	56.73 \pm 2.75	1.1 \pm 0.04
<i>G. lucidum</i> EDTU 11046	56.42–65.10	62.47 \pm 2.78	52.08–60.76	56.75 \pm 2.27	1.1 \pm 0.04
<i>G. molle</i> EDTU 11238	47.74–65.10	58.50 \pm 5.98	43.40–60.76	53.72 \pm 5.36	1.09 \pm 0.06
<i>G. purpureum</i> EDTU 11051	59.67–70.52	64.99 \pm 2.56	56.42–69.44	61.09 \pm 3.27	1.07 \pm 0.04
<i>G. pusillum</i> EDTU 11257	47.74–60.76	51.67 \pm 2.33	43.40–56.42	49.90 \pm 2.31	1.04 \pm 0.03
<i>G. pyrenaicum</i> subsp. <i>pyrenaicum</i> EDTU 11512	60.76–65.10	62.77 \pm 1.95	52.08–65.10	58.74 \pm 3.06	1.07 \pm 0.05
<i>G. robertianum</i> EDTU 11100	60.76–73.78	68.66 \pm 2.37	60.76–69.44	65.10 \pm 1.67	1.05 \pm 0.03
<i>G. rotundifolium</i> EDTU 11118	60.76–73.78	66.06 \pm 3.70	56.42–73.78	63.41 \pm 3.54	1.04 \pm 0.04
<i>G. sanguineum</i> EDTU 11485	91.14–112.84	99.93 \pm 6.17	82.46–99.82	92.78 \pm 4.72	1.08 \pm 0.04
<i>G. tuberosum</i> EDTU 11451	60.76–82.46	67.99 \pm 6.65	47.74–78.12	61.94 \pm 6.97	1.10 \pm 0.05

Table 3. Morphological pollen data of the *Geranium*-II species (values in μm).
 (Abbreviations: M – mean value, V – variation, minimum – maximum values)

	Mesocolpium		Intine		Exine thickness	
	V	M	V	M	V	M
<i>Geranium asphodeloides</i> EDTU 11474	47.74–56.42	53.23 \pm 2.44	0.87–1.75	1.41 \pm 0.22	3.93–6.12	4.93 \pm 0.49
<i>G. columbinum</i> EDTU 11299	39.06–56.42	51.05 \pm 3.78	0.87–1.57	1.33 \pm 0.18	4.90–6.30	5.52 \pm 0.31
<i>G. dissectum</i> EDTU 11426	36.89–47.74	42.94 \pm 3.30	0.87–2.10	1.44 \pm 0.30	3.06–5.25	4.14 \pm 0.65
<i>G. divaricatum</i> EDTU 11450	39.06–47.75	42.63 \pm 2.93	0.87–1.57	1.18 \pm 0.16	3.15–5.25	3.77 \pm 0.65
<i>G. lucidum</i> EDTU 11046	41.23–47.74	44.29 \pm 1.88	0.87–1.75	1.33 \pm 0.32	3.50–5.68	4.74 \pm 0.51
<i>G. molle</i> EDTU 11238	32.55–45.50	38.46 \pm 3.06	0.43–1.75	1.10 \pm 0.31	3.93–5.68	4.76 \pm 0.56
<i>G. purpureum</i> EDTU 11051	36.89–49.91	44.48 \pm 3.43	0.87–1.75	1.36 \pm 0.23	3.50–5.68	4.86 \pm 0.51
<i>G. pusillum</i> EDTU 11257	30.38–41.23	35.80 \pm 2.62	0.43–1.75	1.19 \pm 0.30	2.62–5.07	3.75 \pm 0.53
<i>G. pyrenaicum</i> subsp. <i>pyrenaicum</i> EDTU 11512	41.23–47.74	44.26 \pm 1.89	0.87–1.57	1.27 \pm 0.21	3.67–5.25	4.35 \pm 0.49
<i>G. robertianum</i> EDTU 11100	21.70–52.08	38.86 \pm 7.62	0.87–1.75	1.35 \pm 0.30	3.50–6.12	4.95 \pm 0.44
<i>G. rotundifolium</i> EDTU 11118	39.06–48.82	44.72 \pm 2.43	0.87–1.75	1.23 \pm 0.31	3.50–7.00	5.17 \pm 0.66
<i>G. sanguineum</i> EDTU 11485	56.42–73.78	63.72 \pm 4.11	1.75–3.50	2.22 \pm 0.41	7.00–9.62	7.92 \pm 0.76
<i>G. tuberosum</i> EDTU 11451	47.74–56.42	52.57 \pm 2.41	0.87–1.75	1.49 \pm 0.23	4.37–6.56	5.36 \pm 0.55

