



Pollen Morphology and Generic Delimitation of the Polygonaceae in Egypt

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POLLEN grains of 18 species representing seven genera (*Atraphaxis*, *Calligonum*, *Oxygonum*, *Emex*, *Periscaria*, *Polygonum* and *Rumex*) of Polygonaceae Juss. (Subfamily, Polygonoideae) in Egypt were investigated using light microscopy (LM) and scanning electron microscopy (SEM) to evaluate the taxonomic value of micro-morphological characters of pollen grains in order to differentiate between the genera and species. The study showed that pollen grains are radially symmetrical, isopolar, oblate-spheroidal, spheroidal, subprolate to prolate, with ambtrilobed or circular, tricolporoidate, tricolporate, rarely tetracolporate and pantopolporate. Tectum is striato-reticulate to reticulate, coarse reticulate with double bacula in muri and pilatelumine, rugulo-reticulate, microechinate-foveolate, and microgranulate-perforate. Based on these characters, five groups of pollen types are recognized. The results indicate that pollen morphology characters of shape, aperture and exine ornamentation are useful in generic delimitation. A key for the separation of the different pollen types is provided.

Keywords: Generic delimitation, Pollen morphology, Polygonaceae, Polygonoideae, SEM.

Introduction

The Polygonaceae Juss., are a cosmopolitan family of herbs, shrubs and small trees characterized by simple leaves with sheathing ochreastipules (Ronse Decraene & Akeroyd, 1988; Li et al., 2003; Sanchez & Kron, 2008). It comprises about 59 genera embracing 5,385 species (The Plantlist.org, 2018). According to Boulos (1999), the Polygonaceae are represented in Egypt by 29 species belonging to eight genera.

The classification of Polygonaceae was a matter of debate at the generic and tribal levels since the 19th century (Haraldson, 1987; Ronse Decraene & Akeroyd, 1988). The family was divided into three subfamilies on the basis of morphological evidences (Dammer, 1892; Gross, 1913; Roberty & Vautier, 1964). More recently, Freeman & Reveal (2005) and Burke et al. (2010), recognized two subfamilies (Eriogonoideae and Polygonoideae) on the basis of other criteria. Several studies dealt with the

macro- and micro-morphological characters of Polygonaceae (Simmonds, 1945; Haraldson, 1987; Ronse Decraene & Akeroyd, 1988; Hamed & Tantawy, 1990, 1991; Leresten & Curtis, 1992; Brandbyge, 1993; Partridge, 2001; Li et al., 2003; Tantawy et al., 2005; Mosaferi & Keshavarzi, 2011; Hussein et al., 2012). The Polygonaceae are a eucaryal family among dicotyledons (Wodehouse, 1931). The pollen morphology and taxonomy of Polygonaceae were the subject of several studies (e.g. Gross, 1913; Wodehouse, 1931; Hedberg, 1946; Nowicke & Skvarla, 1977).

The objective of the study is to examine and shed more light on the pollen morphological characters in the family as well as to assess the significance of the pollen characters in the delimitation of genera and species.

Materials and Methods

Pollen grains samples of 18 species belonging to seven genera of the Polygonaceae-

Polygonoideae were collected from their natural habitats or specific herbaria (Table 1). Materials for light microscope were prepared according to Erdtmann (1960). At least 30 pollen grains per taxon were examined by using Zeiss light microscope with an eye-piece micrometer. For SEM studies, non-acetolyzed grains were mounted on aluminum stubs, with double sided adhesive tape, coated for 5min with a thin layer

of gold in a JFC-1100E ion sputtering coating unite, examined at accelerating voltage of 15-25KV. and then photographed with JEOL JSM- 5300 SEM (Electron Microscopic Unit, Faculty of Science, Alexandria University). The terminology used here for pollen description is in accordance with Erdtman (1952) and Punt et al. (2007).

TABLE 1. Collection data of the specimens representing 18 species from 7 genera of the Polygonaceae.

