

## POLLEN MORPHOLOGY OF SOME GERANIUM SUBGENUS ROBERTIUM SPECIES OF IRAN

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**Abstract.** *Geranium* (Geraniaceae) comprises more than 23 annual or perennial species in Iran. There is no study in Iran with pollen morphology emphasize. The main aim of this study is to find diagnostic pollen characters in studied species. Totally 40 accessions of five species (*G. albanum*, *G. molle*, *G. purpureum*, *G. mascatense* and *G. pusillum*) were collected. Pollen grains were studied by use of light and Scanning electron microscopy. To reveal the species relationships different multivariate statistical methods were used. The pollen grains were monad, isopolar, radially symmetric and of spheroid, prolate-spheroid or oblate-spheroid classes. The main ornamentation type was clavate, however reticulate but striate was also observed. All sections are clearly separated by their pollen features except of *Batrachioidea* which show confusion with *Ruberta*. Species relationship is discussed.

**Key words:** *Geranium*, *Robertium*, pollen morphology, Iran

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### Introduction

*Geranium* L. (Geraniaceae) comprises 423 species in the world which are grouped in 3 subgenera and 18 sections (AEDO *et al.* 1998). This genus contains more than 23 species in Iran. These are annual or perennial plants of different habitats in Iran (SCHONBECK-TEMESY 1970).

YEO (1984) was the first who divided *Geranium* into three subgenera based on fruit discharge mechanism. In seed ejection type which is in concordance with subgenus *Geranium*, a single seed is actively discharged by the explosive recurvature of the awn, which remains together with the mericarp attached to the columella. The carpel projection type which is observed in subgenus *Robertium* (Picard) Rouy differs, the whole mericarp containing the seed is dispersed and awn remains with columella (AEDO *et al.* 1998). The fruit discharge method in subgenus *Erodioidea* (Picard) Yeo is identified as “*Erodium*-type”. In this case the mericarp, including the coiled awn, is propelled over a short distance.

In *Geranium* subgen. *Robertium* there are 8 sections and totally 30 species in the world. Pollen grains of 35 Asian *Geranium* species were previously studied by PARK &

KIM (1997). They used light microscopy and scanning electron microscopy to evaluate the classification and phylogenetic aspects of studied taxa. They found high variation in pollen size and exine sculpture.

Geraniaceae is a eurypalynous family which pollen grains for the first time have been studied by BORTENSCHLAGER (1967). Later VERHOEVEN & VENTER (1986), STAFFORD & BLACKMORE (1991), PERVEEN & GAISER (1999), and SHEHATA (2008) had studied pollen grains of different taxa in this family. SHEHATA (2008) pointed to different exine sculpture as reticulate- striate, gemmate- reticulate and reticulate in Geraniaceae. He believed that these features are of diagnostic importance.

In subg. *Geranium*, pollens are larger than in subg. *Robertium* and *muri* are more elaborated with fine lumens which strengthen the probability of reverse evolution in Asian elements of *Geranium*. There is no record of palynological study of *Geranium* species in Iran. The main aim of present study is to use the pollen grains features as a source of diagnostic characters to distinguish different *Geranium* species of subgenus *Robertium*.

## Material and methods

The material used for this study was collected from wild populations and herbarium specimens (Tab. 1). The observations were made with an Olympus DP12 light microscopy, without any pretreatment. The measurements were based on at least 20 pollen grains per population. For SEM studies, the pollen grains suspended in a drop of water were directly transferred by a fine pipette to a metallic stub using double sided cello tape and then coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, 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. The terminology of HESSE *et al.* (2009) for pollen sculptures was followed.

The characters of pollen grains of the studied *Geranium* species are summarized in Tabs 2 & 3. In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, we have used cluster analysis and principal component analysis (PCA) (INGROUILLE 1986). For multivariate analysis, the mean of the quantitative characters was used. Qualitative characters were coded as binary or multistate. Variables were standardized for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of pollen data. In order to determine the most variable characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 20 and PAST ver. 2.17c (2013) softwares were used for statistical analysis.

