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Palynological analysis of genus *Geranium* (Geraniaceae) and its systematic implications using scanning electron microscopy

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Abstract. Pollen morphology of 23 species belonging to *Geranium* have been studied in details, which represent eight sections of two subgenera i.e., *G. sect. Dissecta*, *Geranium*, and *Tuberosa* of subgen. *Geranium*, *Divaricata*, *Lucida*, *Ruberta* and *Trilopha* of subgen. *Robertium*. These plant species were collected from different phytogeographical regions of Iran. The palynological investigation was done using scanning electron microscopy (SEM) techniques. Different palyno-morphological features have been observed, and the closely related species were distinguished. We used different multivariate statistical methods to reveal the species relationships. Ward clustering analyses have been done to check out the relationship among the species. The shapes of pollen grains were monad, radially symmetric, isopolar, apertures were tricolporate, and of spheroid, prolate-spheroid or sub-prolate classes. Three pollen types were recognized on the basis of differences in exine sculpturing pattern: reticulate-clavate, striate-rugulate, reticulum cristatum with clavae. Observed differences were not of diagnostic importance in subgenera and sections level. The main objective of this study is to find distinguish pollen characters in the species of the genus *Geranium* and to elucidate their systematic importance.

Keywords: *Geranium*, pollen morphology, systematics, phytogeographical regions.

RESEARCH HIGHLIGHTS

- Pollen grains of the studied species were investigated by light and scanning electron microscope
- Using scanning electron microscopy techniques for the easy and quick identification of plant species
- Using micro-morphological (SEM) characters for the identification and species delimitations.

INTRODUCTION

The *Geranium* L., is the largest genus of Geraniaceae and contains 325 species. *Geranium* species are distributed worldwide except in lowland tropical areas (Aedo 2017). Most of the species belong to subg. *Geranium* (Aedo 2001). Traditionally the genus *Geranium* was classified into three subgenera: 1- subg. *Geranium*, 2- subg. *Erodioidea* (Picard) Yeo, and 3- subg. *Robertium* (Picard) Rouy. According to Yeo (1984), the subg. *Robertium* primary diagnostic characteristics are carpel projection of fruit discharge. This subgenus has thirty species, and these are distributed in eight sections. The subgenus, *Geranium*, is the largest subgenus containing 300 species. These 300 species were in at least ten sections. The genus is characterized by a seed-ejection fruit type in which the explosive recurvature of the awn carries the mericarp upwards in an arc, throwing the seed out (Marcussen and Meseguer 2017). The family Geraniaceae Juss., is a worldwide family and comprising additionally five genera: *Hypeocharis* J. Remy (subfam. Hypeocharitoideae), *Erodium* L'Herit ex Aiton., *Geranium* L., *Monsonia* L., *Pelargonium* L'Herit. ex Aiton and *California* being successive sisters to *Geranium* L. (subfam. Geranioideae) (Fiz et al. 2008; Weng et al. 2014). These genera have a moderate number of species than *Geranium* and had a more narrow distribution.

The characteristic five-carpelled schizocarp Geranioideae fruit with a prominent central rostrum has a great taxonomic value in this genus. The *Geranium* shows four different fruit discharge methods: carpel-projection type, *Erodium*-type, seed-ejection type, and the inoperative type (Marcussen and Meseguer 2017). These methods have been considered in the delimitation of major groups within the *Geranium*. Marcussen and Meseguer (2017) pointed that phylogenetic relationships within *Geranium* and the evolution of the different seed discharge methods are still unknown. The new classification has shifted some of the studied taxa from subg. *Robertium* to subg. *Geranium*, while a third subgenus (*Erodioidea*) has been reduced to synonymy under subg. *Geranium* (Marcussen and Meseguer 2017).

The Geraniaceae is a eurypalynous family (Baser et al. 2016). The pollen morphological features of the family have a great importance in the discrimination at the generic level (Perveen and Gaiser 1999). The pollen grains are usually isopolar with radial symmetry; generally have oblate-spheroidal shape, rarely sub-oblate; three-lobed, tri-colporate, rarely colpate and the colpi are short; the sexine is thicker than nexine, and the tectum is reticulate with densely baculate or gemmate muri or striate (Perveen and Gaiser 1999).

Various workers described the pollen morphology of different species of Geraniaceae, species delimitation and species relationships in the genus *Geranium* L. Since Bortenschlager's (1967) preliminary study on the pollen morphology of the Geraniaceae, the pollen morphology of *Monsonia* (Verhoeven and Venter 1986), *Sarcocaulon* (Verhoeven and Venter 1988), and *Erodium* (Verhoeven and Venter 1987) of the southern African Geraniaceae has been investigated. *Erodium* pollen grains of the Middle East (EI-Oqlah 1983) and north-western Europe have been studied (Stafford and Blackmore 1991). Stafford and Blackmore (1991) reviewed the pollen grains of *Geranium* species in north-western Europe. Pollen morphology of 35 taxa of the genus *Geranium* in Asia has been observed through light and scanning electron microscopy (SEM) by Park and Kim (1997). They found high variation in pollen grains size and exine sculpture (muri, lumina, excrescences distribution) among the examined taxa. Also, palynological characters were useful to establish the phylogeny within the genus. However, they are not helpful characters for separation at subgenera and sections. Perveen and Gaiser (1999) studied the pollen morphology of Pakistani Geraniaceae taxa and based on exine ornamentations and apertural type. They divided the pollen grains into three groups i.e., *Erodium cicutarium*, *Geranium himalayense*, and *Monsonia senegalensis*-types. They observed that the pollen grains were mostly oblate-spheroidal, rarely prolate-spheroidal or spheroidal, and often sub-oblate in the examined taxa. Shehata (2008) analyzed pollen grains of the Geraniaceae family from Egypt. The results showed that pollen morphological characters, i.e., pollen size, pollen shape, and aperture, were taxonomically significant at the generic level and up to some extent at the specific level. Ilcim et al. (2008) analyzed the morphological and palynological properties of *G. tuberosum*. Palynological investigation of 13 species of genus *Geranium* in Turkey by Deniz et al. (2013), did not reflect significant differences between the sections, except some differences in pollen grains of the subgenus *Geranium*. According to Deniz et al. (2013), all examined species' pollen grains were large, and exine ornamentation was reticulate-clavate; and the apertures were tri-colporate.

In Iran, the only study on pollen morphology of five *Geranium* species was assessed by Keshavarzi et al. (2016). There is no comprehensive palynological investigation of *Geranium* species in Iran. The present study aimed to use the pollen grains morphological features as a source of diagnostic characters to distinguish different Iranian *Geranium* species.