Results

Thirteen species of the genus *Geranium* naturally distributed in Thrace (*G. lucidum*, *G. purpureum*, *G. robertianum*, *G. rotundifolium*, *G. molle*, *G. pusillum*, *G. divaricatum*, *G. columbinum*, *G. dissectum*, *G. tuberosum*, *G. asphodeloides*, *G. sanguineum*, *G. pyrenaicum* subsp. *pyrenaicum*) were analyzed palynologically. The properties of pollen grains of these species are presented in Tables 2 and 3 (Figs 1-5).

Discussion

Analysis has shown that *G. sanguineum* had the largest (Table 2, Fig. 2, 5), while *G. pusillum* (Table 2, Figs 1, 4), had the smallest pollen grains. Although there was no significant difference between the sections in terms of pollen grains, it was found that the grains of species of subgenus *Geranium* were larger than those of subgenus *Robertium*.

Exine ornamentations were found to be reticulate-clavate, apertures were tricolporate, radially symmetric, and equatorial view was circular. *Geranium sanguineum* and *G. columbinum*, with the largest pollen grains, had tectate tectum structure, while the other pollen grains had semitectate tectum structure (Figs 2, 4, 5). Although the form of pollen grains was generally spheroidal-subprolate, *G. asphodeloides*, *G. pusillum* and *G. rotundifolium* were only spheroidal (Figs 1-5).

In their work *The Northwest European Pollen Flora*, vol. VI, Punt & al. (1991) presented an identification key for genus *Geranium*. In this key, genus *Geranium* was divided into species and groups involving some species. This identification took into account the length, density-scarcity of supratectal elements and the size of pollen grains. It was reported that *G. sanguineum* had medium-sized pollen grains, while *G. purpureum*, *G. pusillum*, *G. pyrenaicum* subsp. *pyrenaicum*, *G. robertianum*, *G. rotundifolium*, *G. columbinum*, *G. dissectum*, *G. divaricatum*, *G. lucidum*, and *G. molle* had small to large pollen grains. In the present study, we have found that the pollen grains of *G. pusillum* were from medium to large, while the other species had large pollen grains.

Perveen & Gaiser (1999) carried out a pollen study into the *Geraniaceae* family and divided the family pollen grains into three groups, according to the exine ornamentation: 1. *Geranium himalayense* type, 2. *Erodium sikutarium* type and 3. *Monsonia senegalensis* type. Although identification in that study was at a genus lev-

el, no diagnostic relationship has been found at species level. Although *G. lucidum*, *G. robertianum* and *G. rotundifolium* were found with oblate-spheroidal pollen grains by these authors, we have found that *G. lucidum* and *G. robertianum* had spheroidal-subprolate pollen grains, while *G. rotundifolium* had spheroidal pollen grains (Table 2, Figs 1, 3). These differences may be due to different chemicals used in the two studies.

Shehata (2008) studied pollen morphology of the *Geraniaceae* family in Egypt and similarly identified three types and three subtypes, according to the exine ornamentation. Type 1: Striate/striate-reticulate (includes some *Erodium* species). Type 2: Reticulate/gemmulate (includes some *Geranium* species). Type 3: Reticulate. Subtype A. *Monsonia heliotropioides*, subtype B. *Monsonia senegalensis*. Subtype C. *Pelargonium grandiflorum*. Although in that study it was reported that *G. dissectum* and *G. molle* had prolate-spheroidal pollen grains, we have found that these species had spheroidal-subprolate pollen grains. Shehata (2008) reported *G. dissectum* pollen as $P = 51$ (47–55) $E = 48$ (45–51) μm and *G. molle* pollen grain size as $P = 53$ (49–60) $E = 50$ (48–54) μm . However, in the present study *G. dissectum* and *G. molle* pollen grains were found to be larger [$P = 59.97$ (47.74–75.95) $E = 56.14$ (43.40–69.44) μm] [$P = 58.50$ (47.74–65.10) $E = 53.72$ (43.40–60.76) μm], respectively (Table 2, Figs 1-4). The reason for this difference in Shehata's study (2008) may be due to the application of Erdtman's method. A study by İlçim & al. (2008) reported tectate tectum in *G. tuberosum*, while in the present study we have found that *G. tuberosum* had semitectate tectum.

