



Taxonomic significance of stamens and pollen morphology of some selected taxa of Primulaceae in Egypt

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Abstract

The circumscription study of Primulaceae is still uncertain and remains controversial. Stamens and pollen grains morphological characters of five species and one variety representing five genera viz., *Anagallis*, *Lysimachia*, *Coris*, *Primula* and *Samolus* of Primulaceae in Egypt have been studied using LM and SEM. The specific target of the present study is to evaluate the taxonomic value of the macro and micromorphological characters of stamens and pollen grains in order to distinguish between the studied species. The obtained results showed remarkable variations in anther shape, anther dehiscence, filament attachment and presence of trichomes on the anther filament. Pollen grains are radially symmetrical, isopolar, spheroidal-subprolate to prolate, with amb angulaperturate or fossaperturate and tricolpate or tricolporate. Tectum is microreticulate, reticulate with perforated lumine and clavate exine ornamentation. Out of the studied taxa *Coris monspeliensis* is distinguished by the presence of prominent margo, reticulate exine ornamentation, minute luminal perforations decreasing towards the colpi, orbicular anther shape, latrorse dehiscence of anthers with short longitudinal slit and presence of glandular diseriata trichomes at the base of the filaments. Clavate exine ornamentation as well as lanceolate anther shape, extrorse dehiscence and short filament can distinguished *Samolus valerandi* from the remaining studied taxa. An artificial key for the studied species was constructed based on stamens and pollen morphological criteria is provided.

Keywords: Anther dehiscence, Exine ornamentation, Primulaceae, Pollen grain, Stamen

Introduction

Primulaceae (order Ericales) comprises about 2590 species circumscribed in 58 genera (Stevens, 2001 onwards), distributed in the Northern hemisphere. The family includes herbs, shrubs and trees. The flowers are characterized with sympetalous corolla, stamens epipetalous, opposite the petals, free central placentation, bitegmic tenuinucellate ovules, and nuclear endosperm (Källersjö *et al.*, 2000).

In Egypt, Primulaceae (including Coridaceae) are represented by five genera, two subspecies and two varieties (Täckholm, 1974), while Boulos (2000) added one more subspecies viz.: *Anagallis* L. (with two subspecies and two varieties), *Lysimachia* L., *Coris* L., *Primula* L., and *Samolus* L.

The circumscription study of Primulaceae is still uncertain and remains

controversial. Primulaceae was treated as a sister group of Maesaceae, Myrsinaceae, and Theophrastaceae in the Primulales (Anderberg *et al.* 1998, 2000 and Källersjö *et al.* 2000). Those closely related families based on morphological and molecular data were subordinated as subfamilies within Primulaceae. In this sense, Primulaceae now comprises the subfamilies Maesoideae, Myrsinoideae, Primuloideae, and Theophrastoideae (Stevens, 2001 onwards).

The floral characters have proven to be valuable in defining relationship within Primulaceae (El-Karemy & Hosni, 1993; Oh *et al.*, 2008; Morozowska *et al.*, 2011; Xu *et al.*, 2016 and Luna *et al.*, 2017). Stamens and pollen morphology have been of interest for over one century few studies on these topics have been done (Bonner and Dickinson, 1989, 1990; Keijzer *et al.*,

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1996 and Islam *et al.*, 2008). However, very few reports regarding the taxonomic significance of stamen and pollen morphology as seen by LM and SEM are available of Primulaceae. Accordingly, the present study deals with stamens and pollen grains morphology of six studied species in order to evaluate the importance of these diagnostic characters as criteria for taxonomic delimitation.

Material and methods

Herbarium and living specimens of the studied taxa of Primulaceae were collected from various sources (table 1). For LM investigation, stamens were prepared for LM and SEM investigation. Pollen grains were acetolyzed according to

Erdtman (1960). At least 30 fully developed grains per specimen were examined by using Zeiss light microscope with an eye piece micrometer. For SEM investigation, dehydrated stamens and dry pollen grains were transferred directly on a stub with double-sided tape, coated for five minutes with a thin layer of gold in a polar on JEC-1100E ion sputtering coating unite, examined at accelerating voltage of 20 Kv. and then photographed with JEOL JSM-IT200 series SEM (Electron Microscopic Unite, Faculty of Science, Alexandria University). The terminology for stamens morphology based on Endress and Stumpf (1991) and for pollen morphology on Erdtman (1952) and Punt *et al.* (2007).