MATERIAL AND METHODS

Totally 60 populations were collected and studied from 23 taxa of *Geranium* from different habitats in Iran to explore the pollen features (Table 1). Ten individuals of each location were studied and examined for three qualitative and eight quantitative features (Table 2). For scanning electron microscopy (SEM) examinations, pollen grains were not acetolyzed according to the method of Erdtman (1960). The pollens were suspended in a drop of water for a while and then directly transferred to a metallic stubby a fine pipette, and double-sided cello tape was used. And then the pollens were sputtered in a chamber coated with gold (Sputter Coater BALTEC, SCDOOS). Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. The SEM examination was carried out on a TESCAN microscope. Pollen sculptures were described according to previous terminology (Hesse et al. 2009).

The evaluated and measured characters of the species studied of *Geranium* are summarized in Tables II. To detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. For multivariate analysis, the mean of the quantitative characters was used. Principal Components Analysis (PCA) was performed among the species to determine palynological characters useful for separating the species. In order to group the species, cluster analysis using WARD (Minimum spherical variance) methods and PCA ordination plot were performed by PAST software (Hammer et al. 2001), using Euclidean and taxonomic distance among the species was calculated (Podani 2000).

The qualitative traits were coded as binary or multi-state. Variables were systematized for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficients in the pollen data cluster analysis. Image Tool Version 3.0 (<http://ddsdx.uthscsa.edu/dig/itdesc.html>) was used to carry out the required measurements.

RESULTS

Infrageneric variation

We showed that *G. purpureum*, *G. robertianum* and *G. sylvaticum* possess type I pollen grains. Sub-genus *Geranium* had type II pollens. Species, i.e., *G. biuncinatum*, *G. mascatense* and *G. trilophum* belonging to *Trilophum* section showed the type III of pollen grains.

Factor analysis shows three factors explained more than 77% of the total observed variation in studied pol-

len grains. The first factor revealed equatorial length, pollen shape importance. Aperture condition, colpus length, and exine thickness illustrated more than 44% of the observed variation in the second factor. Principal component analysis (PCA) based on pollen grain qualitative and quantitative traits confirms the cluster analysis results by Ward's method.

General pollen grain features

The majority of *Geranium* species depicted prolate-spheroidal pollen types. For instance, *G. columbinum*, *G. collinum*, *G. sylvaticum*, *G. pratense*, *G. dissectum*, *G. linearilobum*, *G. tuberosum*, *G. kotschyi*, *G. platypetalum*, *G. gracile*, *G. ibericum*, *G. purpureum*, *G. pyrenaicum*, *G. robertianum*, *G. divaricatum*, *G. lucidum*, and *G. molle* had prolate-spheroidal shape (Figures. 2 A1, B1, C1, D1; 3 E1, F1, G1; 4 M1, O1; 5 P1, Q1; 6T1, W1, Y1, Z1; 7 X1, U1). Spheroidal pollen types were observed in *G. rotundifolium*, *G. pusillum*, *G. albanum* (Figs. 4 L1, 3 H1, 4 N1). *G. biuncinatum*, *G. mascatense*, *G. trilophum* species had sub-prolate pollen morphology (Figures. 5 R1, S1; Figures. 7, U4-U6). We found that *G. lucidum* and *G. robertianum* had spheroidal-prolate pollen grains. While *G. rotundifolium* had spheroidal pollen shape (Table II, Figures 4 L1-L2). Our statistical and microscopy analyses depicted that *G. platypetalum* had the largest (Table II, Figure. 6 Z1, Z2) and *G. pusillum* possessed smallest pollen grains. (Table II, Figures 3 H1, H2). Our observations revealed that pollen grains were generally prolate-spheroidal except *G. pusillum*, *G. rotundifolium*, and *G. albanum* (Figures 3H1-H2, Figures 4 L1-L2; N1-N2). Mean polar axis length varied from 38.55 µm (*G. pusillum*) to 104.88 µm (*G. platypetalum*), while the mean of the equatorial axis length varied from 37.55 µm (*G. pusillum*) to 105µm (*G. platypetalum*). The main colpus length varied from 12 µm (*G. molle*) to 58 µm (*G. trilophum*). P/E ratio differed from 0.89 µm (*G. albanum*) to 1.5 µm (*G. trilophum*). The main features of the investigated pollen grains are summarized in Table II. The basic ornamentation of the exine surface in the *Geranium* was reticulate-clavate, striate-rugulate, and reticulum cristatum with clavae. On the basis of differences in exine sculpturing pattern, the following 3 types are recognized: reticulate-clavate, striate-rugulate, reticulum cristatum with clavae. Most of the specimens belong to reticulum cristatum with clavae pattern.

Type I: *Geranium robertianum*- type (“reticulate-clavate”)

Species: *Geranium robertianum* (Figure 5, P3; Figure 4, O3; Figure 3, E2)

Pollen class: Tricolporate

Table 1. List of *Geranium* species in Iran their localities and voucher numbers.

No	Section	Sp.	Locality
1	<i>Dissecta</i> Yeo	<i>G. dissectum</i> L.	Guilan, Lahijan East Azerbaijan, Kaleybar, Cheshme Ali Akbar East Azerbaijan, Kaleybar, Shoj-abad Tehran, Damavand Khorassan, Kashmar-Darvaneh
2	<i>Geranium</i>	<i>G. columbinum</i> L.	Guilan, Siahkal, Ezbaram; Guilan, Langerud, chaff; Guilan, Bandar-e Anzali; Tehran, Damavand; Khorassan, Kashmar-darvaneh
3		<i>G. rotundifolium</i> L.	Tehran, Tuchal Mazandaran, Kandovan-Siahbisheh East Azerbaijan, Kaleybar, Shoj-abad
4		<i>G. collinum</i> Stephan ex Willdenow	Mazandaran, Tonekabon-Jannat Rudbar Hamedan, 20km s of Nahavand
5		<i>G. sylvaticum</i> L.	East Azerbaijan, Kaleybar, Cheshme Ali Akbar
6		<i>G. pratense</i> L.	East Azerbaijan, Kaleybar, Shoj-abad Mazandaran, 40 km Tonekabon to Janat Abad
7	<i>Tuberosa</i> (Boiss.) Reiche	<i>G. platypetalum</i> Fisch. & C. A. Mey.	East Azerbaijan, Kaleybar Razavi Khorasan, Kashmar Tehran, Damavand
8		<i>G. ibericum</i> Cav.	Tehran, Damavand Kordestan, Sanandaj Khorassan, Kashmar-Darvaneh
9		<i>G. gracile</i> Ledeb. ex Nordm.	Mazandaran, Noshahr, Kheyroud Kenar Forest Kerman, Lalehzar, Baghabad
10		<i>G. linearilobum</i> DC.	Tehran, Firuz kuh Mazandaran, 40 km Tonekabon to janat abad
11		<i>G. kotschyi</i> Boiss.	Alborz, Karaj- Qazvin Tehran, Desin
12		<i>G. tuberosum</i> L.	East Azerbaijan, Kaleybar Cheshme Ali Akbar, Tehran, Tuchal
13	<i>Batrachioidea</i> W.D.J. Koch	<i>G. molle</i> L.	East Azerbaijan, Kaleybar, Shojabad East Azerbaijan, Kaleybar, Cheshme Ali Akbar Hamedan, 20km s of Nahavand
14		<i>G. pyrenaicum</i> Burm. f.	East Azerbaijan, Kaleybar, roadside East Azerbaijan, Kaleybar, Cheshme Ali akbar East Azerbaijan, Kaleybar, Shojabad East Azerbaijan, Babak fort Tehran, Damavand
15		<i>G. pusillum</i> L.	East Azerbaijan, Kaleybar, roadside East Azerbaijan, Kaleybar Cheshme Ali Akbar East Azerbaijan, Kaleybar, Shojabad Hamedan, 20km s of Nahavand Tehran, Damavand
16	<i>Ruberta</i> Dumort.	<i>G. purpureum</i> Vill.	East Azerbaijan, Kaleybar, Cheshme Ali Akbar Guilan, Gole roddbar Guilan, Gole roddbar, roadside Guilan, Jirandeh Khorassan, Kashmar-darvaneh Kerman, Lalehzar, Baghabad
17		<i>G. robertianum</i> L.	Guilan, Gole roddbar Esfahan, 50 km Delijan Tehran, Damavand
18	<i>Divaricata</i> Rouy	<i>G. albanum</i> M. Bieb.	Guilan, Sangar, roadside Guilan, Lahijan Guilan, Jirandeh Mazandaran, Siah bisheh to Chalus Golestan, Ramian