## Results

The pollen grains of studied *Geranium* species are monad, isopolar and radially symmetric. They are of spheroid, prolate-spheroid or oblate-spheroid class. Light

micrographs of pollen grains were similar (Fig. 1), therefore scanning electron micrographs were studied to find more differences. All studied taxa were tricolporate. The colpi were short and linear. The basic ornamentation of the studied *Geranium* species was clavate-reticulate or baculate-reticulate, but striate ornamentation was also observed (Figs 2 & 3).

*G. albanum* showed two parted wart-like sculptures while *G. mascatense* showed very different exine sculpture in studied taxa with striate type of ornamentations.

In order to define the diagnostic value of pollen grains in species delimitations in studied *Geranium* species, cluster analysis by Ward's method were performed on the base of 14 qualitative and quantitative features (Fig. 4). As studied species represent each of the sections, it is evident in cluster pattern that all sections are clearly separated except *Batrachioidea* which show confusion with *Ruberta*. *G. mascatense* and *G. albanum* comprise a separate cluster with more similarity in their pollen features.

Factor analysis revealed that there were three factors provided more than 78% of total observed variation in studied pollen grains. Studying the component matrix for each factors it was evident that colpus length and equatorial shape are most important features in the first factor and aperture orientation, while homogeneity of pollen sculpture and exine thickness – in the second factor. In the third factor only equatorial length is responsible for more than 19% of observed variation. PCO based on qualitative and quantitative features of pollen grains (Fig. 5) confirms the results of cluster analysis by Ward's method.

## Discussion

Analysis has shown that *G. albanum* had the largest, while *G. molle* had the smallest pollen grains (Tab. 3). Although DENIZ *et al.* (2013) believed that there was no significant difference between the sections in terms of pollen grains, but studied species of present

**Tab. 1.** Vouchers of *Geranium* species used in this study. Vouchers are deposited at Herbarium of Alzahra University.