Taxa	Locality/Date/Collector/source
<i>Atraphaxis spinosa</i> L.	Saint Catherena , 3181/3/, Gamal El-Ghazaly, ALEX.
<i>Calligonum comosum</i> L'Hér.	Museum Wadi el Rayan, 20/42008/, Mona Shiha, ALEX.
<i>Calligonum polygonoides</i> L.	Kafr El-Sheikh, 42009/4/, Selim Zedan & Samia Rashad, ALEX.
<i>Emex spinosa</i> (L.) Campd.	Matrouh, 312005/3/, Sania Kamal, ALEX.
<i>Oxygonum sinuatum</i> (Meisn.) Dammer	Khar Gwob Red sea, 1066/12/, L. Boulos, Boulos Herb.
<i>Persicaria lanigera</i> (R.Br.) Soják	Amyot canal bank, 699/5/, D.A. Ahmed, H.A. Hosni, Tanta Herb.
<i>Persicaria lapathifolia</i> (L.) Delarbre	Lake Edku, 182003/4/, Tarek Galal, Tanta Herb.
<i>Persicaria salicifolia</i> (Brouss. Ex Willd) Assenov	Edku Lake, 45km before Edku Agnic. Secondary School, 152008/12/, Ream Marzouk & Samia Rashad, ALEX.
<i>Persicaria senegalensis</i> (Meisn.)Soják	Masraf el Gabaroti, Mohatta El-Aslah on the road Alex.-Rosetta,81970/9/, R. El-Ghareeb, ALEX.
<i>Polygonum aviculare</i> L.	Mersa Matrouh, 20/4/2006, L. Boulos, Boulos Herb.
<i>Polygonum bellardii</i> All.	Tanta,2-9-98 , D.A. Ahmed, Tanta Herb.
<i>Polygonum equisetiforme</i> Sm.	North Med. Coastal region, sand dunes ca. 3km from Mayyah tunnel, 122005/4/, L. Boulos, S. El-Darir, M. Shiha & A. Shehata. ALEX.
<i>Polygonum plebeijum</i> R. Br.	Mersa Matrouh, 31994/, Dr. Maged, ALEX.
<i>Rumex bucephalophorus</i> L.	Elsalom Plateau, 3/78, L. Boulos, Boulos Herb.
<i>Rumex cyprius</i> Murb.	Sinai, 295/4/, Sania Kamal, M. Fawzy, ALEX.
<i>Rumex dentatus</i> L.	Burg El- Arab, 25/ 32017/, M. Shiha, ALEX.
<i>Rumex pictus</i> Forssk.	El Omaid, 878/3/, L. Boulos, Boulos Herb.
<i>Rumex vesicarius</i> L.	Wadi El-Ghadir, 41961/2/, Vivi Täckholm, M. Kassas, M. Samy, CAI.

Results

Variation in pollen morphological characteristics as revealed by LM & SEM is presented in Table 2 and illustrated by SEM micrographs in Figs. 1-36. Five pollen types are easily recognizable in the following key:

- 1a. pollen grains polyantoporate.....
Persicariatype
- 1b. Pollen grains 3–4 colporate.2
- 2a. Pollen grains oblate-spheroidal.....*mex-Rumex* type
- 2b. Pollen grains prolate to subprolate 3
- 3a. Tectum microechinate - foveolate.....*Polygonum* type
- 3b. Tectumrugulo-microreticulate to reticulate*Calligonum – Oxygenum* type
- 3c. Tectum striate–reticulate *Atraphaxistype*

There follows a detailed description of the five pollen types with names of taxa characterized by each type.

1- Atraphaxis type: Pollen grains isopolar, radiosymmetric, tricolporate, prolate; Polar axis (P)= 11.73-13.84µm, Equatorial diameter (E)= 7.69-8.26µm, P/E= 1.5-1.7. Ambtrilobed.Colpi length (L)=10.57-11.92µm, slit-like, narrow, distinctly sunken, ends acute, ora usually not distinctly delimited, lalongate. Exine sculpture striate–reticulate, the striae running parallel to the colpus. This pollen type characterizes *Atraphaxis spinosa*. (Figs. 1, 2).

2- Calligonum and Oxygenum type: Pollen grains isopolar, radiosymmetric, tricolporate, 20.18- 21x11.21-12.30µm in *Calligonum* spp. and 26.66x12.85µm in *Oxygenum sinuatum*. Pollen shape prolate, P/E= 1.6-1.9 with trilobed amb. Ectoaperture colpus, narrow, slit-like, long, sunken, with acute ends. Endoaperture porus, lolongate. Exine sculpture rugulo microreticulate to reticulate. This type characterizes three species: *Calligonum comosum*, *Calligonum polygonoides* (Figs. 3-6) and *Oxygenum sinuatum* (Figs. 7 and 8).

3- Persicaria type: All *Persicaria* spp. have this type of grains. Pollen grains isopolar, radiosymmetric, pantoporate, with average diameter in the range of 15.62-42.0µm. Spheroidal in shape P/E± 1. Grains of *Persicaria salicifolia* (15.62µm) appeared to be the smallest in diameter while those of *P. lanigera* (42µm) are the largest. *P. senegalensis* (18.75µm) and *P. lapathifolia* (27.1µm) grains are intermediate in size. Exine sculpture clearly visible in the four *Persicaria* species, tectum appeared coarse reticulate in pattern. Pori circular, distributed in a single lumen of the reticulum, lumina variable in size, angular, usually forming 4-6 sided polygonal, the sides straight to slightly wavy, granules numerous inside the lumina. Muri thin dupli-columellate, columella usually opposite or alternate.(Figs. 9-16).

4- Polygonum type: All *Polygonum* investigated species have this type of grains. Pollen grains are isopolar, radiosymmetric, tricolporate, pollen size varies from 10.95- 26.5x 7.89-13.5µm. *Polygonum bellardii* (10.95x 8.45µm) appears to be smallest in size, while *P. equisetiforme* (27.1x 13.5µm) has the maximum size among the studied species. Subprolate to prolate, P/E= 1.29-1.91, fossaperturate amb. Aperture ectoaperture colpus, narrow, slit-like, long and sunken, with acute ends. Endoaperture pore lolongate. Exine sculpture micro echinate–foveolate tectum (Figs. 17-24).