Table 1. Collection data of the studied species of Primulaceae

No	Taxa	Locality/ Source / Date and Collector
1.	<i>Anagallis arvensis</i> L. var. <i>caerulea</i> Gouan.	Mediterranean Coastal region, Burg El- Arab, 2018. M. Shiha & M. Megahad s.n.(ALEX)
2.	<i>A. pumila</i> Sw.	Mediterranean Coastal region, Saloum., 21-4-2007, L. Boulos and A. Hegazy s.n.(ALEX)
3.	<i>Lysimachia linum-stellatum</i> L. (= <i>Asterolinon linum-stellatum</i> (L.) Duby	Western Mediterranean Coastal region: Amria , 25-4-2007, L. Boulos s.n.(ALEX)
4.	<i>Coris monspeliensis</i> L.	Mediterranean Coastal region, Ras El- Hekma, 24-4-1997, L.Boulos s.n. (ALEX)
5.	<i>Primula boveana</i> Decne.	Shagg Musa, Sinai, 8-5-2004, K. Shaltout s.n. (Southern Sinai Herbrrium)
6.	<i>Samolus valerandi</i> L.	Siwa Oasis: Gebel G'afar, along an irrigation canal in the farm,15-3-1998, K. Shaltout s.n. (Environmental Quality international Herb.)

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Table 2. Stamen and pollen characters of the studied taxa of Primulaceae

Taxa Characters	Stamens characters						Pollen grains characters							
	A.L. (μ m)	A.W. (μ m)	Anther Shape	Filament attachment	T.	A.D.	P.A. (μ m)	E.A. (μ m)	P/E	Shape	Amb	Type	C.L.	Exine sculpture
<i>Anagallis arvensis</i> var. <i>caerulea</i>	868.8	409.7	Sagittate	Dorsifixed long filament	G.A.M.	L.S.	28.88-30.87 (30.53)	17.22-18.01 (17.99)	1.67	Prolate	F.A.	Tricolpate	25.01-28.87 (28.62)	Microreticulate
<i>Anagallis pumila</i>	787.2	453.1	Sagittate	Dorsifixed long filament	G.A.M.	L.S.	19.01-19.93 (19.58)	14.01-14.93 (14.76)	1.32	Subprolate	A.A.	Tricolporate with Bridge	13.23-13.97 (13.85)	Microreticulate
<i>Lysimachia linum-stellatum</i>	364.4	288.9	Kidney	Basifixed short Filament	—	L.S.	21.8- 23.1 (22.51)	10.90- 11.5 (11.17)	2.01	Prolate	F.A.	Tricolpoate	18.1-19.0 (18.96)	Microreticulate
<i>Coris monspeliensis</i>	500	560	Orbicular	Basifixed- long Filament	G.D.M.	S.L.S.	24.93-25.78 (25.41)	16.92-17.83 (17.51)	1.43	Prolate	F.A.	Tricolporate	20.87-21.43 (21.25)	Reticulate - perforate lumin, double baculate muri
<i>Primula boveana</i>	2.481 mm	623.7	Lanceolate	Basifixed short filament	—	L.S.	21.83-22.67 (22.24)	18.92-19.45 (19.15)	1.16	Spheroidal - Subprolate	A.A.	Tricolporate With Bridge	16.22-17.05 (16.97)	Microreticulate (Heterobrochate)
<i>Samolus valerandi</i>	1.368 mm	378.9	Oblong	Basifixed short filament	—	L.S.	41.8-42.9 (42.35)	27.87-28.78 (28.23)	1.5	Prolate	F.A.	Tricolpate	16.9-18.9 (18.5)	Clavate

Abbreviation: A.A.= Angula Aperturate, A.D.= Anther Dehiscence, A.L. = Anther length, A.W. = Anther width, C.L. = Colpus length, E.A. = Equatorial axis, F.A.= Foss Aperturate, G.A.M.= Glandular Articulate multicellular, G.D.M.= Glandular Diserriate multicellular, L.S.= Longitudinal slit, P.A. = Polar axis, P/E= relation between polar axis and equatorial axis, S.L.S.= Short Longitudinal Slit, T.= Trichome.