No	Section	Sp.	Locality
			Esfahan, 50 km delijan Khorasan, Birjand Tehran, Darakeh Hamedan, 20km s of Nahavand
19		<i>G. divaricatum</i> Ehrh.	East Azerbaijan, Kaleybar Tehran, Darband Khorasan, Birjand Tehran, Darakeh Kerman, Lalehzar, Baghabad
20	<i>Lucida</i> R. Knuth	<i>G. lucidum</i> L.	East Azerbaijan, Kaleybar Cheshme Ali Akbar
21	<i>Trilophum</i> Yeo	<i>G. trilophum</i> Boiss.	Hormozgan, Amani village, Kushk-e Nar Rural Hamedan, 20km s of Nahavand
22		<i>G. mascatense</i> Boiss.	Hormozgan, Qeshm, Bakho Mountain
23		<i>G. biuncinatum</i> Kokwaro	Khuzestan, Shushtar- Masjed soleyman

Table 2. Evaluated characters of pollen grains in *Geranium* species studied (values M ± SD μm). M- Mean value; SD- Standard deviation. Aperture: At the same level =2, protruding = 1; Pollen shape: prolate-spheroidal =1, spheroidal= 2, subprolate=3; Exine ornamentation type: reticulate-clavate=1, striate-rugulose =2, reticulum cristatum with clavae= 3

Taxon	Polar axis length (μm)	Exine thickness (μm)	Equatorial axis length (μm)	P/E ratio	Apocolpium length (μm)	Mesocolpium length (μm)	Colpus length (μm)	Pore diameter (μm)	Aperture	Pollen shape	Exine ornamentation type
<i>G. dissectum</i>	55.67±2.1	4.5	55.87±3.3	1.05±0.05 ±0.03	23.67	35.66	36.44	6.87	1	1	3
<i>G. columbinum</i>	62.76±3.4	5.88	62.66±2.9	1.00±0.03	35.87	52.54	22	15	1	1	3
<i>G. rotundifolium</i>	66.44±3.8	5.87	65.54±2.3	1.01±0.02	37.22	35.77	25	8.5	1	2	3
<i>G. collinum</i>	86.5±2.8	5.34	77.32±4.8	1.11±0.04	48.55	34.33	55	12	1	1	3
<i>G. platypetalum</i>	103.32±4.1	4.45	101±2.7	1.01±0.05	68.87	54.77	33	24	1	1	3
<i>G. sylvaticum</i>	78.44±2.9	5.87	75.55±1.4	1.04±0.04	54.22	42.32	25	7	2	1	1
<i>G. pretense</i>	75.55±3.7	5.88	66.77±2.4	1.06±0.02	55.77	60.22	32	7	2	1	3
<i>G. ibericum</i>	81.44±3.9	4.34	78.33±3.6	1.03±0.05	47.22	54.55	28	13	2	1	3
<i>G. gracile</i>	76.44±2.4	5.45	74.56±2.9	1.02±0.03	37.55	53.77	27	16	1	1	3
<i>G. linearilobum</i>	71.88±2.8	5.65	61.55±4.2	1.13±0.06	38.43	38.34	39	14	1	1	3
<i>G. kotschyi</i>	63.66±4.1	5.8	59.55±4.5	1.06±0.03	42.66	35.77	39	11	1	1	3
<i>G. tuberosum</i>	81.55±2.8	5.3	75.66±2.2	1.08±0.02	48.77	46.44	33	12	1	1	3
<i>G. molle</i>	49.44±1.6	4.55	47.34±1.3	1.04±0.04	31	35	13	10	1	1	3
<i>G. pyrenaicum</i>	64.22±2.8	4.3	59.34±2.2	1.08±0.05	33	30	23	4	2	1	3
<i>G. pusillum</i>	39.65±1.4	3.7	38.22±5.3	1.02±0.03	26-27	24	15.66	7.87	1	2	3
<i>G. purpureum</i>	58.27±1.9	4.2	54.55±2.5	1.1±0.04	37.85	29.01	27.68	7.4	2	1	1
<i>G. robertianum</i>	61.76±4.1	4.7	51.54±1.8	1.02±0.02	30	22-28	21-22	7-8	2	1	1
<i>G. albanum</i>	63.44±2.5	5.45	66.34±2.6	0.95±0.03	38.76	39.44	26.33	14	1	2	3
<i>G. divaricatum</i>	59.57±3.7	4.55	51.66±3.9	1.13±0.01	33.66	35.33	32	9	1	1	3
<i>G. lucidum</i>	58.55±5.2	4.76	49.65±3.3	1.09±0.04	33.77	25.44	32	6	1	1	3
<i>G. mascatense</i>	64.66±6.1	5.88	60.33±2.9	1.1±0.05	23.77	47.55	50	14	1	3	2
<i>G. trilophum</i>	65.44±2.6	5.34	54.45±2.5	1.2±0.03	21.45	43.55	56	9	1	3	2
<i>G. biuncinatum</i>	63.25±5.1	4.35	63.76±7.9	1.1±0.03	22.65	45.98	59	12	1	3	2