5- Rumex and Emex type: Pollen grains are isopolar, radiosymmetric, oblate spheroidal to subprolate in shape P/E=0.93-1.4, circular to quadangular in polar view. Apertures 3-4, colporate in *Rumex dentatus*, tricolporoidate in *Rumex cyprius*; tricolporate in *R. bucephalophorus*, *R. pictus* and *R. vesicarius* and 3-brevicolpate in *Emex spinosa*. Ectoaperture colpus, narrow, long, usually sunken, with acute ends in *Rumex*, while narrow, very short in *Emex spinosa*. Endoaperture porous, lalongate or ± lolongate. Exine sculpture granular–microgranulate, perforated tectum in the investigated *Rumex* spp. and microreticulate-foveolate with granules (spinules) on the surface in *Emex spinosa*, *Rumex bucephalophorus*, *R. cyprius*, *R. dentatus*, *R. pictus* and *R. vesicarius*. Based on variation in the pollen micro-morpholglcal characters, an artificial key was constructed to facilitate the discrimination between the five pollen types.

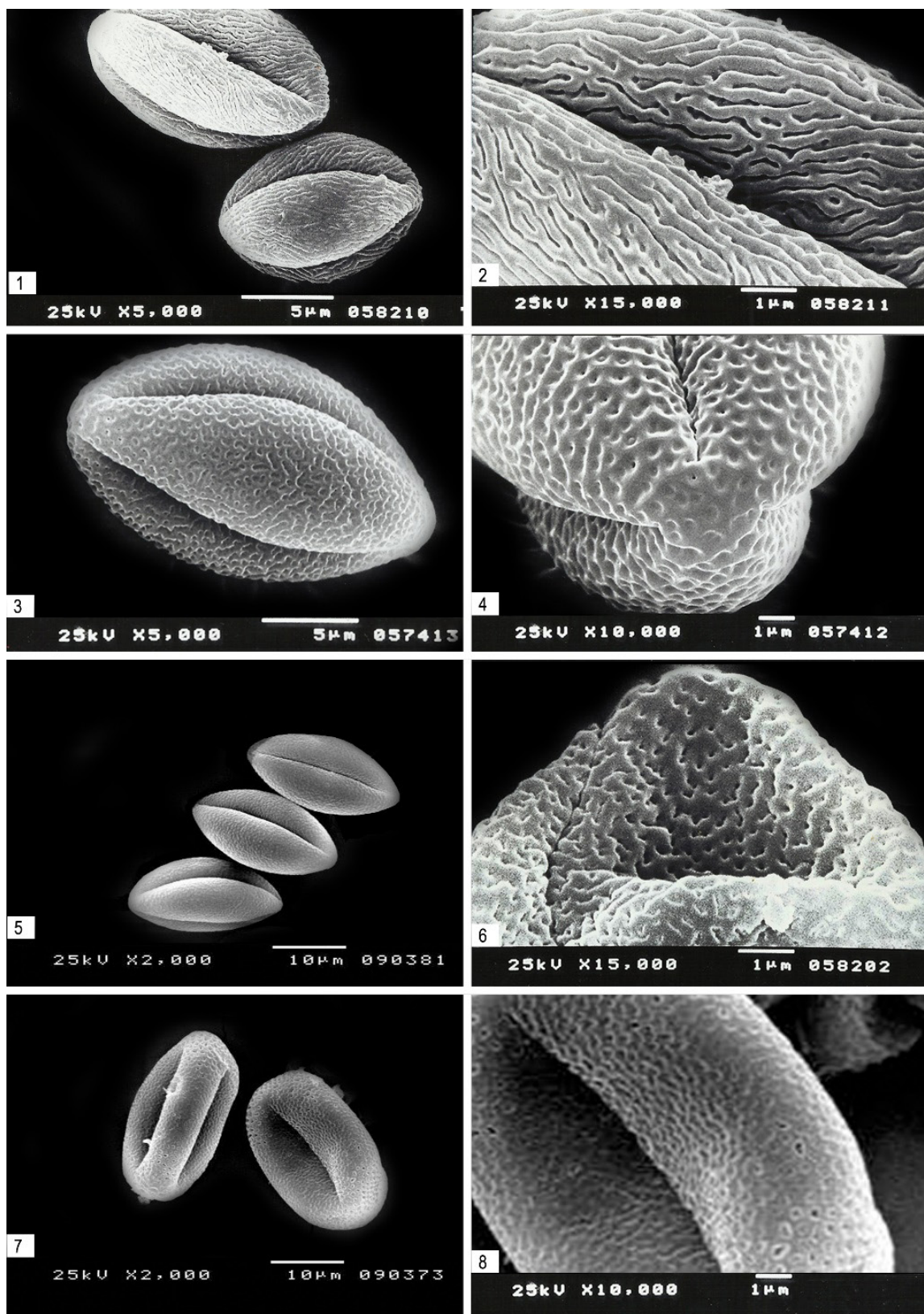
TABLE 2. Pollen characters of the studied taxa of the Polygonaceae (LM & SEM).