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Results

A summarized stamen and pollen morphological characteristics as revealed by LM & SEM were presented in table 2. Microphotographs by SEM to show specific structures were illustrated in Figs. 1-27. The stamens and pollen morphology of the studied taxa examined using SEM show considerable variations in anther shape, filament attachment, way of anther dehiscence, pollen type, pollen aperture and exine ornamentation.

1. *Anagallis arvensis* var. *caerulea*

Stamens five, epipetalous arranged opposite the petals. Anthers sagittate shaped, 868.8 X 409.7 μm (L x W), latrorse dehiscent (the anther split positioned to the side, towards other anther) and by longitudinal slit. Filaments long dorsifixed with long glandular articulate trichomes. Pollen grains isopolar, radiosymmetric, tricolpate, prolate in shape, P/E = 1.6. The polar axis (P) length is 30.53 μm and the equatorial axis (E) is 17.99 μm ., with fossaperturate amb. Ectocolpi elongate, nearly equal to the polar axis in length, presence of margo (smooth exine area around the colpus). The exine sculpturing (tectum ornamentation) is microreticulate. Lumina regular rounded in shape with minute granules. (figs. 1, 2, 14, 15).

2. *Anagallis pumila*

Stamen anthers sagittate shaped, anther length is 787.2 μm X 453.1 μm (L X W), extrorse dehiscent (the anther face lies towards center of the flower by longitudinal slit). Filament very long and dorsifixed. Glandular articulate trichrome spread all over the longitudinal axis of the filaments. Pollen grains isopolar, radiosymmetric, tricolporate, subprolate in shape P/E = 1.33. The polar axis length is 19.58 μm and the equatorial axis 14.76 μm , with angulaperturate amb. Aperture; the endoaperture is an equatorial lalongate. Ectocolpi elongated, nearly equal to the polar axis in length (CL= 16.85). The

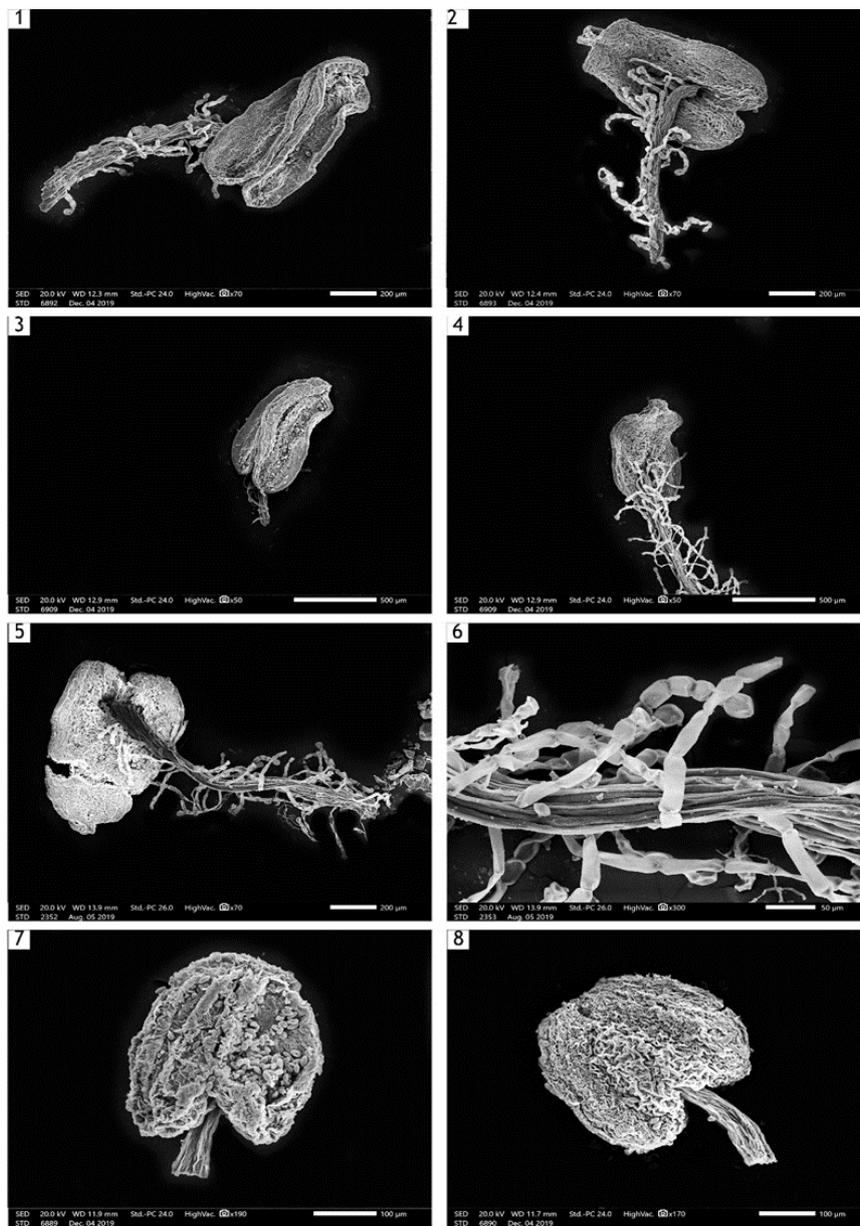
colpus margin is distinct and often raised at the equatorial forming a bridge covered the pore. The exine sculpture is microreticulate (heterobrochate). Lumina vary in shape, muri are latimurate i.e. muri are thicker than the distance cross lumina (figs. 3, 4, 5, 6, 16, 17).

