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Pollen Morphology of Egyptian Geraniaceae: An Assessment of Taxonomic Value

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Abstract: Pollen morphology of sixteen species including two subspecies and representing four genera: *Geranium* L., *Erodium* L., *Monsonia* L. and *Pelargonium* L´ Hér. of Geraniaceae in Egypt were investigated by the aid of light and scanning electron microscopy. The study showed that pollen grains were radially symmetrical, isopolar, prolate spheroidal to oblate spheroidal, tricolp (ate) orate. Tectum was generally striate/reticulate, reticulate/gemmate or reticulate. On the basis of pollen morphological characters, principally aperture types and exine sculpture, pollen grains were separated into three types and three subtypes representing different taxonomic categories. A key to the different pollen types and subtypes was provided. The results demonstrated that pollen morphological characters, as pollen shape, size, aperture characters as well as exine sculpture and structure, were taxonomically significant at the generic level and to some extent at the specific level. Moreover, the results confirmed the eurypalynous nature of the family.

Key words: Egypt, exine sculpture, Geraniaceae, key, palynology, pollen grains, SEM, taxonomy

INTRODUCTION

Geraniaceae is a cosmopolitan family of mostly temperate and subtropical annual or perennial herbs and a few small shrubs, comprising about 750 species belonging to five genera: *Erodium* L´ Hér., *Geranium* L., *Monsonia* L., *Sarcocaulon* (DC.)Sweet and *Pelargonium* L´Hér. (Hutchinson, 1969; Mabberley, 1997). The family is subdivided by Hutchinson (1969) into two tribes: Geranieae with primarily actinomorphic flowers (*Erodium*, *Geranium*, *Monsonia* and *Sarcocaulon*) and Pelargonieae with zygomorphic flowers (*Pelargonium*). Geraniaceae has been studied by several authors utilizing different criteria, as floral anatomy (Al-Nowaihi and Khalifa, 1973); nectaries (Link, 1991); chloroplast DNA variations (Price *et al*., 1990); rbcL sequence comparisons (Price and Palmer, 1993); comparative karyological studies (Albers, 1990); Floral morphological characters (Aldasoro *et al*., 2002) etc.

The application of pollen morphology in the recognition, identification and interpretation of relationships of plants at various hierarchical levels in plant taxonomy and production of pollen keys has been reported in many angiosperm families (Erdtman, 1966; Mulder, 2003; Huysmans *et al*., 2003; Al-Quran, 2004). Moreover, the advent of scanning techniques has greatly enabled the study of pollen morphology with great precision, thereby making their application much more efficient and dependable.

The pollen morphology of Geraniaceae or some of its representatives has been studied by several researchers Bortenschlager (1967), El-Oqlah (1983, 1989), Verhoeven and Venter (1987), Stafford and Blackmore (1991) and Stafford and Gibby (1992). Recently, Aedo *et al*. (2007) have used the palynological data in combination with morphological data in their taxonomic revision of genus *Geranium*.

In Egypt, Geraniaceae is represented by twenty five species belonging to three genera namely: *Erodium*, *Geranium* and *Monsonia* (Tackholm, 1974; Boulos, 2000). As far as the author is aware, the systematic investigation of the pollen morphology of Geraniaceae in Egypt had not been studied comprehensively, although some of its pollen was occasionally identified from neighboring countries (El-Ghazaly, 1991). The main objective of the present investigation was to investigate and describe the pollen morphology of some of the taxa of wild and cultivated Geraniaceae growing in Egypt and to show how far the pollen morphological variations could be used to distinct between the taxa studied.

MATERIALS AND METHODS

The study is based on the pollen morphology of 16 species including two subspecies of wild and cultivated Egyptian representatives of Geraniaceae.

Pollen samples of each studied species were collected from living collections as well as from herbarium