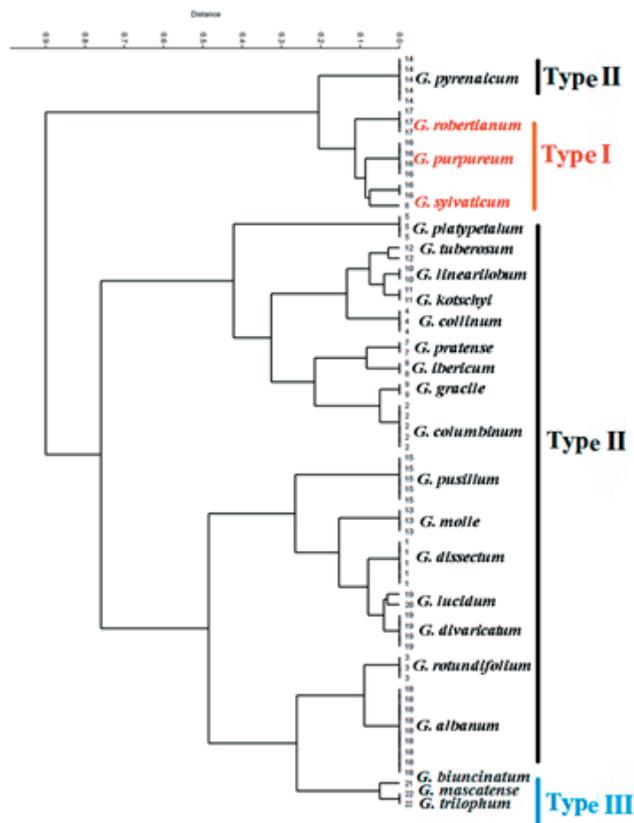


Figure 1. Ward clustering of *Geranium* species based on observed pollen data.

Shape: Prolate-spheroidal

Apertures: Ectoaperture-colpi small

Ornamentation: Tectum coarsely reticulate with clavae
 Measurements: polar length (P) (56-75 μm), equatorial diameter (E) (55-78 μm), colpi (20-25 μm) in diameter. Mesocolpium (20-40 μm). Apocolpium (33-59 μm). Exine 4.9-5.9 μm

Species included: *G. robertianum*, *G. purpureum*, *G. sylvaticum*

Type II: *Geranium molle* -type (“reticulum cristatum with clavae”)

Species: *G. molle* (Figures 2, A3, B3, C3, D3; Figures 3, F3, G3, H3; Figures 4, L3, M3, N3; Figure 5, Q3; Figures 6, T3, W3, Y3, Z3; Figures 7, X3, U3)

Pollen class: Tricolporate

Shape: Prolate- Spheroidal

Apertures: Ectoaperture - colpus short (approx. 1/5 to 1/8 of polar axis)

Ornamentation: Tectum reticulum cristatum with clavae
 Measurements: Polar length (P) (38-105 μm), Equatorial diameter (E) (38-105 μm), colpi (10-58 μm) in diameter. Mesocolpium (22-67 μm). Apocolpium (22-69 μm) . Exine 3.7-5.8 μm

Species included: *G. gracile*, *G. ibericum*, *G. pyrenaicum*, *G. divaricatum*, *G. lucidum*, and *G. molle*, *G. pusillum*; *G. columbinum*, *G. collinum*, *G. pratense*, *G. dissectum*, *G. linearilobum*, *G. tuberosum*, *G. kotschyi*, *G. platypetalum*

Pollen type III: *Erodium*-type (“striate-rugulose”)

(Figures. 5, R3, S3; Figures. 7, U4-U6)

Pollen class: Tricolporate

Shape: Sub- Prolate

Apertures: Ectoaperture-colpi large

Ornamentation: Tectum striate-rugulose

Measurements: Polar length (P) (68-69 μm), Equatorial diameter (E) (50-64 μm), colpi (53-59 μm) in diameter. Mesocolpium (40-49 μm). Apocolpium (20-25 μm). Exine 5.5-5.9 μm

Species included: *G. mascatense*, *G. trilophum*, *G. biuncinatum*

DISCUSSION

Geranium species are relatively challenging to study due to the overlapping of morphological characters (Aedo and Pando 2017; Ji et al. 2020; Wang et al. 2020; Sun et al. 2021). Therefore, an attempt was carried out to investigate pollen grains of *Geranium* species by scanning electron microscopy. Our approach also included the usage of principal component analysis to verify our findings. The current study showed that *Geranium* species have eurypalynous pollens morphology. Present results corroborate with a previous study conducted on the Geraniaceae family (Baser et al. 2016). Most of the species showed prolate-spheroidal pollen types. This pattern of pollen type is previously reported in the Geraniaceae family (Baser et al. 2016). We observed monad, isopolar, radial symmetry, tricolporate, and short linear colpi pollen grains in *Geranium* species. Similar pollen types have been reported in Iran while working on five *Geranium* species (Keshavarzi et al. 2016). Our result recorded spheroidal-prolate pollen grains in *G. lucidum* and *G. robertianum* species. However, previously oblate-spheroidal pollen grains were reported in *G. lucidum*, *G. robertianum* (Perveen and Gaisar 1999). We argue that such a difference in pollens is due to sampling site and habitat (Brodschneider et al. 2019). Annual or biennial species, i.e., *G. lucidum*, *G. pusillum*, *G. molle*, *G. dissectum*, *G. rotundifolium* showed the smaller pollen grains (from 39 to 66 μm). *G. collinum*, *G. sylvaticum*, *G. pratense*, *G. platypetalum*, *G. gracile*, and *G. ibericum* had larger (63 to 103 μm) pollen grains. Such variation in pollen grains has been described in the past (Aedo 2001; Aedo et al. 2007; Jurgens et al. 2012; Hao et al. 2020).