3. *Lysimachia linum-stellatum*

Stamen small sized, reniform shaped, 364.4 μm X 288.9 μm (L x W), extrorse dehiscence by longitudinal slit. Filaments short, basifixed and trichomes wanting. Pollen grains isopolar, radiosymmetric, tricolpate, prolate to preprolate in shape P/E = 2.01. The polar axis length is 22.51 μm and the equatorial axis 11.17 μm , with fossaperturate amb. Ectocolpi elongated, distinctly sunken, nearly equal to the polar axis in length (CL= 18.96 μm) with acute ends. The exine sculpture is microreticulate - foveolate (heterobrochate), margo absent. Lumina vary in shape with minute granules, muri latimurate, i.e. muri thicker than the distance cross lumina (figs. 7, 8, 18, 19).

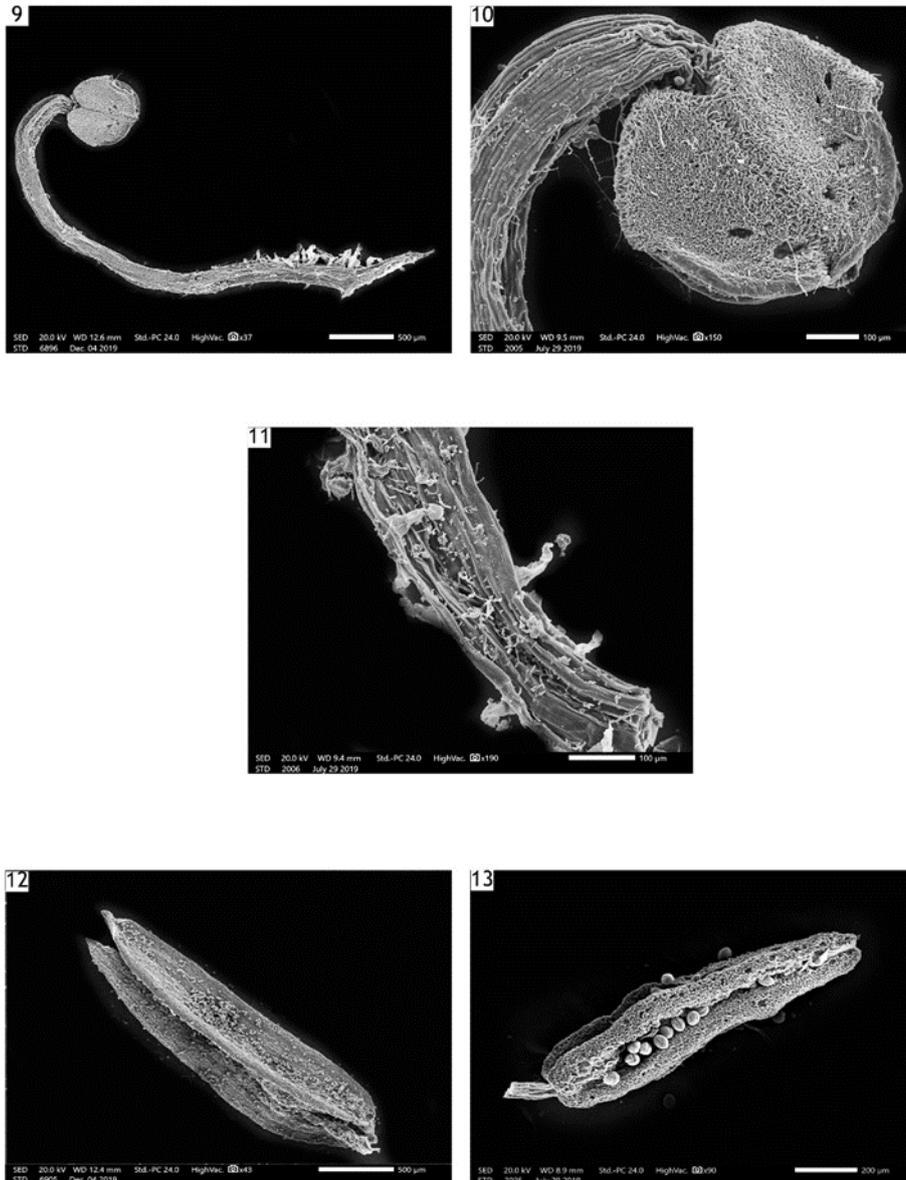
4. *Coris monspeliensis*

Stamens small, orbicular, 500 μm X 560 μm (L x W), latrorse and dehisced by short longitudinal slit. The filaments very long and basifixed. Few glandular, multicellular, diseriante trichomes detected at the base of the filaments. (figs 9, 10, 11). Pollen grains isopolar, radiosymmetric, tricolporate, prolate in shape, P/E = 1.43. The polar axis length is 33.76 μm and the equatorial axis is 23.33 μm , with angular-aperturate amb. Ectocolpi elongated, nearly equal to the polar axis in length (CL= 30.0 μm) with acute ends and small apocolpi. The colpus margin is distinct, often raised at the equator. The endoaperture lalongate, extending beyond the boundaries of the ectocolpi and covered with conspicuous operculum. The exine sculpture at mesocolpi reticulate with minute luminal perforation. However, the exine area bordering the colpi called the