Conclusion

Analysis has shown that the pollen grains of all species have reticulate-clavate ornamentation. *G. asphodeloides*, *G. pusillum* and *G. rotundifolium* have spheroidal, while the other species have spheroidal-subprolate form. In all species, aperture is tricolporate, although the exine structure of *G. columbinum* and *G. sanguineum* is tectate, while the other species are semitectate in structure (Figs 1-5).

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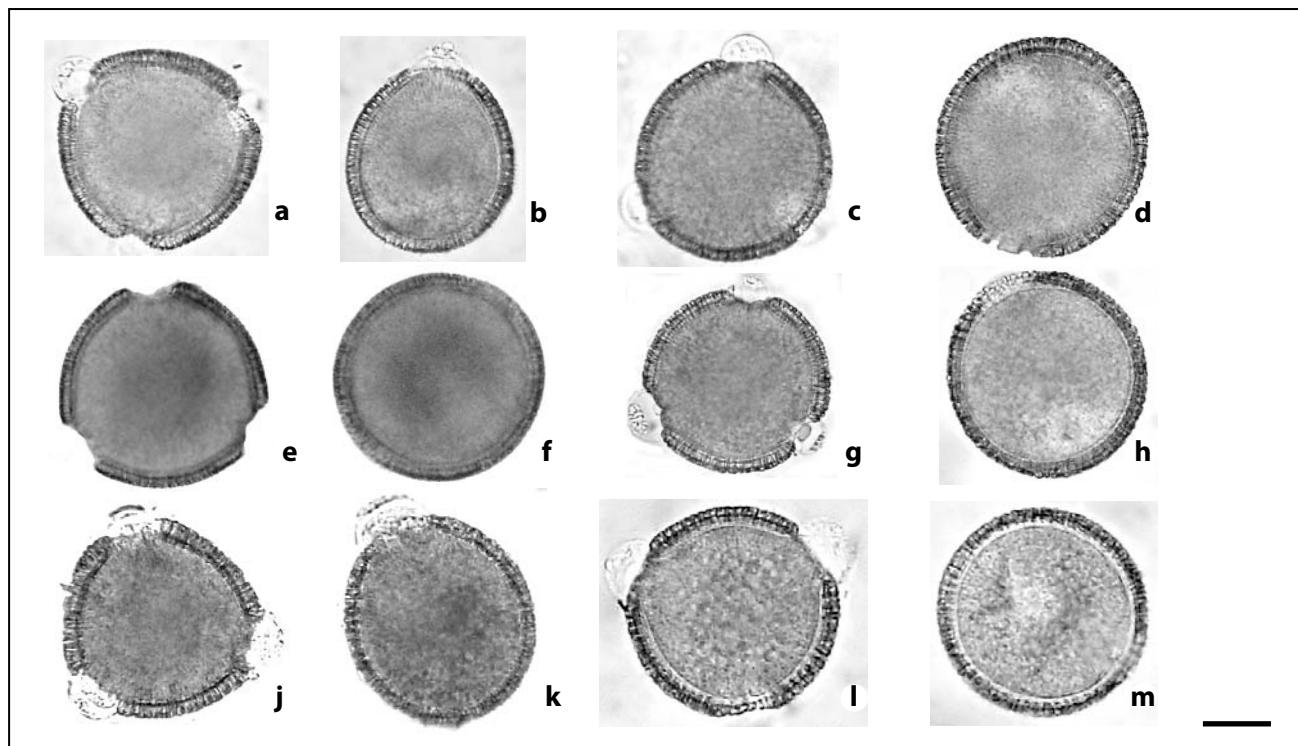


Fig. 1. a, b. *Geranium lucidum*, c, d. *G. purpureum*, e, f. *G. robertianum*, g, h. *G. rotundifolium*, j, k. *G. molle*, l, m. *G. pusillum* (a, c, e, g, j, l: polar view, b, d, f, h, k, m: equatorial view) (LM). (Scale bar: 10 µm).