Table 1: Collection data of the investigated specimens

Taxon	Localities and Herbarium
Erodium cicutarium (L.) L´Hér.	Burg El Arab, Mariut. Herbarium of Alexandria University
E. ciconium (L.) L´Hér.	Burg El Arab, Mariut. Herbarium of Alexandria University.
E. crassifollium L´Hér.	64 km along the highway from Mersa Matruh to Sollum. Herbarium of Alexandria University.
E. glaucophyllum (L.) L´Hér.	Burg El Arab, Maruit. Herbarium of Alexandria University.
E. gruinum (L.) L´Hér.	37 km west of Alexandria along the inner road to Burg El-Arab. Herbarium of Alexandria University.
E. laciniatum (Cav.) Willd.	Coastal region. Sand dunes, East of Idku.
subspecies *laciniatum*	Herbarium of Alexandria University.
E. laciniatum (Cav.) Willd.	The road between Damanhour and Itai El Baroad,
subspecies *pulverulentum* (Bioss.) Batt.	Herbarium of Alexandria University.
E. malacoides (L.) L´Hér.	Saint Caterine, Sinai. Herbarium of Alexandria University.
E. moschatum (L.) L´Hér.	Burg El Arab, Maruit, Herbarium of Alexandria University.
Geranium dissectum L.	Rashid. Herbarium of Alexandria University.
Geranium molle L.	Alamen, Maruit. Herbarium of Agricultural Museum.
Geranium trilophum Bioss.	Gebel Elba. Herbarium of Alexandria University.
Monsonia heliotropioides (Cav.) Bioss.	Gebel Elba. Herbarium of Agricultural Museum.
Monsonia nivea (Decne.) Webb.	Noebaa, Cathrine road, Sinai.
Monsonia senegalensis Guill. and Perr.	Gebel Elba. Herbarium of Agricultural Museum.
Pelargonium grandiflorum Willd.	Botanic garden, Faculty of science, Alexandria University

specimens kept in the herbaria of Alexandria University and the Agricultural Museum (Table 1). Identification and plant name followed Boulos (2000). The pollen grains were prepared for light (LM) and Scanning Electron Microscopy (SEM) by the standard method described by Erdtman (1960). For LM studies, pollen grains were mounted on glycerin jelly onto glass slides. For examination by SEM, dried pollen grains were mounted onto stubs with double sided tape. The specimens were coated with gold using a Fine Coat Sputter JFC 1100 and scanned using the JEOL JSM 5300 SEM, at 15 and 25 KV accelerating voltage, at Electron Microscopy Unit, Alexandria University.

Pollen diameter, polar axis (P) and equatorial diameter (E) were measured on at least 20 mature pollen grains with LM using oil objective lens and P/E was calculated. Lumina and muri were measured in the middle of the mesocolpia, chiefly from digital SEM images; by using a minimum sample of two sets of 10 measurements from each SEM graphs. The terminology employed in this investigation follows that of Erdtman (1969) and Punt *et al*. (1994).

RESULTS

The palynological data of the investigated taxa are shown in Table 2.

General pollen morphological characters: The present results revealed that pollen grains were usually monad, isopolar, radially symmetrical. The mean value of polar axis (P) ranged from 44 μm in *Pelargonium grandiflorum* to 111 μm in *Erodium gruinum*, while the mean value of equatorial diameter (E) ranged from 32 μm in *Pelargonium grandiflorum* to 106 μm in *Erodium gruinum*. The grains were circular to slightly triangular with convex sides in polar view and circular to elliptic in equatorial view. Apertures in the studied taxa were tricolp(ate)orate. Tectum showed variable sculptures (reticulate, striate/reticulate and reticulate/gemmate).

The studied pollen grains were found to be categorized in terms of exine sculpture into three pollen types and three subtypes summarized as follows:

Type I: Striate/Striate-Reticulate type:

Species: *Erodium cicutarium*, *E. ciconium*, *E. crassifollium*, *E. glaucophyllum*, *Erodium gruinum*, *E. laciniatum* subspecies *laciniatum* and subspecies *pulverulentum*, *E. malacoides* and *E. moschatum*.

Shape: Oblate spheroidal to prolate spheroidal.

Pollen class: Tricolporate.

Exine ornamentation: Complex and consists of three layers. The first layer corresponding to the sexine (1) is formed by the apices of the columellae, above which there is a semitectate reticulum (sexine 2). The uppermost layer (sexine 3) consists of long or short winding striae which form a distinct separate layer. In *E. moschatum*, the striate elements appear to be more interwoven, curved and fused with the underlying muri of the reticulum beneath. In *E. malacoides*, the striae were unbranched and closely spaced, while they were branched, sparsely spaced and running at various directions in *E. cicutarium*, *E. ciconium*, *E. laciniatum* subspecies *laciniatum* and subspecies *pulverulentum*, *E. gruinum*, *E. crassifollium* and *E. glaucophyllum*.

Within this pollen type, it was not possible to sharply distinguish between the species (Fig. 1-16).