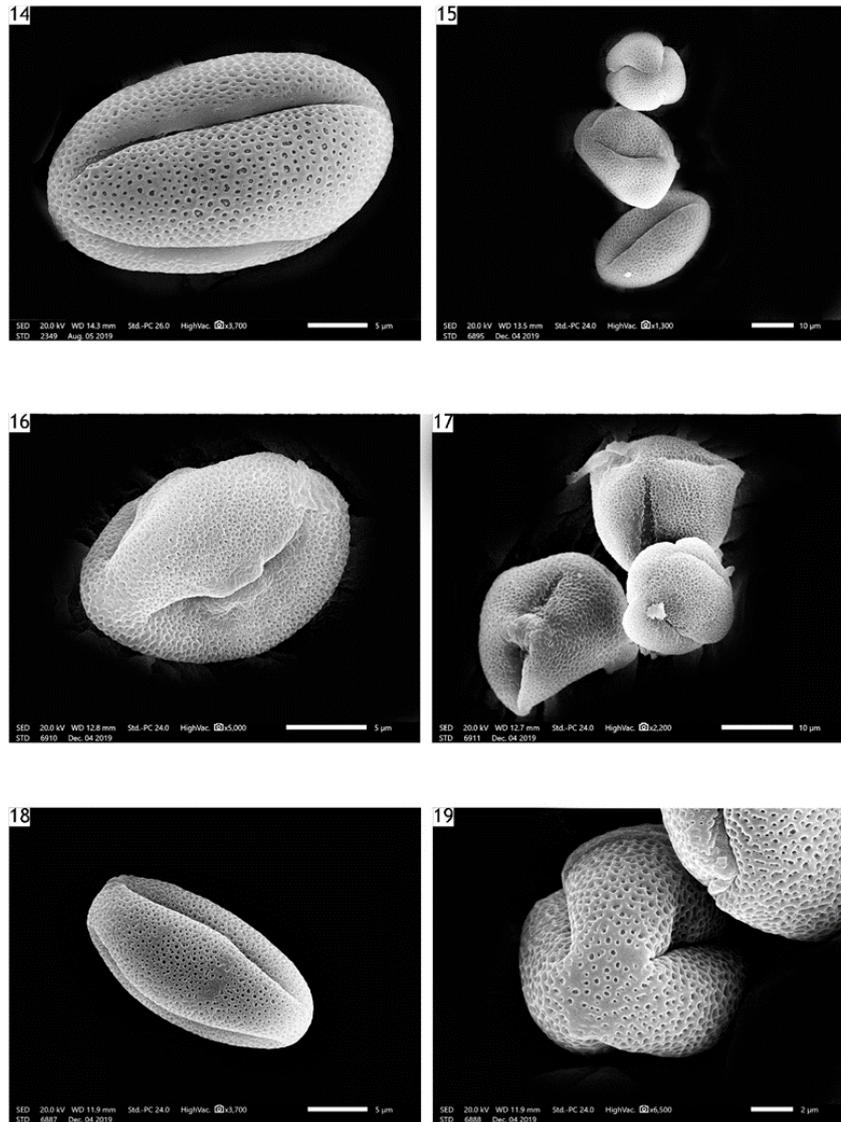


Figs 1-8. SEM micrographs of stamens in Primulaceae Figs 1, 2 *Anagallis arvensis* var *caerulea*; 1-adaxial showing longitudinal slit ,2- abaxial surface showing dorsifixed attachment of the anther, articate multicellular trichomes scattered allover the filament. Figs 3-6 *Anagallis pumila*; 