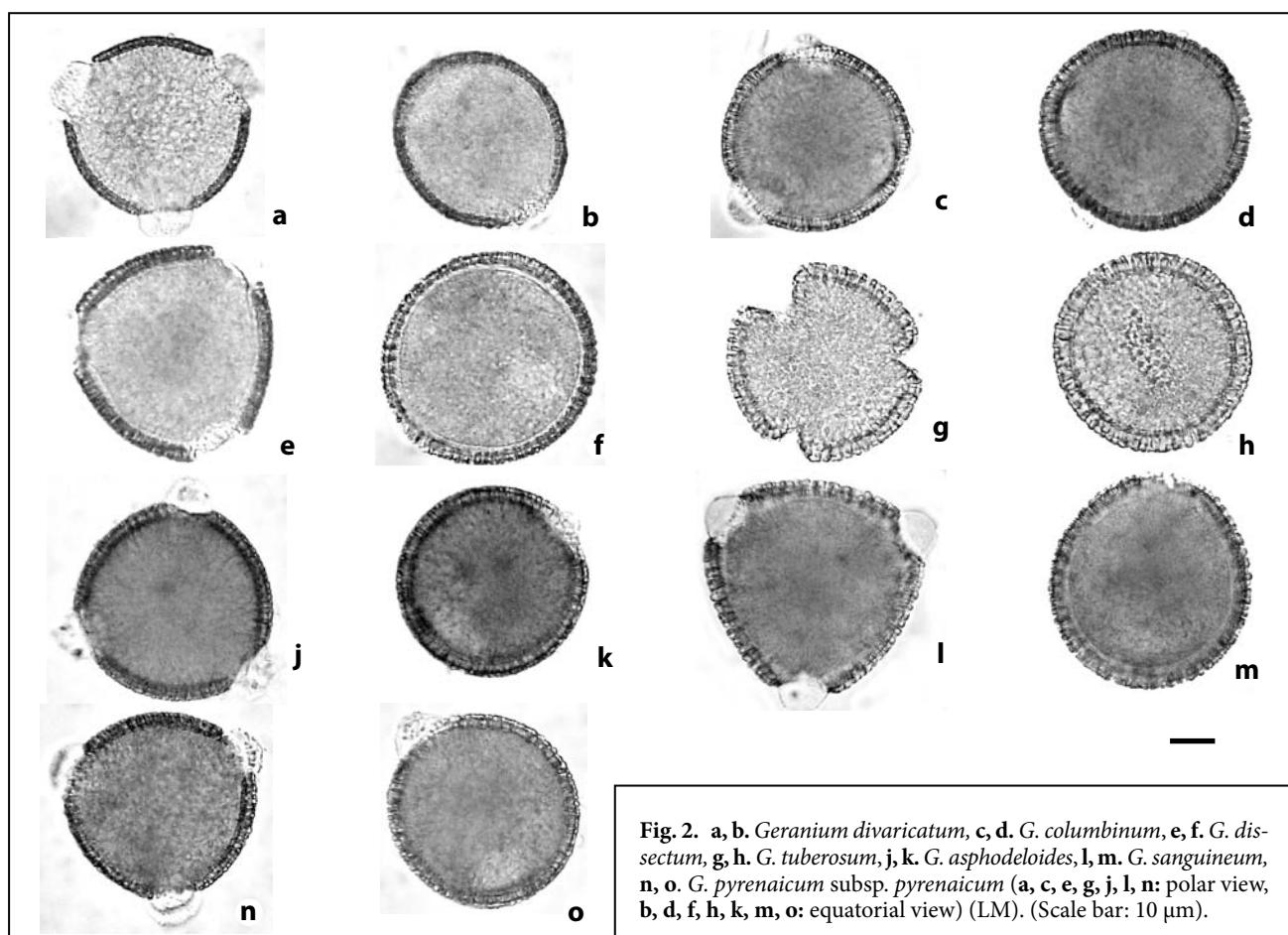


Fig. 2. a, b. *Geranium divaricatum*, c, d. *G. columbinum*, e, f. *G. dissectum*, g, h. *G. tuberosum*, j, k. *G. asphodeloides*, l, m. *G. sanguineum*, n, o. *G. pyrenaicum* subsp. *pyrenaicum* (a, c, e, g, j, l, n: polar view, b, d, f, h, k, m, o: equatorial view) (LM). (Scale bar: 10 µm).