Table 2: Pollen morphological data of the studied taxa of Geraniaceae

Taxa	*Polar axis P (μm)	*Equatorial axis E (μm)	Shape	Exine sculpture	Pollen type
Erodium cicutarium	77(70-84)	68(59-70)	Prolate spheroidal	Striate reticulate	I
E. ciconium	58(52-60)	55(53-59)	Prolate spheroidal	Striate reticulate	I
E. crassifollium	74(68-78)	76(70-80)	Prolate spheroidal	Striate reticulate	I
E. glaucophyllum	69(65-72)	78(75-84)	Oblate spheroidal	Striate reticulate	I
E. gruinum	111(101-121)	106(96-109)	Prolate spheroidal	Striate reticulate	I
E. laciniatum subspecies *laciniatum*	54(50-59)	55(54-60)	Oblate spheroidal	Striate reticulate	I
E. laciniatum subspecies *pulverulentum*	67(65-73)	60(55-66)	Prolate spheroidal	Striate reticulate	I
E. malacoides	75(67-83)	67(63-70)	Prolate spheroidal	Striate reticulate	I
E. moschatum	60(56-64)	56(53-67)	Prolate spheroidal	Striate reticulate	I
Geranium dissectum	51(47-55)	48(45-51)	Prolate-spheroidal	Reticulate Gemmate	II
G. molle	53(49-60)	50(48-54)	Prolate-spheroidal	Reticulate Gemmate	II
G. trilophum	60(57-62)	55(51-60)	Oblate spheroidal	Reticulate Gemmate	II
Monsonia heliotropioides	70(68-76)	78(71-80)	Oblate spheroid	Reticulate	III
M. nivea	73(68-76)	76(72-80)	Oblate spheroid	Reticulate	III
M. senegalensis	85(71-89)	86(71-89)	Oblate spheroidal	Reticulate	III
Pelargonium grandiflorum	44(42-46)	32(36-38)	Prolate	Reticulate	III

*: Mean values followed by range in parenthesis

Type II. Reticulate/Gemmate type:

Species: *Geranium molle*, *G. trilophum* and *G. dissectum*

Shape: Oblate spheroidal to prolate spheroidal

Pollen class: Tricolporate

Exine ornamentation: Tectum reticulates. However it is heavily ornamented with bacula and gemmae. Supratectal processes were of variable sizes. Two main forms of gemmae were distinguished: Bifid large gemmate projections with distinct shallow striations and smaller rod like bacula with slightly swollen head. Intermediate-sized processes occur between these two forms but were relatively few in number. *G. dissectum* is distinguished from the rest of species by its small size range (Table 2) (Fig. 17-22).

Type III: Reticulate type: In this type, tectum was simple and consisting of a closely defined reticulum which was hetero-or homobrochate. Lumina were regular or irregular in shape with sparsely or closely spaced baculae. Muri that were linking tops of columellae were either smooth or beset with supratectal elements of various shapes. According to the nature of muri the reticulate pollen type is subdivided into three subtypes (Fig. 23-30).

Subtype A: *Monsonia heliotropioides* subtype:

Species: *Monsonia heliotropioides* and *M. nivea*

Shape: Oblate spheroidal

Pollen class: Tricolpate

Exine ornamentation: Coarsely reticulate. The reticulum was heterobrochate. The average area of lumina was 5.22 μm^2 for *Monsonia heliotropioides* and 2.47 μm^2 for *M. nivea*. The surface of nexine was perforated. Muri was articulate, fragmentimurate, simplibaculate. Baculae were closely spaced (sometimes fused), relatively stout and interconnected by muri which were provided with knob like elements of varying shape and size. The mean value of muri width was 1.01 μm for *M. heliotropioides* and 0.97 μm for *M. nivea* (Fig. 23-26).

Subtype B: *Monsonia senegalensis* subtype:

Species: *Monsonia senegalensis*.

Shape: Oblate spheroid.

Pollen class: Tricolpate.

Exine ornamentation: Coarsely reticulate. The reticulum was generally homobrochate, characterized by five to six angled wide lumina. The mean of lumina area was 4.42 μm^2, each muri was supported by one to four columellae. The surface of nexine was slightly granular at high magnification in SEM. Baculae were interconnected by straight sided and smooth muri. The mean of muri width was 0.63 μm. In some parts, the reticulum was generally level, but some muri may dip in the centre forming peaks at some of the supporting columellae (Fig. 27 and 28).