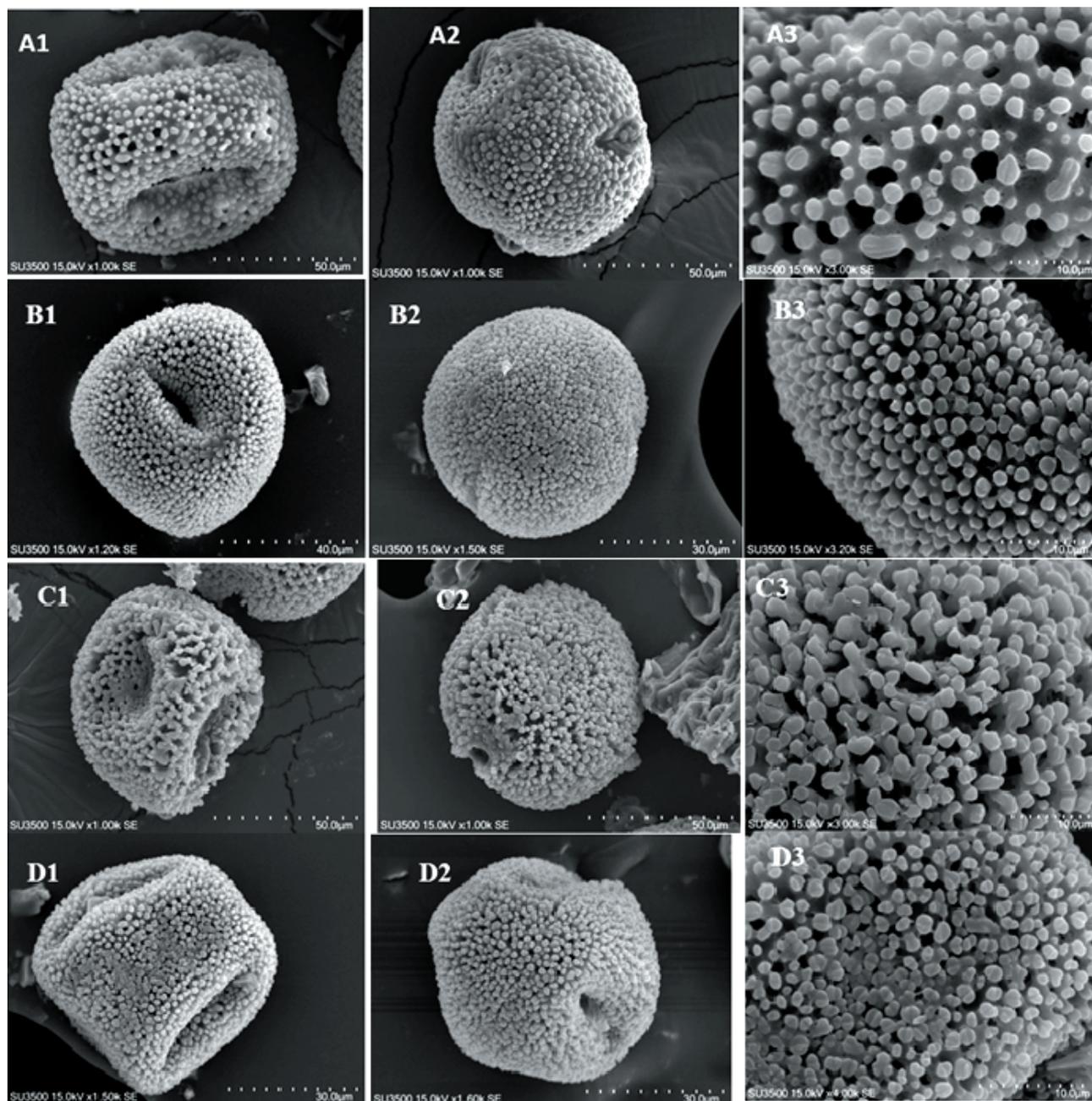


Figure 2. Pollen micrographs of *Geranium* species: A1-A3: *G. collinum*, B1-B3: *G. columbinum*, C1-C3: *G. pratense*, D1-D3: *G. dissectum*; A1, B1, C1, D1 – equatorial view; A2, B2, C2, D2 - polar view; A3, B3, C3, D3 -exine sculpture.

Clustering results revealed pollen grain type III in section *Trilopha*. Henceforth, type III pollen is the diagnostic feature to identify *Trilopha* Section.

Deniz et al. (2013) observed the exine ornamentation to be reticulate-clavate in *Geranium* taxa. While in the present study, exine ornamentations were reticulate-clavate, striate-rugulose, reticulum cristatum with cla-

vae. Most of the specimens belong to reticulum cristatum with clavae pattern. The pollen of all species of *Geranium* sect. *Trilopha* is of the Erodium-type (Aedo et al. 2016), which is congruent with the present findings. The striate ornamentation is considered a diagnostic feature to differentiate *Trilopha* section. This distinguishing feature may support the close relationship between *G.* sect.

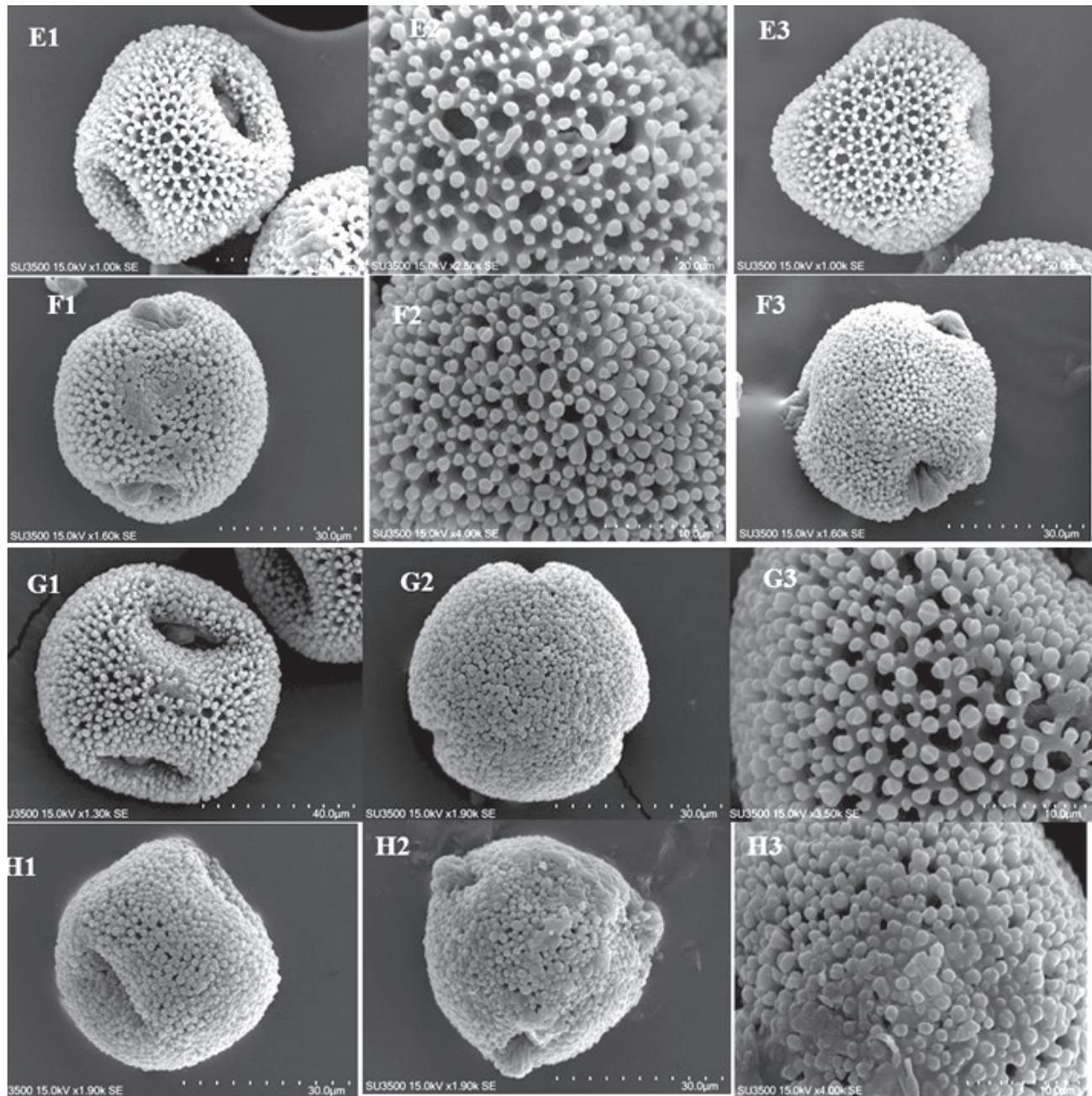


Figure 3. Pollen micrographs of *Geranium* species: E1-E3: *G. sylvaticum*, F1-F3: *G. molle*, G1-G3: *G. pyrenaicum*, H1-H3: *G. pusillum*; E1, F1, G1, H1 – equatorial view; E2, F2, G2, H2 – polar view; E3, F3, G3, H3 – exine sculpture.