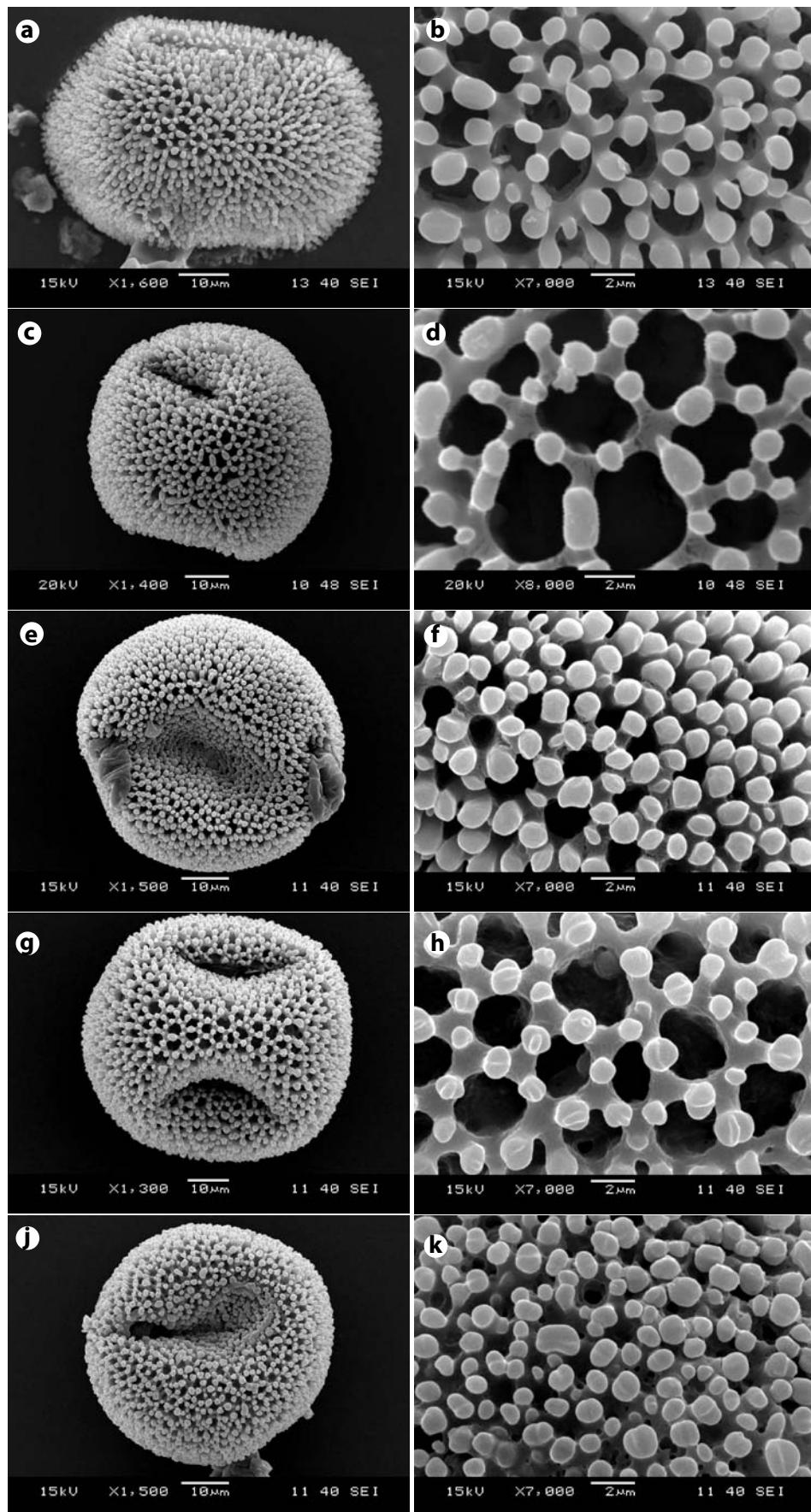


Fig. 3. a, b. *Geranium lucidum*,
c, d. *G. purpureum*,
e, f. *G. robertianum*, g, h. *G. rotundifolium*, j, k. *G. molle*
(a, c, e, g, j: general view,
b, d, f, h, k: ornamentation) (SEM).