Subtype C: *Pelargonium grandiflorum* subtype:

Species: *Pelargonium grandiflorum*

Shape: Prolate

Pollen class: Tricolpate

Exine ornamentation: Reticulate, lumina generally angular in shape. The measure of lumina area was 1.5 μm^2. The surface of nexine was smooth. The mean of muri width was 0.60 μm. Muri were laterally compressed and somewhat undulating giving the surface a slightly striate appearance (Fig. 29 and 30).

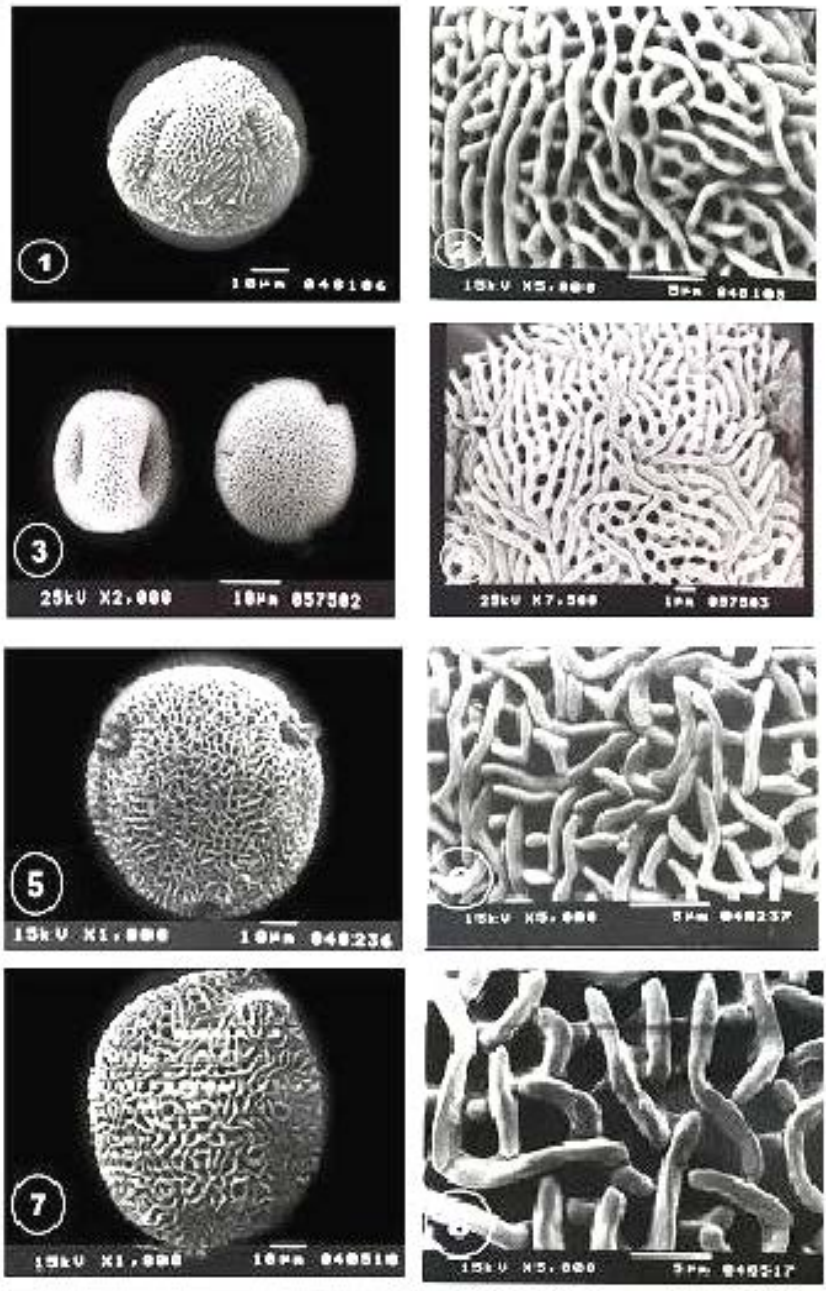

Fig. 1-8: SEM micrographs of pollen morphology and exine sculpture of studied taxa of Geraniaceae
Fig. 1: *Erodium cicutarium*, equatorial view
Fig. 2: *E. cicutarium*, exine pattern
Fig. 3: *E. ciconium*, equatorialand polar view
Fig. 4: *E. ciconium*, exine pattern
Fig. 5: *E. crassifolium*, polar view
Fig. 6: *E. crassifolium*, exinepattern
Fig. 7: *E. gruinum*, polar view
Fig. 8: *E. gruinum*, exine pattern