3- showing longitudinal slit, 4,5 abaxial surface showing dorsifixed attachment of the anther, 6- showing articate multicellular trichomes. Figs 7,8 *Lysimachia linum-stellatum*; 7- adaxial showing longitudinal slit ,8- showing basifixed attachment.

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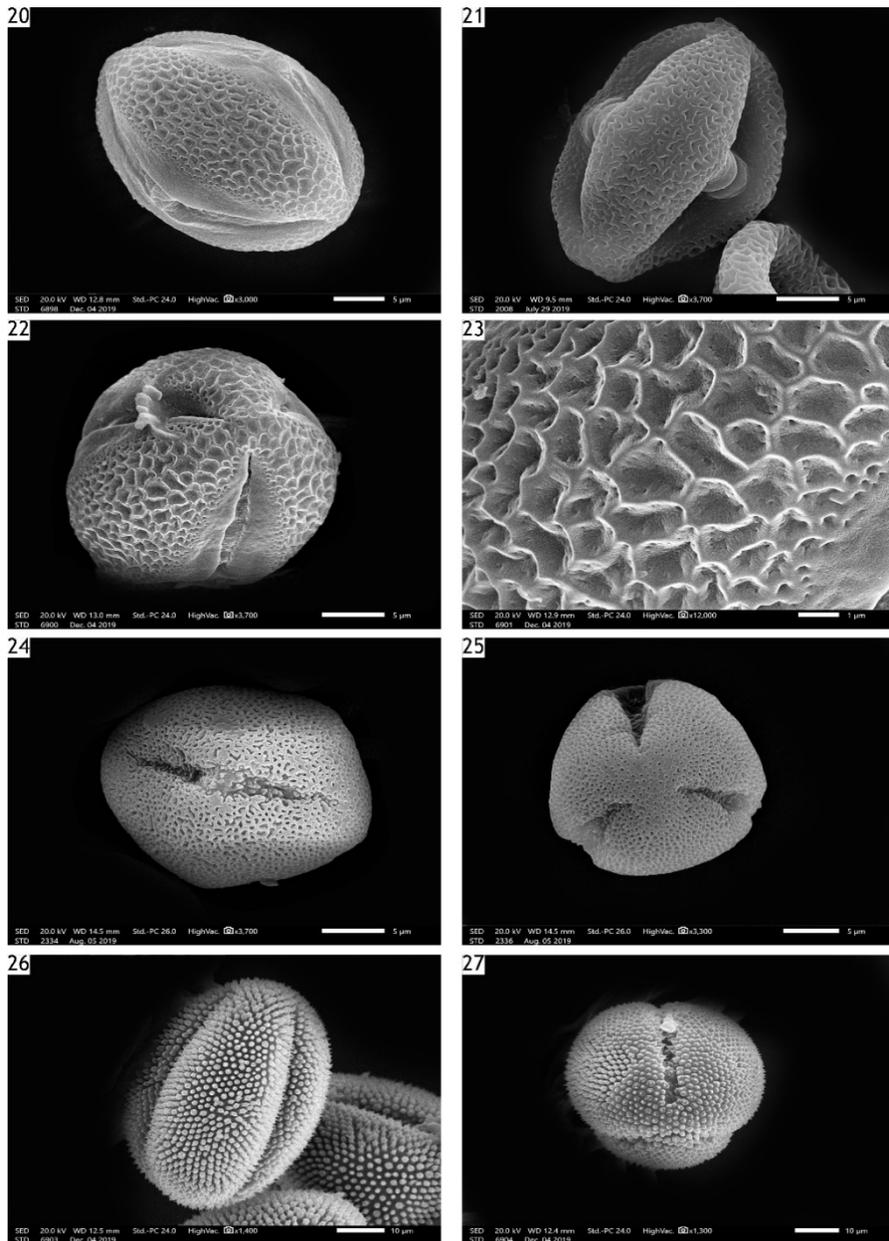