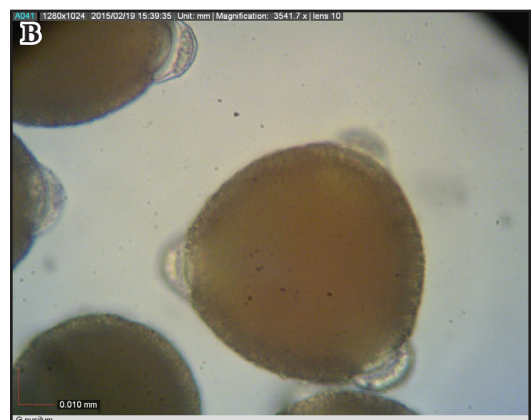
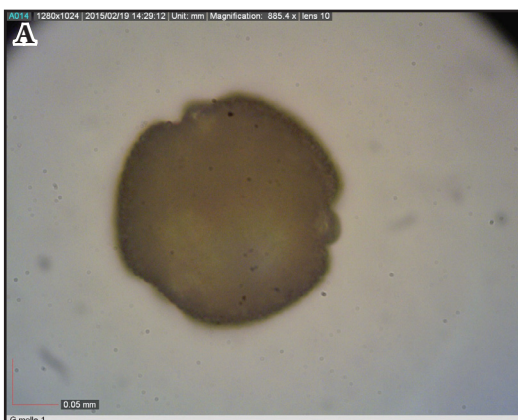
Section	Species	Voucher specimen		
<i>Batrachioidea</i> W.D.J.	<i>G. molle</i> L.	1. Mazandaran: Chalous, Shariatabad Village, Nataj 350AUH		
		2. Tehran: Damavand to Roudehen, Veskareh Village, Ghasemian 352AUH		
		3. Mazandaran: Sari to Ghaemshahr, Seydi 353AUH		
		4. Golestan: Gorgan, Naharkhoran Jungle, Soleimani 354AUH		
		5. Mazandaran: Ghaemshahr, Fasahat & Rezaii 355AUH		
		6. Guilan: Saravan Forest, Mohamadjani, 356 AUH		
		7. Mazandaran: Babolsar, Sari road km 5, Rezapour 357AUH		
		8. Mazandaran: Kelardasht, Bayat 258AUH		
		9. Golestan: Gorgan, Motalebi 359AUH		
		10. Khuzestan: Dezfuoil, Near Dez river, Bagheri 360AUH		
		11. Mazandaran: Ghaemshahr, Jamshidi 361AUH		
		12. Guilan: Lahijan district, Pedram 362AUH		
		13. Mazandaran: Ghaemshahr, Talar Forest, Cheniani 363AUH		
		14. Mazandaran: Babolsar, Sari to Babolsar road, km 5, Rezapour 364AUH		
		15. Mazandaran: Amol, Siahbishe Forest, Behzadifar & Pourabdollah 365AUH		
		16. Guilan: Astara, Heyran, Sayadlar, Behzadifar & Pourabdollah 366AUH		
<i>G. pusillum</i> L.		17. Mazandaran: Javaherdeh, Mosaferi 367AUH		
		18. Guilan: Jirandeh, Keshavarzi 368 AUH		
		19. Guilan: Khalesar, Behzadifar 369 AUH		
<i>Divaricata</i> Rouy	<i>G. albanum</i> M. Bieb.	20. Golestan: Ziarat, Pakravan 370AUH		
		21. Guilan: Masoule, Alebouyeh-ansari 371AUH		
		22. Mazandaran: Amol, Siabishe to Chalous road, Behzadifar & Pourabdollah 373AUH		
		23. Mazandaran: Jirandeh, Keshavarzi 372AUH		
		<i>Trilopha</i> Yeo	<i>G. mascatense</i> Boiss.	24. Fars: Kazeroun, Keshavarzi, 375AUH
				25. Khuzestan: Dezful, Keshavarzi, 376AUH
<i>Ruberta</i> Dumort.	<i>G. purpureum</i> Vill.	26. Guilan: Lahijan District, Pedram 377AUH		
		27. Mazandaran: Ramsar, Javaherdeh, Gholizadeh 378AUH		
		28. Guilan: Langeroud, Mehrvarz 379AUH		
		29. Mazandaran: Ramsar, Tavakoli 380AUH		
		30. Mazandaran: Ramsar, Irani 381AUH		
		31. Mazandaran: Chalous, Shariat abad Village, Nataj 382AUH		
		32. Golestan: Minodasht, Alfajr Village, Habibi 383AUH		
		33. Guilan: Anzali, Mohamadjani 384AUH		
		34. Tehran: Darband, Baghaiipour 385AUH		
		35. Tehran: Darband, Mohamadjani 386AUH		
		36. Tehran: Darband, Keshavarzi 387AUH, 388AUH		
		37. Golestan: Gorgan, Ghorogh forest, Mosaferi 389AUH		
38. Guilan: Baharestan forest, Behzadifar & Pourabdollah 390AUH				
39. Guilan: Gisom forest, Donya jan to Kharmakin, Behzadifar & Pourabdollah 391AUH				
40. Mazandaran: Siahbishe to Chalous, Behzadifar & Pourabdollah 392AUH				