Taxa	Pollen measurements in μm and shapes				Aperture characters			Exine Sculpture	Gr.	
	P.A.	E.A.	P/E	Shape	No	Type	C.L. μm			Mesocolpia μm
<i>Atraphaxis spinosa</i>	11.73-13.84 (13.63 μm)	7.69-8.26 (7.89 μm)	1.70	Prolate	3	colporate	10.57-11.92 (11.11 μm)	4.24-4.80 (4.62 μm)	Striate Reticulate	1
<i>Calligonum comosum</i>	19.0-20.76 (20.18 μm)	12.11- 12.40 (12.30 μm)	1.64	Prolate	3	colporate	17.30-18.26 (18.07 μm)	7.44-7.69 (7.52 μm)	Rugulo-microreticulate	2
<i>C. polygonoides</i>	20.0-21.90 (21.20 μm)	10.55-11.42 (11.21 μm)	1.92	Prolate	3	colporate	19.04-19.52 (19.17 μm)	6.66-7.24 (7.14 μm)	Rugulo microreticulate	2
<i>Emex spinosus</i>	7.88-8.46 (8.07 μm)	7.88-8.46 (8.07 μm)	1.02	Spheroidal	3	Colporate- brevicolpate	1.7-1.9 (1.9)	-	Microreticulate-foveolate, with scattered spinules	5
<i>Oxygonum sinuatum</i>	25.71-27.14 (26.19)	12.85-15.23 (14.28)	1.8	Prolate	3	colporate	17.61-19.52 (19.04)	5.60-5.97 (5.71)	Reticulate to microreticulate	2
<i>Periscaria lanigera</i>	39.0-42.0 (39.0 μm)	39.5-42.6 (41.0 μm)	0.95	Spheroidal	Poly	Porate	-	-	Iregular coarse reticulate with pila in lumina	3
<i>P. lapathifolia</i>	26.66-27.2 (27.1 μm)	26.66-27.2 (27.1 μm)	1.0	Spheroidal	Poly	Porate	-	-	Regular coarse reticulate with pila in lumina	3
<i>P. salicifolia</i>	15.62-16.05 (15.78 μm)	15.72- 16.05 (15.78 μm)	0.98	Spheroidal	Poly	Porate	---	----	Regular coarse reticulate with pila in lumina	3
<i>P. senegalensis</i>	18.12-19.37 (18.75 μm)	18.12-19.37 (18.75 μm)	1.07	Spheroidal	Poly	Porate	----	----	Regular coarse reticulate with pila in lumina	3

TABLE 2. Cont.

Taxa	Pollen measurements in μm and shapes				Aperture characters				Exine Sculpture	Gr.
	P.A.	E.A.	P/E	Shape	No	Type	C.L. μm	Mesocolpia μm		
<i>Polygonum aviculare</i>	13.10-13.45 (13.37 μm)	7.89- 8.20 (8.01 μm)	1.67	Prolate	3	colporate	10.89- 1.20 (11.01 μm)	3.53-3.85 (3.75 μm)	Microechinate foveolate	4
<i>P. bellardii</i>	10.76-11.15 (10.95 μm)	8.07-8.65 (8.45 μm)	1.29	Subprolate	3	colporate	8.84-9.03 (8.93 μm)	5.32-5.89 (5.76 μm)	Microechinate foveolate	4
<i>P. equisetiforme</i>	26.5-27.10 (27.1 μm)	13.01-13.9 (13.5 μm)	1.91	Prolate	3	colporate	21.5-22.5 (22.0 μm)	5.5-6.5 (5.85 μm)	Microechinate foveolate	4
<i>P. plebijum</i>	18.0-18.98 (18.88 μm)	10.89-11.23 (11.11 μm)	1.69	Prolate	3	colporate	14.53-14.85 (14.72 μm)	6.10-6.82 (6.66 μm)	Microechinate foveolate	4
<i>Rumex Bucephalus</i>	8.65-9.0 (8.95)	8.0-8.25 (8.13)	1.03	Spheroidal	3	colporate	4.87-5.13 (5.0)	2.66-2.85 (2.73)	Microgranulate perforate	5
<i>R. cyprius</i>	8.10-8.75 (8.52 μm)	9.01-9.32 (9.11 μm)	0.93	Oblate- Spheroidal	3	colporoidate	6.98-7.25 (7.05 μm)	3.25-4.11 (3.60 μm)	Microgranulate perforate	5
<i>R. dentatus</i>	17.23-17.96 (17.69 μm)	14.12-14.62 (14.42 μm)	1.22- 1.4	Subprolate- Prolate	3-4	colporate	6.31-6.84 (6.57)	--	Microgranulate perforate	5
<i>R. pictus</i>	20.10-21.2 (20.3 μm)	16.17-17.05 (16.45 μm)	1.23	Subprolate	3	colporate	14.70-17.05 (15.88 μm)	8.25-9.87 (8.82 μm)	Microgranulate perforate	5
<i>R. vesicarius</i>	12.63-13.68 (13.42 μm)	8.68-10.96 (10.0 μm)	1.4	Subprolate- Prolate	3	colporate	10.52-11.05 (10.78 μm)	7.56-7.89 (7.63 μm)	Microgranulate perforate	5

L.= Colpus length, E.A. = Total breadth in equatorial view, P.A. = Polar axis, P/E= Relation between polar axis and total breadth of the grain in equatorial view.