Figs 9-13. SEM micrographs of stamens in Primulaceae. Figs 9-11 *Coris monspeliensis*; 9- showing stamens with trichomes at the base of the filament, 10- showing orbicular anther shape with short longitudinal slit and basifixed attachment, 11- showing glandular diseriate trichomes. Fig 12-*Primula boveana*; showing lanceolate anther, longitudinal slit and basifixed attachment. Fig 13- *Samolus valerandi*; showing oblong anther, longitudinal slit and basifixed attachment.



Figs 14-19. SEM micrographs of pollen grains of Primulaceae. Figs 14,15 *Anagallis arvensis* var. *caerulea*;14- oblique equatorial view showing colpate, microreticulate ornamentation and presence of margo. 15- polar view showing fossaperturate amb. Figs 16,17 *Anagallis pumila*; 16- oblique equatorial view showing colporate pollen and ectexine bridge, 17- showing triangular aperture amb. Figs 18,19 *Lysimachia linum-stellatum*;18-equatorial view showing colpate and microreticulate exine ornamentation,19- polar view showing fossaperturate amb and microreticulate sculpture in the amb.

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Figs 20-27. SEM micrographs of pollen grains of Primulaceae. Figs 20-23 *Coris monspeliensis*; 20- equatorial view showing colpate pollen, reticulate exine ornamentation and presence of margo. 21- showing ectexine bridge, 22- angulaperturate amb, 23- reticulate exine with perforated lumina and double baculate muri. Figs 24, 25 *Primula boveana*; 24- equatorial view showing colpate pollen, microreticulate exine, 25- polar view showing angulaperturate amb. Figs 26, 27 *Samolus valerandi*; 26- equatorial view showing colpate pollen, clavate exine ornamentation, 27- oblique polar view showing fossaperturate amb.

margo smooth. Muri straight, polygonal, double baculate and angustimurate, i.e. muri narrower than the distance across the lumina (figs. 20, 21, 22, 23).

5. *Primula boveana*

Stamens large in size, lanceolate shape, 2.481mm X 623.7µm (LxW), extrorse and dehiscent by longitudinal slit. Filaments short and basifixed. Trichomes wanting (fig. 12). Pollen isopolar, radiosymmetric, tricolporate, spheroidal to subprolate in shape P/E= 1.16-1.3. Polar axis (P) = 22.24µm, and equatorial axis E=19.15µm with angulaperturate amb. Ectocolpi granulated and elongated with acute or rounded end. The colpus margin distinct, often raised at the equator. The endoaperture lalongate, extending beyond the boundaries of the ectocolpi and covered with conspicuous operculum. The exine sculpture microreticulate (figs. 24, 25).