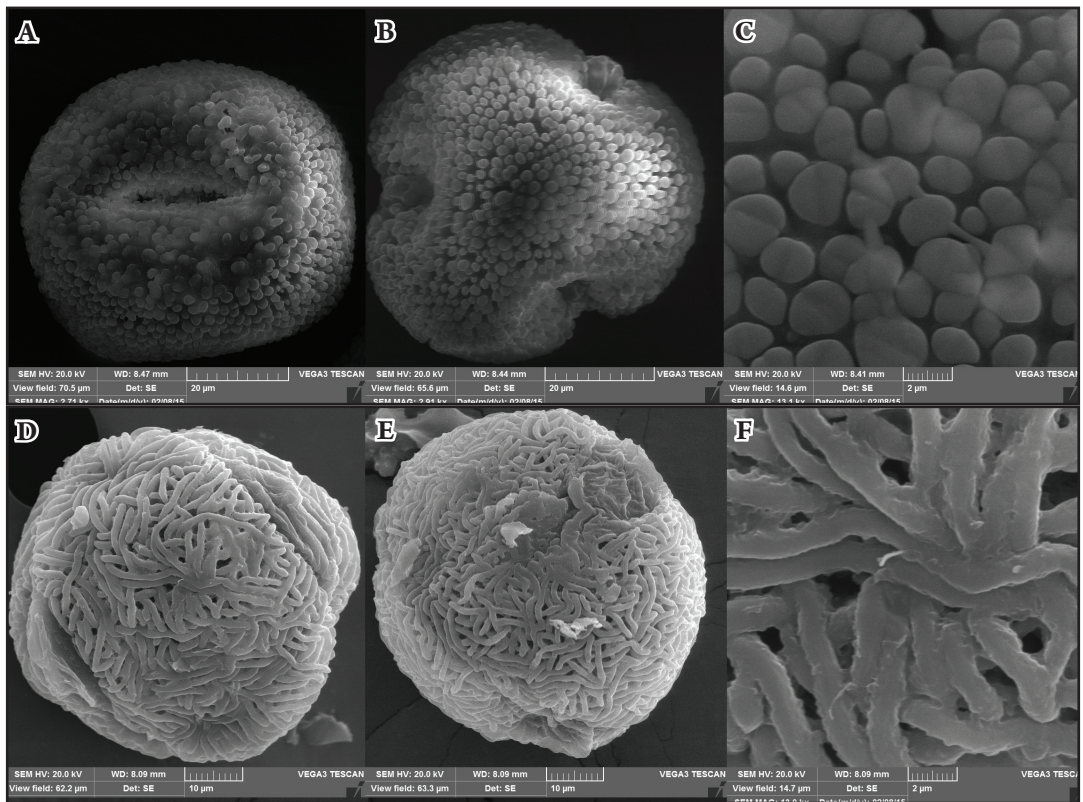
**Tab. 2.** Studied pollen features in *Geranium* species of Iran.

Characters	Characters
Polar axis length	Exine thickness
Equatorial axis length	Nr of aperture
Apocolporium	Colpi length
Mesocolporium	Pore diameter
Qualitative characters	State of characters
Exine ornamentation type	Clavate-baculate-reticulate = 0, striate = 1
polar outline	Triangular = 0, orbicular = 1
Equatorial outline	Quadrangular = 0, rounded = 1, elliptic = 2
Aperture orientation	At the same level = 0, sunken = 1
Exine homogeneity	Present = 0, absent = 1
Pollen shape	Prolate-spheroid = 0, oblate-spheroid = 1, spheroid = 2

**Tab. 3.** Evaluated characters of pollen grains in studied *Geranium* species. \* – codes are mentioned in Tab. 2.

Character	<i>G. albanum</i>	<i>G. mascatense</i>	<i>G. molle</i>	<i>G. purpureum</i>	<i>G. pusillum</i>
Polar axis	66.13	61.67	51.54	62.02	52.7
Equatorial length	61.6	61.54	48.76	58.85	59.08
Exine thickness	0.002	0.005	0.005	0.004	0.006
Colpus length	41.23	59.68	25.83	18.68	21.76
Pore diameter	16.48	18.47	11.87	7.42	11.02
Apocolpium length	32.185	33.09	33.07	42.85	42.46
Mesocolpium length	34.16	39.04	37.95	38.01	21.31
Ornamentation *	1	2	1	1	1
Homogeneity of pollen sculpture *	1	1	1	1	1
Polar shape *	1	2	2	2	1
Equatorial shape *	1	1	1	2	3
Aperture orientation *	1	2	2	1	1
Pollen shape *	0	2	0	0	1

**Fig. 1.** Light micrographs of pollen grains in studied *Geranium* species: **A** – *G. molle*; **B** – *G. pusillum*.



**Fig. 2.** Pollen micrographs of *Geranium* species: **A-C** – *G. albanum*; **D-F** – *G. mascatense*. **A, D** – equatorial view; **B, E** – polar view; **C, F** – exine sculpture.