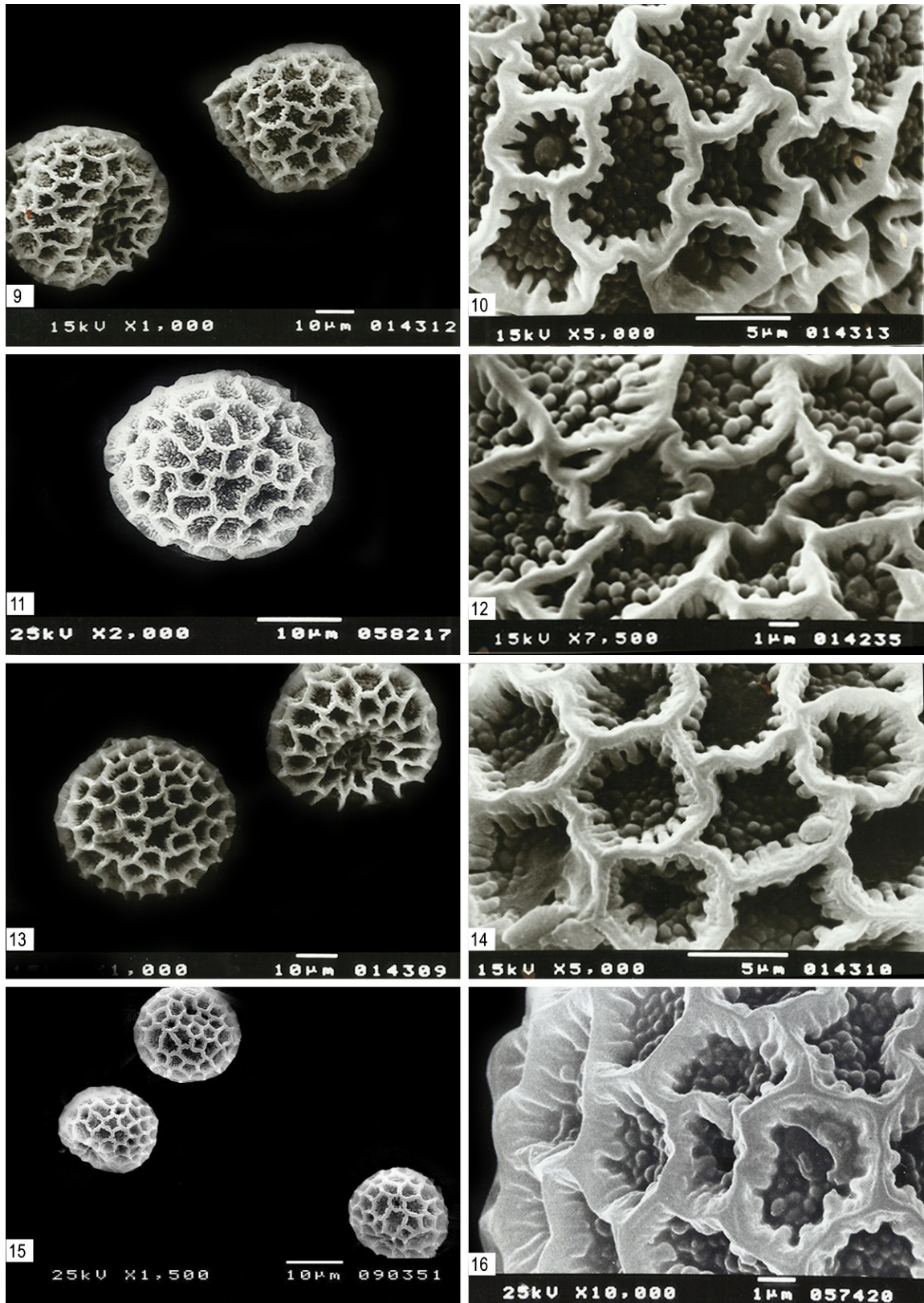
Figs. 1-8. SEM micrographs of pollen grains.

(1, 2) *Atraphix spinose*.