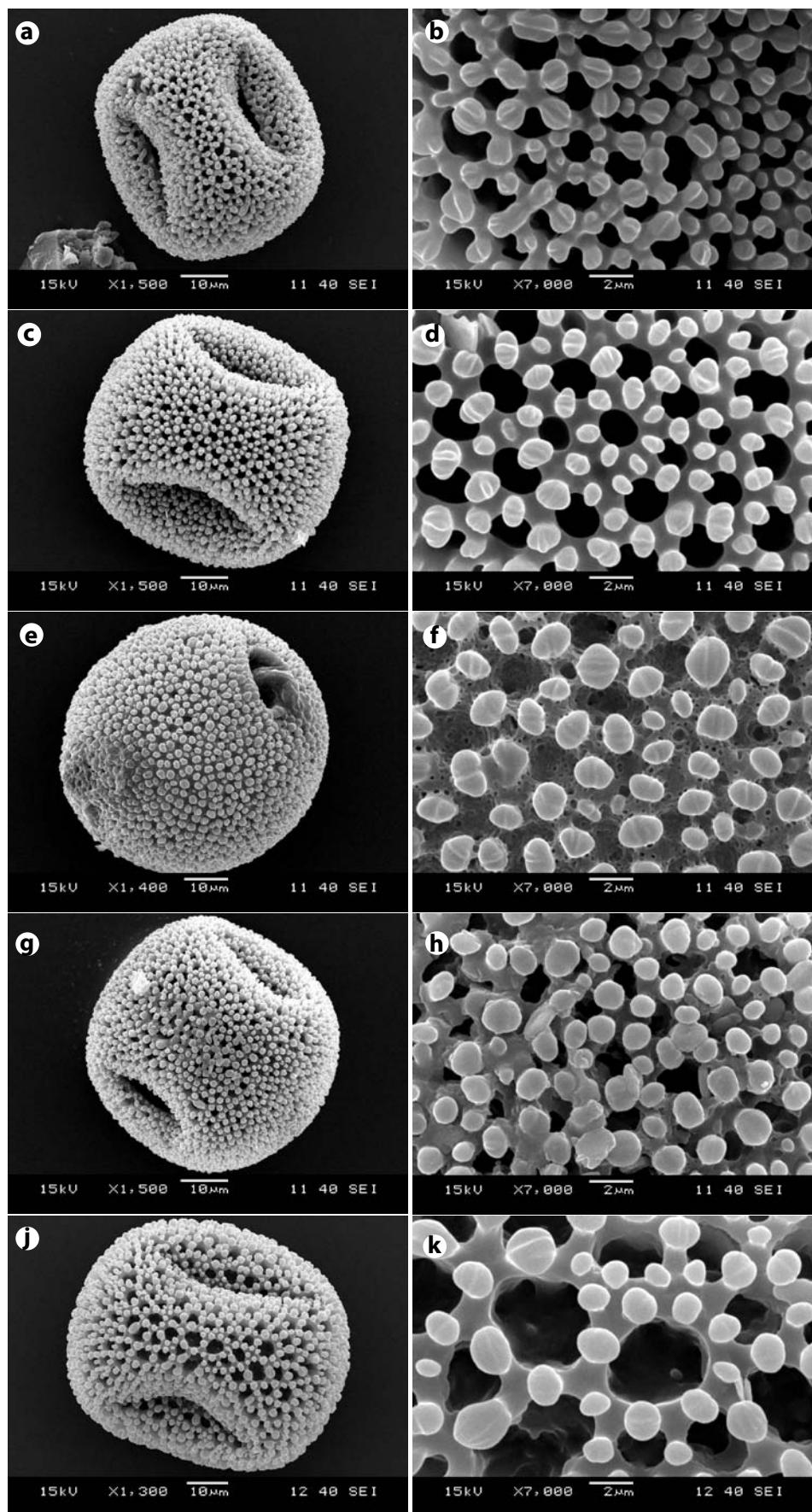


Fig. 4. a, b. *Geranium pusillum*,
c, d. *G. divaricatum*,
e, f. *G. columbinum*,
g, h. *G. dissectum*,
j, k. *G. tuberosum*
(a, c, e, g, j: general view,
b, d, f, h, k: ornamentation) (LM).