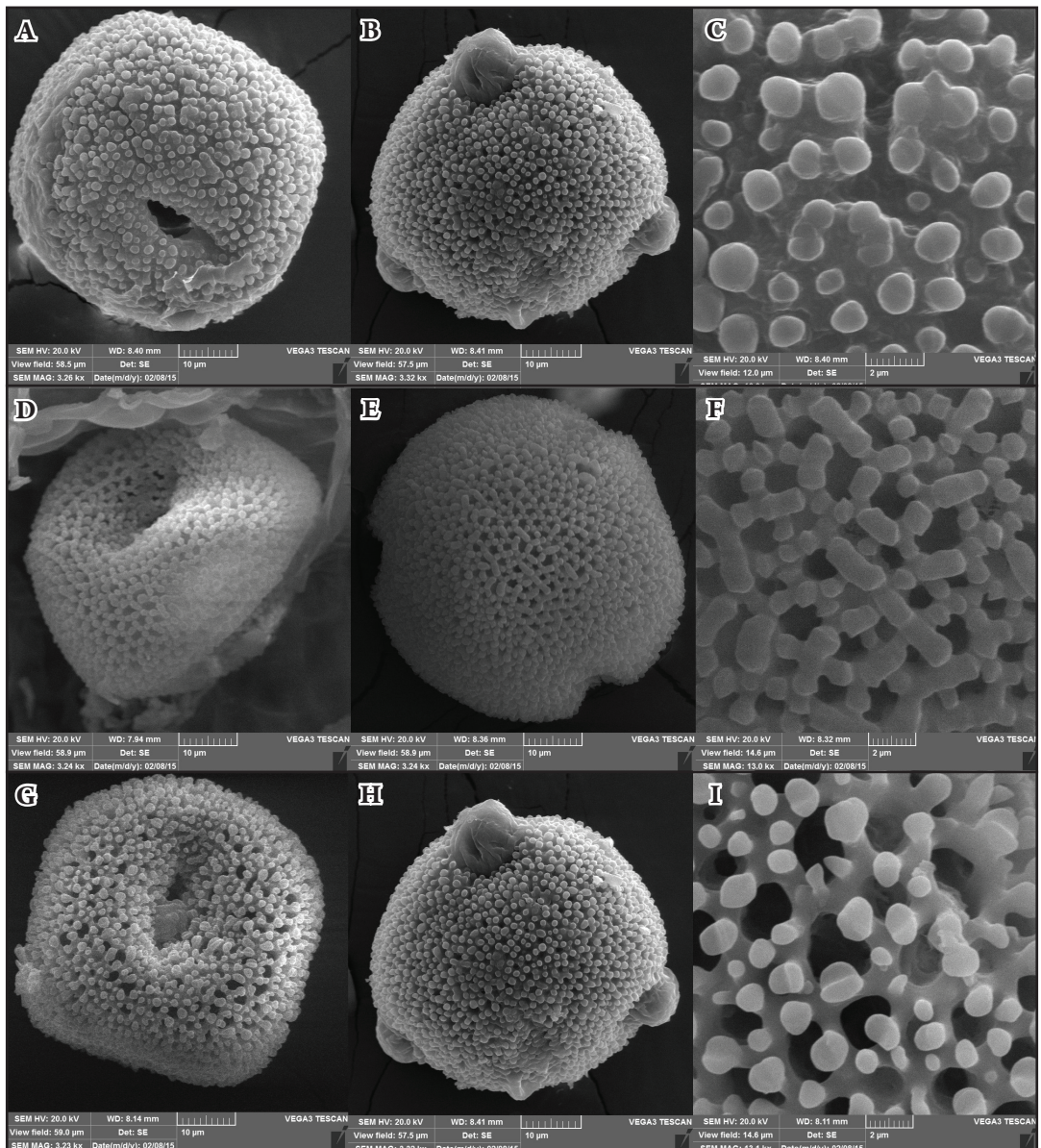
project showed that elements of each section are clearly separated by use of selected set of features.