Polyantha and *G. sect. Trilopha*.

The scanning electron microscopy (SEM) technique has a crucial role in observing the minute biological structure (Ullah et al. 2019). Several taxonomic and plant systematics questions are also addressed through the application of SEM. For instance, seed morphology in Caryophyllaceae was studied by scanning electron

microscopy to identify Caryophyllaceae species correctly (Ullah et al. 2019; Zou et al. 2019). Besides this, detailed plant morphology, anatomy, and pollens in *Spergula fallax* and *Spergula arvensis* revealed the significance of SEM to resolve taxonomic complexity (Ullah et al. 2018). Authors developed identification key and distinguishing features of *Spergula fallax* and *Spergula arvensis* species

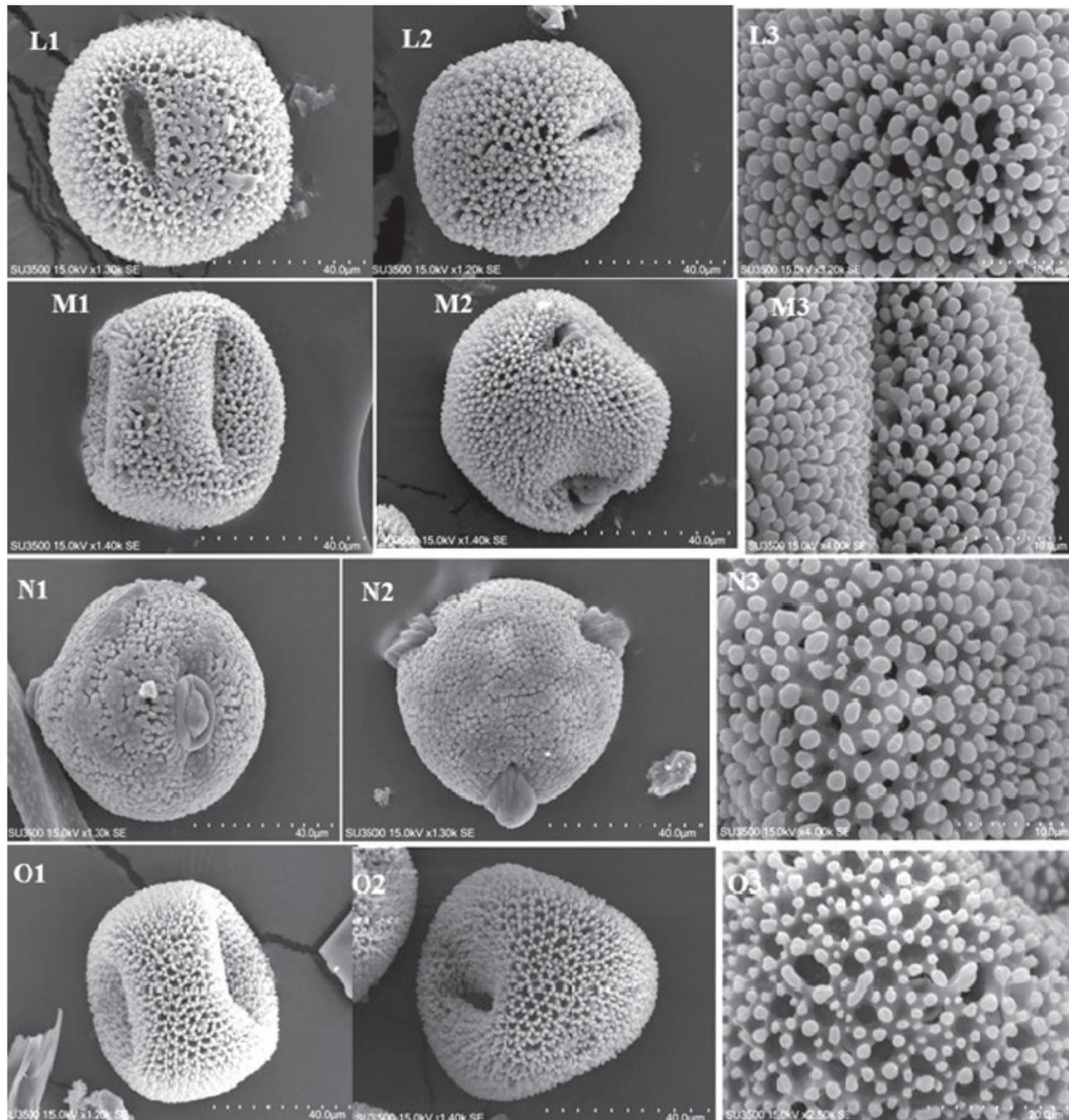


Figure 4. Pollen micrographs of *Geranium* species: L1-L3: *G. rotundifolium*, M1-M3: *G. lucidum*, N1-N3: *G. albanum*, O1-O3: *G. purpureum*; L1, M1, N1, O1 – equatorial view; L2, M2, N2, O2 - polar view; L3, M3, N3, O3 -exine sculpture.

(Ullah et al. 2018). This clearly shows the utilization of new techniques to solve complex questions in plant systematics and biology (Pathan et al. 2010; Ullah et al. 2018; Ullah et al. 2019).

Cluster revealed groupings of *G. uncinatum*, *G. mascatense*, *G. trilophum* into the *Trilopha* section. Similar

results at the section level was reported. Phylogenetic relationship based on chloroplast (*rbcL*, *trnL-trnF*) and nuclear (ITS) DNA sequences highlighted the grouping of *G. uncinatum*, *G. mascatense* into the *Trilopha* section (Marcussen and Meseguer 2017). The phylogenetic tree proved the Geraniaceae family as a monophyletic.