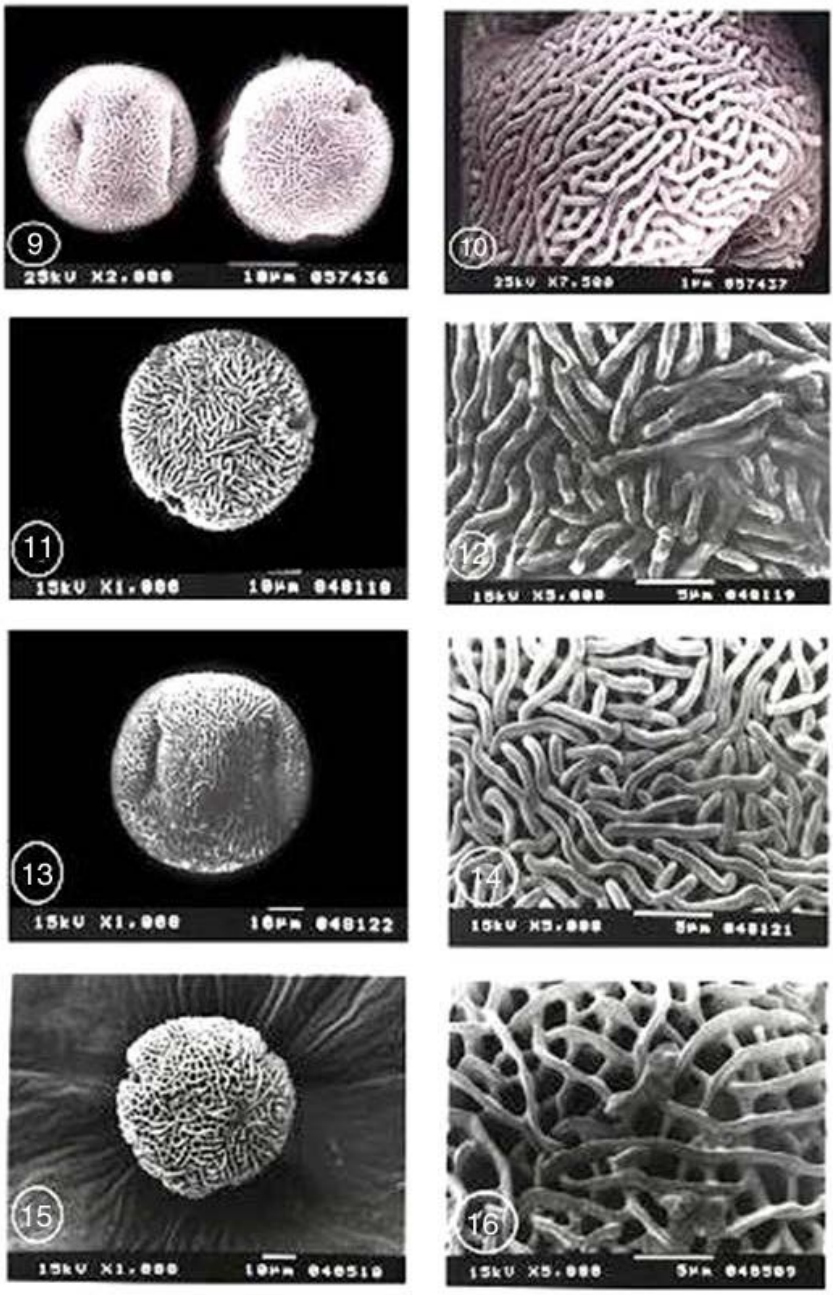

Fig. 9-16: SEM micrographs of pollen morphology and exine sculpture of studied taxa of Geraniaceae
Fig. 9: *E. laciniatum* subspecies *laciniatum*, equatorial and polar view
Fig. 10: *E. laciniatum* subspecies *laciniatum*, exine pattern
Fig. 11: *E. laciniatum* subspecies *pulveruntum*, equatorial view
Fig. 12: *E. laciniatum* subspecies *pulveruntum* exine pattern
Fig. 13: *E. malocoides*, polar view
Fig. 14: *E. malocoides*, exine pattern
Fig. 15: *E. moschatum*, polar view
Fig. 16: *E. moschatum* exine pattern