(3, 4) *Calligonum comosum*

(5, 6) *Calligonum polygonoides*

(7, 8) *Oxygonum sinuatum*



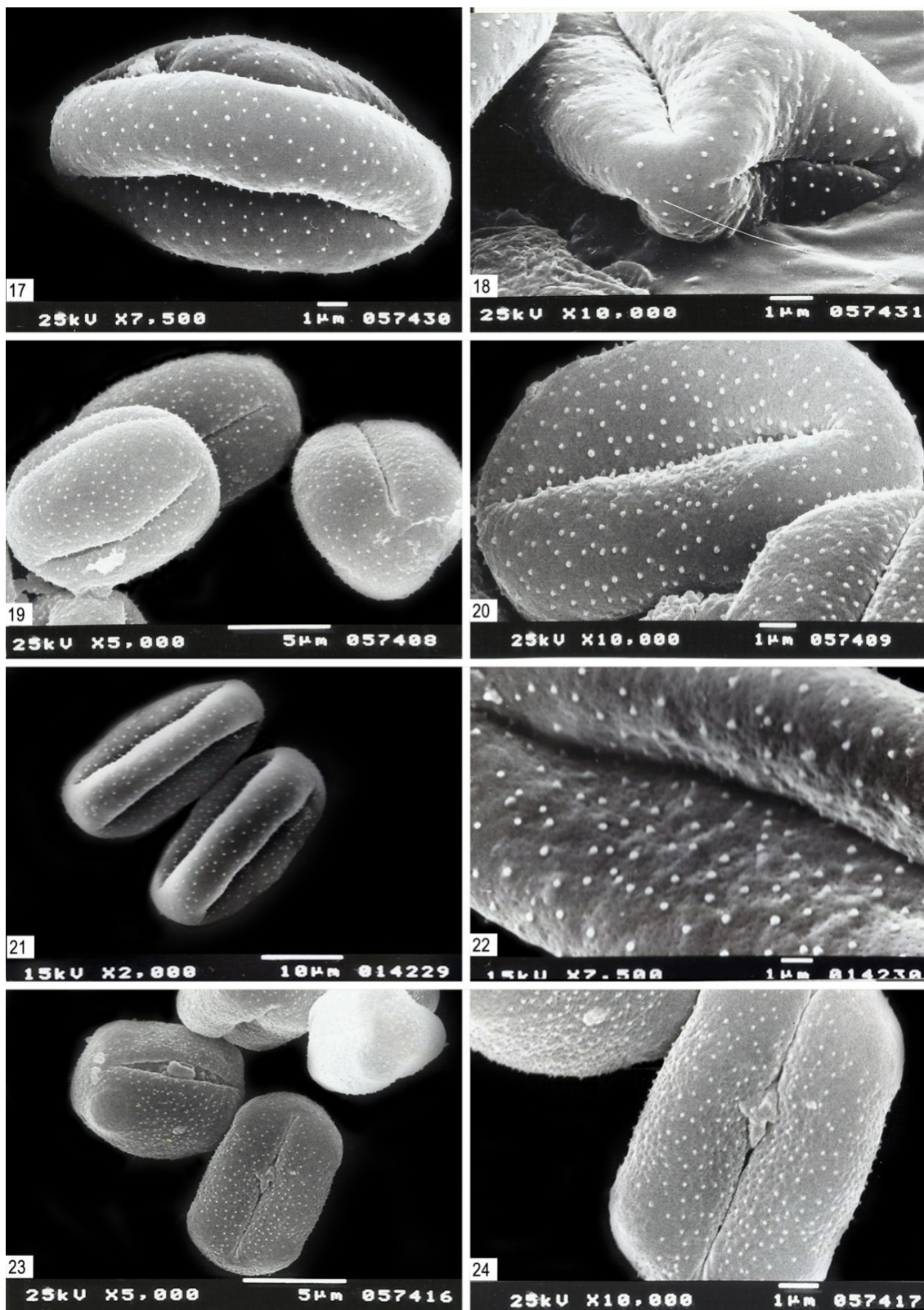
Figs. 9-16. SEM micrographs of pollen grains.