As was pointed by SHEHATA (2008) for the pollen morphology of Geraniaceae family in Egypt, one of different ornamentation types in Geraniaceae is striate and in present study the *G. mascatense* show striate type while others showed clavate-reticulate or baculate-reticulate types. *G. albanum* and *G. molle* showed tectate tectum but other had semitectate one.

DENIZ *et al.* (2013) mentioned spheroidal-subprolate shape for *G. pusillum* while in present study prolate spheroidal shape was observed. SHEHATA (2008) in his studies on pollen morphology of the Geraniaceae identified some types by use of exine ornamentation features viz striate/striate-reticulate, reticulate/gemmate, and

reticulate. He described prolate spheroidal pollen grains in *G. molle* which is in concordant with findings of the present study. Analysis has shown that the pollen grains of all studied *Geranium* species are tricolporate.

Previous study showed that *G. purpureum* is separated from other species morphologically by having glandular hairs in inflorescence with colorless stalks and red glands. *G. albanum* has a discharge mechanism as inoperative but *molle* and *G. pusillum* has an operative one with blue pollen while operative mechanism is accompanied by not carinate calyx is the characteristic of *G. mascatense*. As it is evident in PCO graph species are clearly separated by their pollen features. Further studies are needed to reveal the morphologically similar species.



**Fig. 3.** Pollen micrographs of *Geranium* species: **A-C** – *G. molle*; **D-F** – *G. purpureum*; **G-I** – *G. pusillum*. **A, D, G** – equatorial view; **B, E, H** – polar view; **C, F, I** – exine sculpture.

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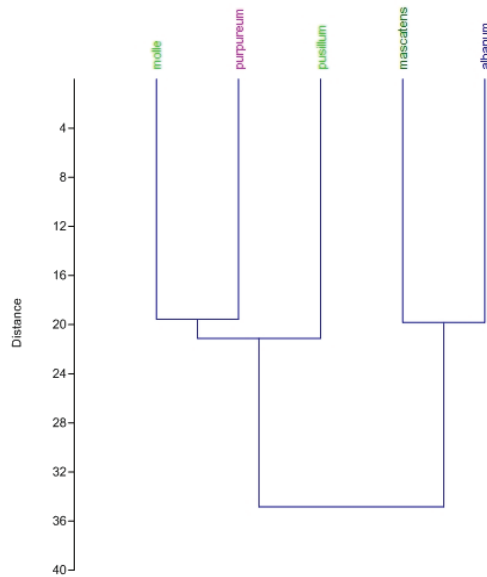


Fig. 4. Cluster analysis (Ward's method) based on pollen features of *Geranium*.

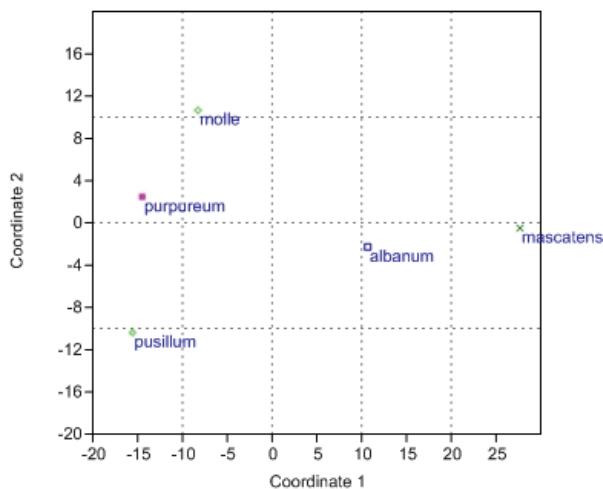


Fig. 5. PCO plot of *Geranium* species based on observed pollen data.

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