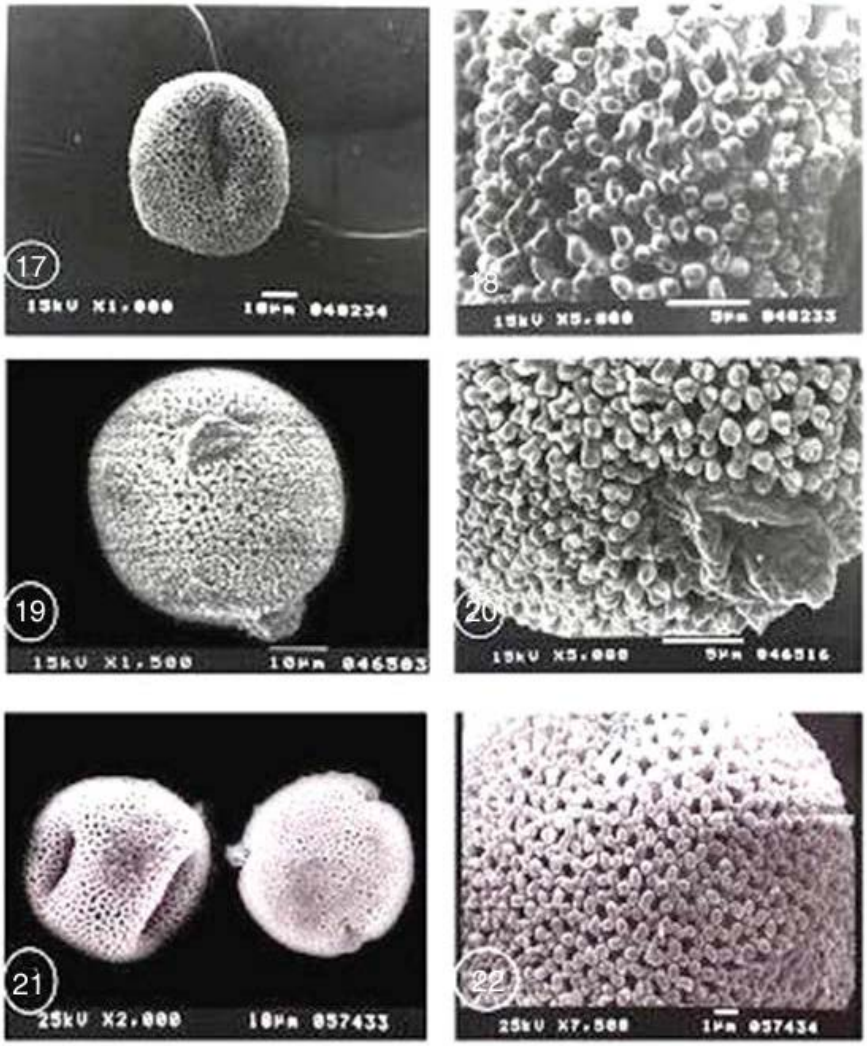

Fig. 17-22: SEM micrographs of pollen morphology and exine sculpture of studied taxa of Geraniaceae
Fig. 17: *Geranium dissectum* general view
Fig. 18: *G. dissectum* exine pattern
Fig. 19: *G. molle* general view
Fig. 20: G. *molle* exine pattern
Fig. 21: *G. trilophum*, equatorial and polar view
Fig. 22: *G. trilophum*, exine pattern

Key to the different pollen types and subtypes:

1 a. Exine sculpture reticulate/gemmate ..... type II
b. Exine sculpture not so..... 2
2 a. Exine sculpture striate to striatereticulate..... type I
b. Exine sculpture reticulate (type III)..... 3
3 a. Muri articulate..... *Monsonia heliotropioides* subtype
b. Muri not so..... 4
4 a. Lumina area 4.42 µm^2..... *Monsonia senegalensis* subtype
b. Lumina area 1.5 µm^2..... *Pelargonium grandiflorum* subtype