(9,10) *Periscaria lapathifolia*

(11,12) *P. longeria*

(13,14) *P. senegalensis*

(15,16) *P. salicifolia*



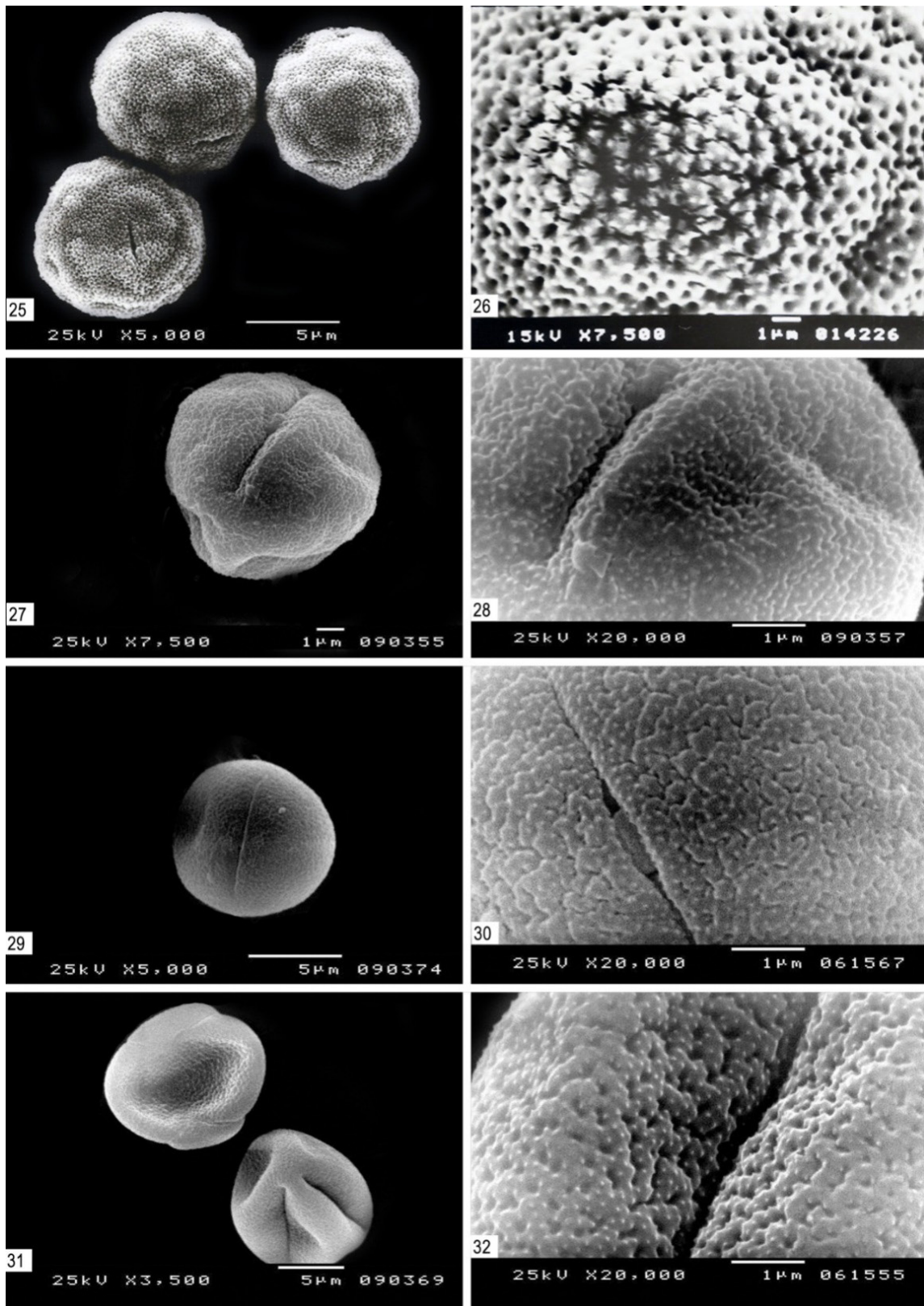
Figs. 17-24. SEM micrographs of pollen grains.

(17-18) *Polygonum auvicular*

(19-20) *P. bellarii*

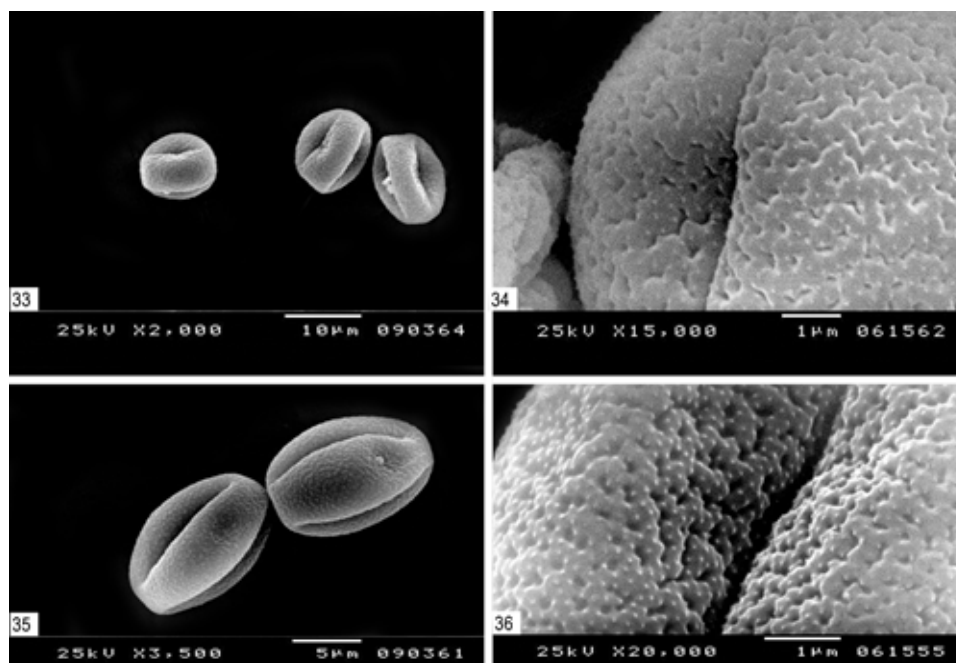
(21-22) *P. equistiform*

(23-24) *P. plebijum*



Figs. 25-32. SEM micrographs of pollen grains.

- (25-26) *Emex spinosa* (27-28) *Rumex bucephalus*
 (29-30) *R. cyprius* (31-32) *R. dentatus*



Figs. 33-36. SEM micrographs of pollen grains.

(33-34) *Rumex pictus* (35-36) *R. vesicarius*

Discussion

The Polygonaceae are evidently an europalynous family. Their pollen morphological characters were successfully used in the taxonomy of the family, especially at generic level (Nowicke & Skvarla, 1977; Van Leeuwen et al., 1988; Yurtseva, 2014, 2016).