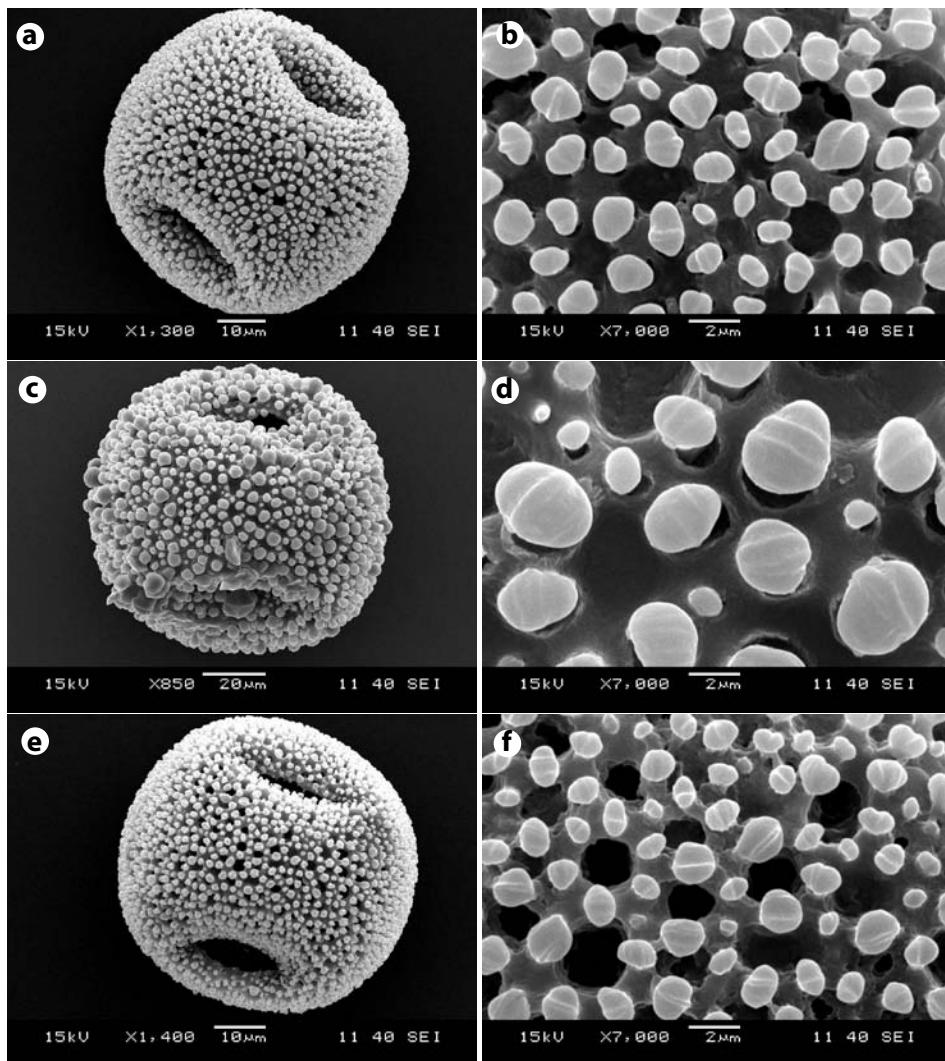


Fig. 5. a, b. *Geranium asphodeloides*,
c, d. *G. sanguineum*,
e, f. *G. pyrenaicum* subsp. *pyrenaicum*
(a, c, e: general view, b, d, f: ornamentation) (SEM).

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