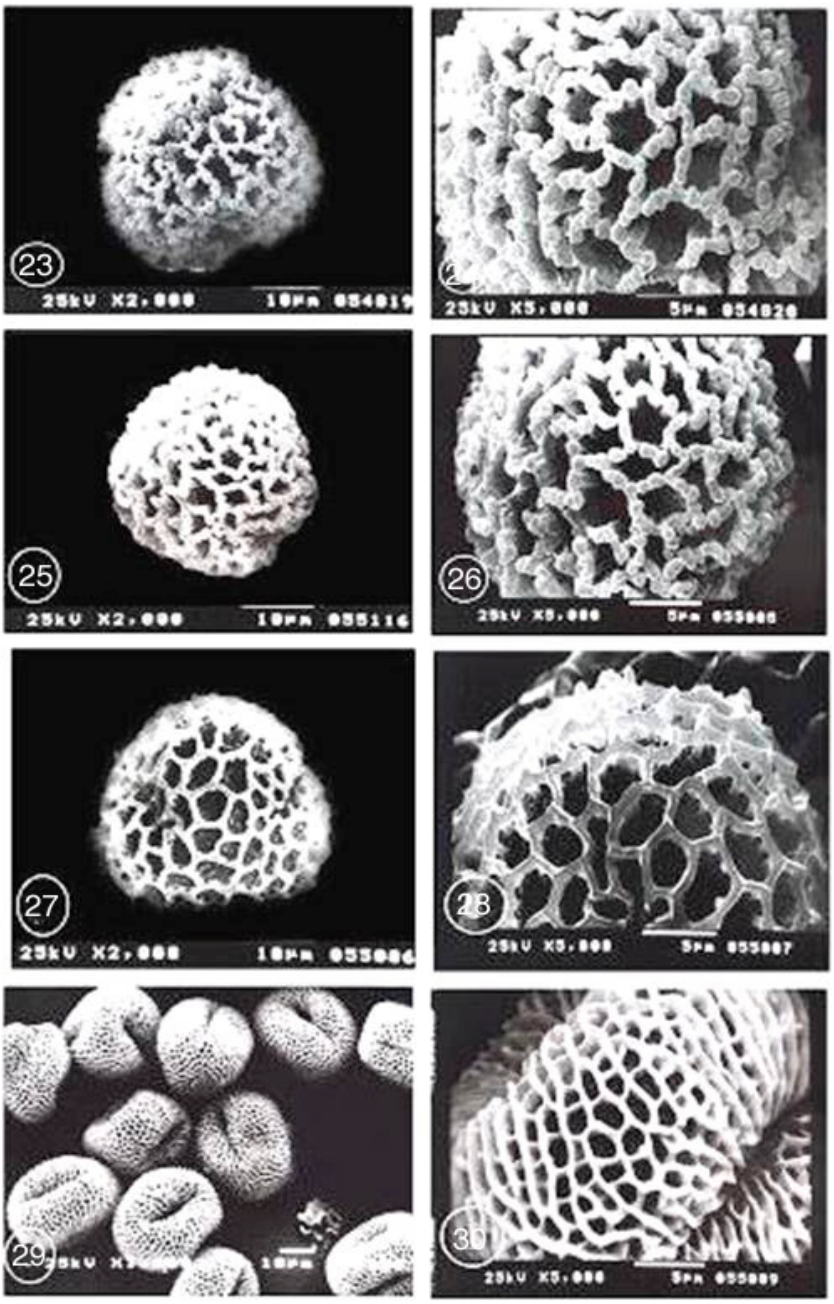

Fig. 23-30: SEM micrographs of pollen morphology and exine sculpture of studied taxa of Geraniaceae
Fig. 23: *Monsonia heliotropioides*, polar view
Fig. 24: *M. heliotropioides*, exine pattern
Fig. 25: *M. nivea*, polar view
Fig. 26: *M. nivea*, exine pattern
Fig. 27: *M. senegalensis*, general view
Fig. 28: *M. senegalensis*, exine pattern
Fig. 29: *Pelargonium grandiflorum*, equatorial and polar view
Fig. 30: *P. grandiflorum*, exine pattern

DISCUSSION

The variation of pollen described and documented above illustrates diverse pollen morphologies in Geraniaceae. These results were generally in accordance with those of Stafford and Blackmore (1991) as well as Perveen and Gaiser (1999), who described Geraniaceae as a eurypalynous family. Moreover the present study revealed that each genus has more or less its own characteristic pollen type. Based on the current study, three distinct pollen types in terms of surface ornamentations and exine structures were recognized viz., striate/reticulate, reticulate/gemmate and reticulate-type.