The striate-reticulate exine sculpture is unique to *Atraphaxis spinosa* and was not encountered in any of the other 17 species in the present study or in the whole family (Nowicke & Skvarla, 1977, 1979). This result is in agreement with Yurtseva et al. (2014) who examined pollen morphology of 23 *Atraphaxis* spp. According to Bao & Li (1993) and Hong (1995), the striate ornamentation of pollen exine in *Atraphaxis* is different from other patterns of ornamentation recorded in the rest of the family.

The *Calligonum-Oxygonum* type is characterized by two patterns of exine ornamentation: rugulo-microreticulate in *Calligonum* species. Hong (1995), studying pollen morphology of *Calligonum comosum* and *C. polygonoides* beside other taxa of Polygonaceae, reported similar type of pollen surface. Nevertheless, the present finding regarding the *Calligonum* species is not in agreement with Nair

et al. (1976), since he described the pollen grains of *Calligonum* species as “3-zonocolpate with reticulate exine”. The reticulate to microreticulate pattern is found in *Oxygonum sinuatum*. El-Naggar & El-Husseini (2001), studying pollen morphology of Polygonaceae, reported the same pollen type in the genus *Oxygonum*.

Palynological evidence from the present study indicates the naturalness of *Persicaria* spp. with their spheroidal poly-pantoporate grains, coarsely reticulate tectum with regularly distributed hexagonal lumina filled with free bacula. The *Persicaria* pollen type was also reported by Wodehouse (1931), Hedberg (1946), Perveen (1993), Yasmin et al. (2010), and Mosaferi & Keshavarzi (2011).

The *Polygonum* type of tricolporate pollen with microechinate-foveolate surface was present in all the studied species of *Polygonum*. This pollen type is comparable to the *avicularia* type proposed previously by Wang & Feng (1994), Zhang & Zhou (1998) and Hong et al. (2005).

The *Rumex-Emex* type is the characteristic feature of *Rumex* spp. and *Emex* spp. Pollen shape varied from oblate spheroidal to subprolate. The grains are tricolporate in *R. bucephalophorus*, *R. pictus* and *R. vesicarius*, tricolporoidate in *Rumex cyprius*, tri- tetra-

colporate in *Rumex dentatus* with microgranulate-perforate tectum. The present observation is similar to that of Zhang & Zhou (1998), Wodehouse (1931) concerning the distribution of apertures and exine sculpture. However, the present finding disagrees with Perveen (1993), who described pollen grains of *Rumex dentatus* as pantocolporate.

Conclusion

Based on the present study, it would appear that there is quite considerable variation in pollen micro-morphology characters especially as shape, aperture types and number as well as exine ornamentation that make the pollen grains of systematic value and a distinct structure which can be employed for the delimitation of the genera in the Polygonaceae.

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الشكل الخارجى لحبوب اللقاح والفصل بين الأجناس فى الفصيلة الحماضية فى مصر

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تمت دراسة الصفات الظاهرية الدقيقة لحبوب لقاح ثمانية عشر نوعا تنتمى إلى سبع أجناس من الفصيلة الحماضية بمصر بواسطة كلا من المجهر الضوئى والمجهر الماسح الالكترونى فى محاولة لإظهار قيمة هذه الدراسة فى الفصل بين الأنواع المدروسة. وقد تم تمييز خمس مجاميع من حيث الشكل ومن حيث عدد فتحات الأنبات بالإضافة إلى الزخرفة السطحية لحبوب اللقاح. وقد اتصفت المجموعة الأولى: بحبوب لقاح ثلاثية الفتحات tricolporate والزخرفة السطحية (striate- reticulate) و ميزة هذه المجموعة جنس *Atraphaxis spinose* spp. والمجموعة الثانية: أمتازت بحبوب لقاح ثلاثية الفتحات والزخرفة السطحية على حبوب اللقاح كانت (reticulate to rugulo -microreticulate)، و ميزت هذه المجموعة الأنواع spp. (*Calligonum, Oxygonum sinuatum*)، المجموعة الثالثة: أتصفت بعديدة الفتحات pantoporate، ذات شكل كروى بالإضافة إلى coarse reticulate tectum وميزت هذه المجموعة أنواع *Periscaria* قيد الدراسة. أما المجموعة الرابعة تميزت بحبوب لقاح ثلاثية الفتحات والزخرفة السطحية -microechinate- foveolate وضمت هذه المجموعة أنواع *Polygonum* قيد الدراسة. المجموعة الخامسة والاخيرة فقد ضمت على أنواع *Emex and Rumex* فى مجموعة واحدة وأتصفت بحبوب لقاح ثلاثية الفتحات فى جنس *Rumex* بينما تميز جنس *Emex* ب brevicolpate بالإضافة إلى زخرفة لأسطح micro-granulate perforated tectum.

وقد أوضحت هذه الدراسة دور حبوب اللقاح فى الفصل بين الأنواع قيد الدراسة على مستوى النوع والجنس. وقد أمكن من هذه الدراسة تصميم مفتاح تعريفى للأجناس قيد الدراسة مستخدما أهم الصفات محل البحث.