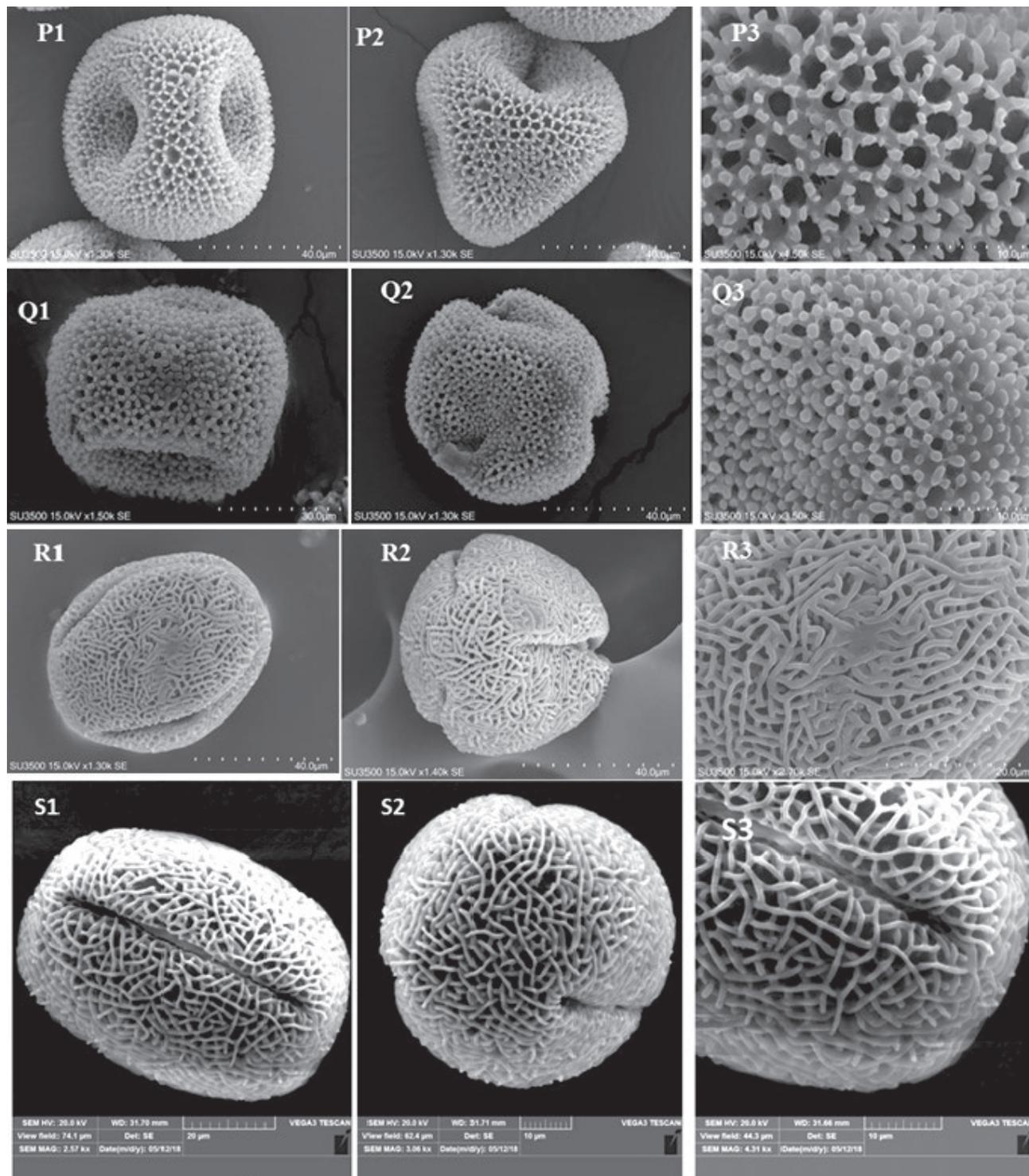


Figure 5. Pollen micrographs of *Geranium* species: P1-P3: *G. robertianum*, Q1-Q3: *G. divaricatum*, R1-R3: *G. trilophum*, S1-S3: *G. mascatense*; P1,Q1, R1, S1 – equatorial view; P2,Q2, R2, S2 – polar view; P3,Q3, R3, S3 –exine sculpture.

The discrepancy in the phylogenetic results obtained by different molecular markers may be the reason for unresolved species relationship in the genus *Geranium*. Evo-

lutionary relationships between *Geranium*, *Erodium*, and are unclear because of the low support for the alternative topologies inferred from both markers (*trnL-F* and *rbcl*)