Pollen type-I (Striate/reticulate), is characterized by a striate reticulate tectum. All investigated *Erodium* species were found to fall into a general striate/reticulate ornamentation type, within which it was not possible to distinguish between the species. In the course of the current study, detailed SEM investigations have demonstrated a continuous range of sculptural variation occurs within pollen type I; thus, making it impossible to enable a subdivision on a non arbitrary basis. Bortenschlager (1967) carried out an extensive survey on the range of pollen types in the Gerniaceae. He examined the pollen morphology of 31 species of *Erodium*, of which 9 species were restudied in the course of this investigation. Bortenschlager recognized two basic pollen types, *Geranium multiflorum* and *Erodium*. According to the present results, pollen type I (Striate-Reticulate) is similar to the *Erodium* pollen type reported previously by Bortenschlager (1967). The latter distinguished three subtypes within his *Erodium* type based on the details of the pollen wall sculpture. The results of the present study, revealed no subtypes of pollen type I, in contrast to the findings of Bortenschlager (1967). On the other hand, the present results are in agreement with the finding of El-Oqlah (1983) who investigated the pollen morphology of 35 species of *Erodium* and pointed to the absence of pollen subtypes. Verhoeven and Venter (1987) reported a similar pollen type in *Erodium* species (except *Erodium oxyrrhynchum* ssp.) and more recently, Perveen and Gaiser (1999) studied the pollen morphology of 16 species of the family Geraniaceae from Pakistan and described the same pollen type in *Erodium* species.

Pollen type II (Reticulate/gemmate), is readily distinguished by the reticulate tectum which is heavily ornamented with bacula and gemmae. This pollen type is characteristic of *Geranium* species. The distinction between *Geranium* species included under this pollen type seemed to be difficult. *Geranium trilophum* in particular may be differentiated by larger size range 60 μm (57-62 μm) and oblate spheroid pollen. Otherwise the other two *Geranium* species were found to be quite similar to each other; both species showed the same pollen shape (prolate spheroid) and nearly the same size range (51-53 μm). Pollen type II is similar to the *Geranium multiflorum* type that described previously by Bortenschlager (1967) and also to the pollen grains of *Geranium* species described. On the other hand, the present result does not support El-Ghazaly (1991), who reported that the exine sculpture of *Geranium molle* is striato-reticulate while the present study showed a reticulate/gemmate exine sculpture for *G. molle*.

Pollen type III (Reticulate), is easily recognized by a coarsely reticulate tectum. Three species of *Monsonia* (*Monsonia heliotropioides*, *M. nivea* and *M. senegalensis*) beside *Pelargonium grandiflorum* were included under this pollen type. Venter (1979, 1983) as well as Verhoeven and Venter (1986) reported a similar type of pollen in several species of *Monsonia*. In addition, Stafford and Gibby (1992) in their extensive work in pollen morphology of *Pelargonium* recognized the same pollen type in many species of *Pelargonium* (as *P. longifolium*, *P. luridum*, *P. patulum*, *P. pinnatum*, *P. crassipes* and others). Pollen type III, was found to be variable both in the characters of exine sculpture, shape and size of pollen. These differences are sufficient for them to be categorized in separate subtypes: subtypeA (*Monsonia heliotropioides*), subtype B (*Monsonia senegalensis*) and subtype C (*Pelargonium grandiflorum*).

The first subtype A is characterized by heterobrochate reticulum, perforated nexine, articulate, fragmentimurate pollen and the presence of supratectal processes. This subtype is characteristic of *Monsonia heliotropioides* and *M. nivea*. The present work revealed that the distinction between *M. heliotropioides* and *M. nivea* palynologically is quite difficult as both species showed more or less similar pollen grains. This finding is in agreement with El-Ghazaly (1991), who pointed to the great similarity of their pollen grains. The second subtype B, with homomorphic reticulum, wide lumina and relatively straight and smooth muri was found to be characteristic to *M. senegalensis*. Venter (1979), on his monograph of *Monsonia* as well as El-Ghazaly (1991) who studied this species among others reported a similar type of pollen in the genus *Monsonia*. The species included under pollen subtypes A and B, could also be distinguished from each other not only by the variations in the pollen morphological characters, but also by the number of pollen content per anther. *M. nivea* and *M. heliotropioides* (pollen subtype A) showed a strikingly low number of pollen content comparing with that of *M. senegalensis* (pollen subtype B). A similar

observation had been reported by Aldasoro *et al*. (2001); they studied the pollen morphology in combination with other morphological characters and gave an account on the phylogenetic relationships among 25 species of the old-world genus *Monsonia*.

The third pollen subtype C is distinguished by the reticulate exine sculpture, small lumina and laterally compressed undulating muri as well as prolate shape. This subtype is characteristic for *Pelargonium grandiflorum*. This type of pollen is similar to that described by Stafford and Gibby (1992).

CONCLUSION

The pollen morphological characters in the current study are considered to be diagnostic at the generic level and to some extent at the specific level. Moreover the present study confirmed the eurypalynous nature of the family. More studies are still needed in the future, utilizing more cosmopolitan material, to achieve more conclusive results.

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