6. *Samolus valerandi*

Stamen large, oblong shaped, its 1.368mm X 378.9µm (L X W), extrorse and dehiscent by longitudinal slit. Filament short and basifixed. Trichomes wanting (fig. 13) Pollen grains isopolar, radiosymmetric, tricolpate, prolate in shape P/E = 1.5. The polar axis length is 42.35 µm and the equatorial axis 28.23µm with fossaperturate amb. Ectocolpi granulate and elongated, nearly equal to the polar axis in length (CL= 35.29µm) with acute ends and small apocolpi. Exine sculpture clavate, free standing columella (figs.26, 27).

Discussion

From the foregoing data it was considered that stamens and pollen morphological characters are considered diagnostic at the generic and specific level among the studied six species of Primulaceae as examined by light LM & SEM. The taxonomic significance of pollen morphology in some members of Primulaceae has already been evident

(Anderberg and El- Gazaly, 2000; Aboel Atta and Shehata, 2003; Morozowska *et al.*, 2011 and Luna *et al.*, 2017).

Stamen morphology of the studied taxa showed great variation with regards to anther dehiscence (long or short longitudinal suture), anther attachments (basifixed and dorsifixed), anther shape (sagittate, reniform, orbicular, oblong or lanceolate) filament indumentum such as glandular articulate multicellular or glandular diserriate multicellular. Moreover the pollen grains show considerable variation with respect to the type of apertures, as well as size of pollen and exine ornamentation. The type of apertures generally two; tricolpate and tricolporate. The pollen grains shape varies from spheroidal - subprolate to prolate, radially symmetrical and isopolar, ranging in size from 19.58µm in *Anagallis pumila* to 42.35µm in *Samolus valerandi*. The colpi nearly equal the polar pollen length. The colpi well defined lack margo as in *Anagallis primula*, *Lysimachia linum-stellatum*, *Primula boveana*, and *Samolus valerandi*. Margo well defined as in *Anagallis arvensis* var. *caerulea* and *Coris monspeliensis*. The present finding is in accord with Punt *et al.* (1974) and Carrion *et al* (1993) since they described pollen morphology of *Coris* species.

Coris stamen is characterized by nearly round anther short longitudinal slit and very long filament provided with short biseriate glandular trichomes at the filament base. Pollen can be distinguished from the remaining of studied taxa of Primulaceae by the conjunction of relatively large pollen grains, prominent margo, and reticulate exine pattern with the peculiarly perforate tectum. The position of *Coris* in the Primulaceae has been acknowledged by most authors as a separate tribe Corideae (Pax, 1897 and Chant, 1978) or Coridoideae (Takhtajan, 1980) but not unanimously so. However, the family Coridaceae were accepted near the Primulaceae or inter- mediate between the

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Primulaceae and the Lythraceae (Sattler, 1962; Willis 1973; Dahlgren, 1983 and Takhtajan, 2009). Indeed, *Coris* differs in a number of conspicuous characters from the Primulaceae, such as the sub-shrubby habit, zygomorphic flower, and toothed calyx tube. Sattler (1962) found sufficient differences in the floral development of *Coris* to support the acceptance of a family Coridaceae. According the present result in addition with the previous finding of Sattler (1962), Willis (1973) and Dahlgren (1983) studying its floral development support the exclusion of *Coris* in a separate family Coridaceae.

Based on the data in the present study,

1A. Pollen grain tricolpate

1B. Exine ornamentation microreticulate

1C. Trichomes present, glandular articulate, multicellular..... *Anagallis arvensis*
var. *caerulea*

2C. Trichomes absent *Lysimachia linum-stellatum*

2B. Exine ornamentation clavate *Samolus valerandi*

2A. Pollen grain tricolporate

1D. Exine ornamentation microreticulate

1E. Anther shape sagittate, presence of glandular trichomes *Anagallis pumila*

2E. Anther shape lanceolate, absent of trichomes..... *Primula boveana*

2D. Exine ornamentation reticulate with perforated lumina..... *Coris monspeliensis*

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Samolus valerandi is characterized by the presence of staminodes opposite the sepals, antipetalous five fertile stamens, extrorse anther dehiscence and oblong anther shape and unique clavate exine ornamentation. The present result is in accord with Caris and Smets (2004) who proposed to keep *Samolus* separated from the genera of Primulaceae and Theophractaceae and suggest inclusion *Samolus* in a separate family Samolaceae.

So far as the data of the present work are concerned, the subsequent artificial key based on the stamens and pollen characters are provided to enable the different taxa of Primulaceae to be distinguished.

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