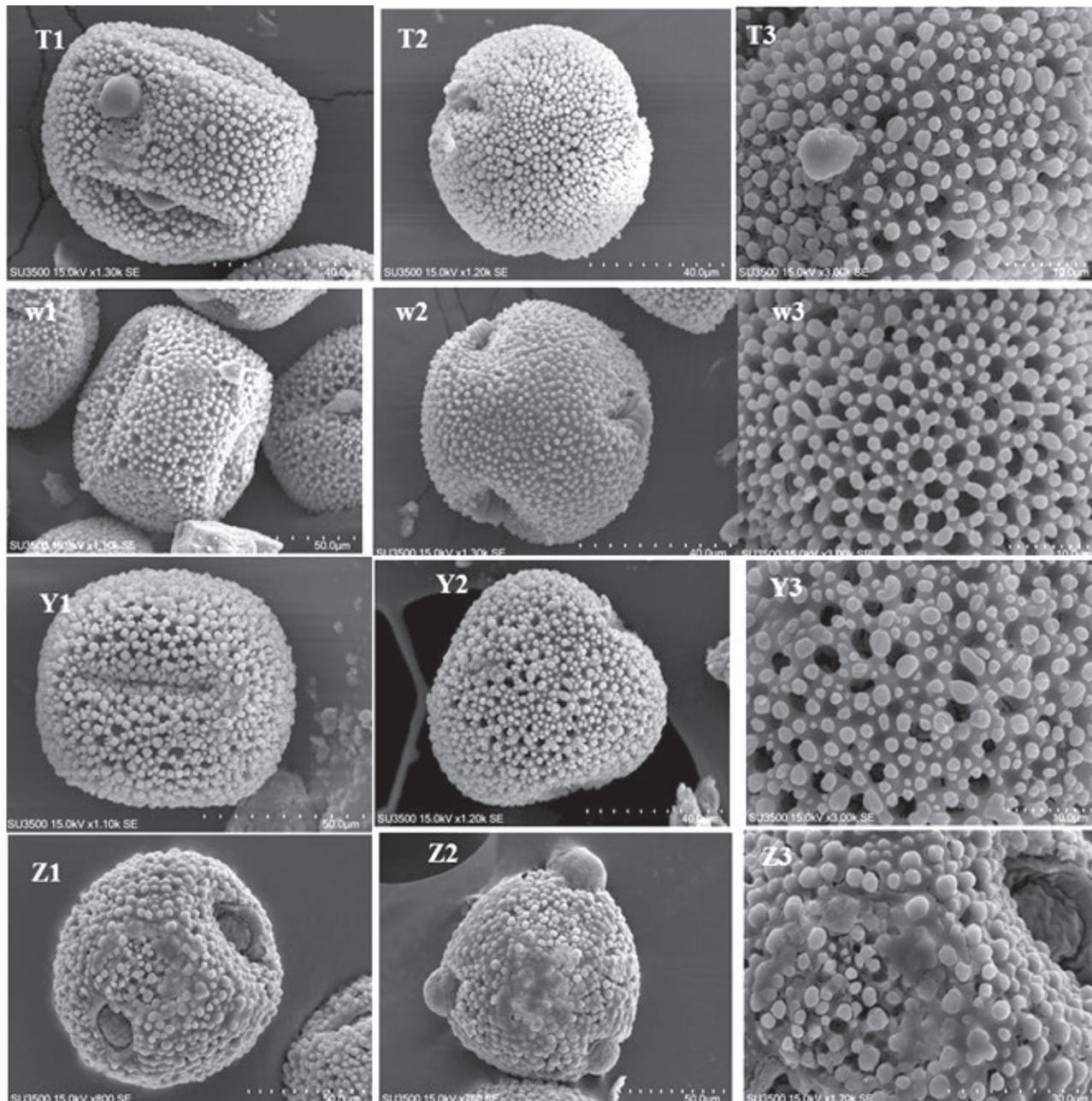


Figure 6. Pollen micrographs of *Geranium* species: T1-T3: *G. kotschyi*, W1-W3: *G. linearilobum*, Y1-Y3: *G. tuberosum*, Z1-Z3: *G. platypetalum*; T1,W1,Y1, Z1 – equatorial view; T2,W2,Y2, Z2 – polar view; T3,W3,Y3, Z3 – exine sculpture.

(Fiz et al. 2008). Inter-simple sequence repeat (ISSR) markers separated *Geranium* species (Esfandani-Bozchaloji et al. 2018). The present study showed that pollen features are not capable of distinguishing different sections in *Geranium*. Two subgenera elements were mixed.

Nonetheless, we found and linked the diagnostic character (Pollen type III) of the *Trilopha* section. Future studies may incorporate phylogenetic data and

chemotaxonomy methods to decipher genus and species level evolutionary relationship in *Geranium*. Linking the morphological and phylogenetic traits may further aid our apprehension in plant evolution and systematics (Cohen 2011).

In this research, we studied different palynological characteristics of the genus *Geranium*. Various characters were crucial for the taxonomic identification of the

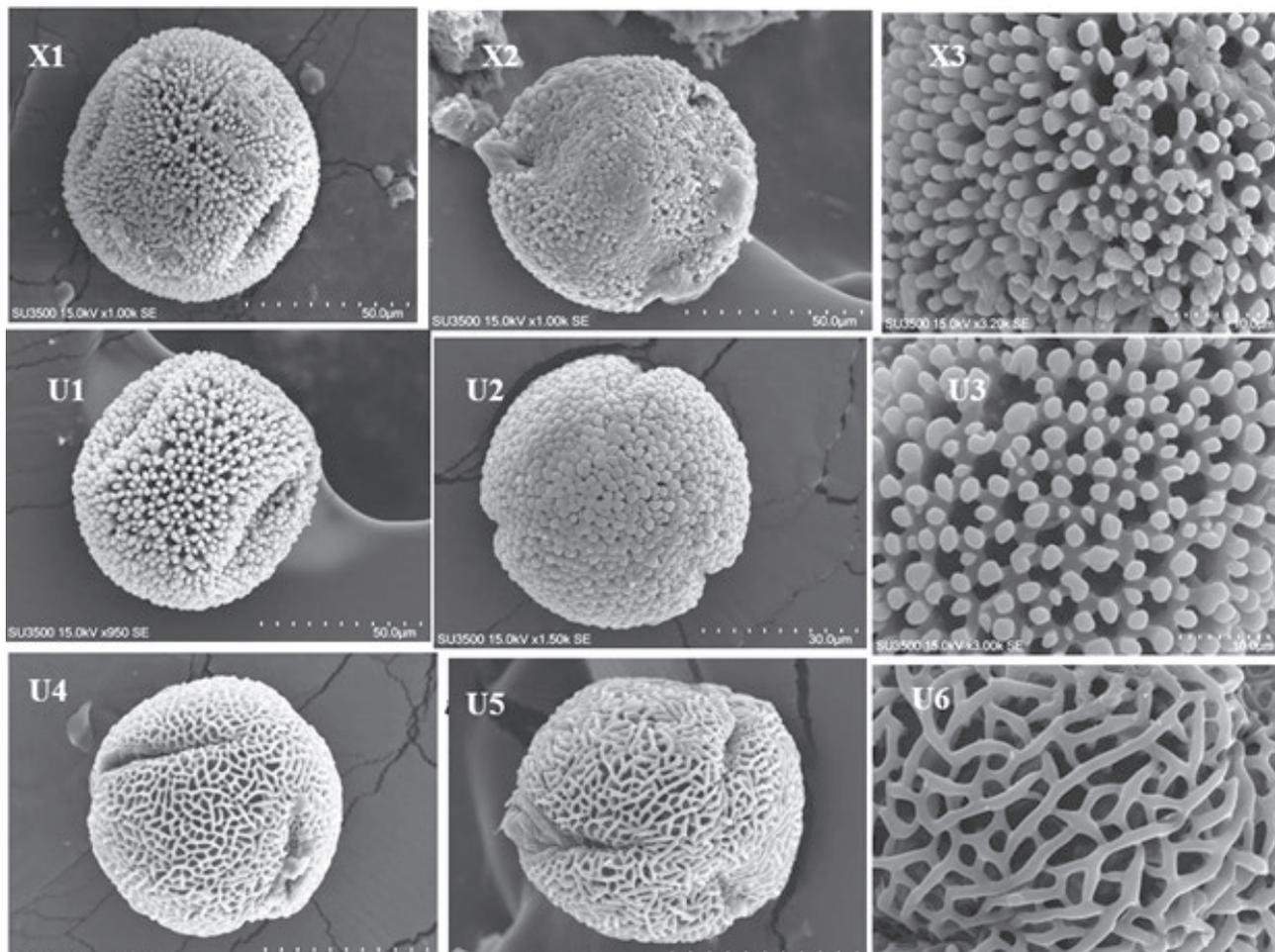


Figure 7. Pollen micrographs of *Geranium* species: X1-X3: *G. ibericum*, U1-U3: *G. tuberosum*, U4-U6: *G. biuncinatum*. X1,U1, U4 – equatorial view; X2, U2, U5- polar view; X3, U3, U6-exine sculpture.

species delimitations. This study could serve better to understand the pollen morphology of *Geranium* species in Iran. The pollen characters studied here were useful for the taxonomic identification of the species of the genus.

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