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A Contribution of the Palynological Criteria in Evaluating The Relationships among Some Species of Apocynaceae *Sensu Lato*

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APOCYNACEAE *sensu lato* is an interesting family with respect to pollen diversification. The pollen grains of 19 species (representing four out of five subfamilies and 18 genera) of Apocynaceae were collected and investigated using LM & SEM. The specific objective of the present study is to shed light on the different pollen morphological characters of the species under investigation and to discuss whether pollen morphology is considered an additional fundamental tool that helps in explanation of the evolutionary trend within the family. The obtained pollen data are considered diagnostic at the specific level *viz*. pollen association, class, polarity, sculpture, orientation and attachment of the translator. *Carissa macrocrpa* (Rauvolfioideae) gathered with the studied species of Apocynoideae based on the presence of porate pollen. In *Cryptostegia grandiflora*, the pseudo-pollinarium is considered a link between monads (as in Rauvolfioideae and Apocynoideae) and the true pollinia (as in Asclepiadoideae). Pollen criteria facilitate the construction of dendrogram, a tentative presentation of phylogenetic relationship and an artificial key to achieve further delimitation between the species under investigation and discussing the interrelationships in the view of dicta of advancement. The palynological criteria in the present study reinforced the treatment of Apocynaceae *s.l.* as two distinct taxonomic families (Apocynaceae and Asclepiadaceae).

Keywords: Apocynaceae, Asclepiadaceae, Eurypalynous, Pollen morphology, Pollinarium.

Introduction

Apocynaceae *s.l.* is a family of flowering plants that contains about 424 genus and 5100 species of evergreen trees, shrubs and herbs (Watson & Dallwitz, 1992; Li Ping-tao et al., 1995, Nazar, 2012; A.P.G., 2016). Tropical, subtropical rain forests or tropical arid environments are the suitable climatic conditions of these species; however, few species grow in temperate areas (Endress, 2000).

A controversy over the taxonomic relationship between the Apocynaceae and Asclepiadaceae persisted ever since. Cronquist (1981, 1988), Dahlgren (1983), Takhtajan (1987) and Rosatti (1989a, 1989b) treated Apocynaceae and Asclepiadaceae as two distinct families, while Judd et al. (1994), Sennblad & Bremer (1996), Struwe et al. (2004) and A.P.G. (2016) considered them as one large family (Apocynaceae).

The phylogenetic studies supported the reunion of the Apocynaceae *s.s.* and Asclepiadaceae *s.s.* in one family; the Apocynaceae; with five subfamilies *viz.* Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Sennblad & Bremer, 1996; Endress & Bruyns, 2000; Potgieter & Albert, 2001; Endress, 2004; Endress et al., 2007; Livshultz, 2010; A.P.G., 2003, 2009, 2016; Simoes et al., 2010; Nazar, 2012). Rauvolfioideae and Apocynoideae, were previously allocated in Apocynaceae *s.s.*, while Periplocoideae, Secamonoideae and Asclepiadoideae in Asclepiadiaceae *s.s.* based on androecium morphological features and system of pollen transfer (Brown, 1811; Cronquist, 1981, 1988; Takhtajan, 1987).

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In Apocynaceae there are great variation in pollen morphological features (Eurypalynous) *viz*. monad as in Rauvolfioideae and Apocynoideae, tetrad pollinarium (Pseudo- pollinarium) as in Periplocoideae or true pollinarium as in Secamonoideae and Asclepiadoideae (Van der Weide & Van der Ham, 2012; Chatterjee et al., 2014; El-Gazzar et al., 2018a, b). Monads, colporate as in Rauvolfioideae, porate as in Apocynoideae (Endress & Bruyns, 2000) or with psilate-perforate sculpture as in Apocynaceae *s.s.* (Middleton, 2007).

Pseudo-pollinarium is the arrangement of pollen cells in sticky masses of multiporate tetrads forming free pollinia without an outer wall or translator, while the true pollinia are the arrangement of pollen cells in compact sticky masses surrounded by an outer wall (Schill & Jakel, 1978; Kunze, 1993; Swarupanandan et al., 1996; Verhoeven & Venter, 2001; Wyatt & Lipow, 2007). Fishbein (2001) mentioned that Periplocoideae (pseudo-pollinarium) is more advanced than Rauvolfioideae and Apocynoideae (monad pollen), and Asclepiadoideae (true pollinia) is more advanced than Rauvolfioideae, Apocynoideae (monad) and Periplocoideae, (tetrad and pseudo-pollinarium). Pollinium characters *viz.* size, shape, colour, breadth and length of pollinium sac, breadth and length of corpusculum, pollinium orientation and attachment of translator (caudicle) are diagnostic features in Asclepiadaceae *s.s.* (Yaseen & Anjum, 2014).

Walker & Doyle (1975) and El-Atroush et al. (2015) have recognized the importance of pollen morphology in the identification and classification of flowering plants. Kuijt & Van der Ham (1997) and Erik & Raymond (2006) recognized some specific pollen characters within 42 *Alstonia* species and 19 species of Apocynaceae respectively. Several authors checked the classification for genera and species of angiosperm by analyzed their results using different numerical programs, e.g. Moawed et al. (2015). El-Gazzar et al. (2018a, b) used the numerical analysis to reveal the relationships between Apocynaceae and Asclepiadaceae according to morphological and pollen grain characters.

The specific objective of the present study is to throw light on the different pollen morphological characters of the species under investigation, to discuss whether pollen morphology is considered additional fundamental tool that can be of help in explanation of the evolutionary trend within the family.

Materials and Methods

In the present study 19 wild and ornamental species representing four out of five subfamilies of Apocynaceae *s.l.* were collected from different localities and botanical gardens from Egypt and Libya (Table.1, according to Endress et al., 2014; A.P.G., 2016).

The wild species were identified by the aid of Täckholm (1974) and Boulos (2000), while the ornamental species by Bailey (1949). Synonyms were derived from the plant list (working list of all known plant species (http://www.theplantlist.org/), GRIN (Germplasm Resource Information Network, http://www.ars-grin.gov/cgibin/npgs/html/index.pl), and IPNI (The International Plant Names Index, http://www.ipni.org/ipni/plantnamesearchpage.do). For LM investigation (BEL: B103 T-PL light microscope), un-acetolyzed pollen grains were taken from the flowers of the species under investigation, fixed in 70% alcohol, the anthers were crushed or opened carefully by needle, mounted on glass slide with few drops drop of glycerin and stained with 4% safranine solution. The microphotographs were taken using digital camera (Canon power-shot A720, 8.0 mega pixels). About seven to ten pollen grains per species were subjected to the measurements. For Scanning Electron Microscope (SEM) investigation, pollen grains were dried, mounted onto stubs then coated with gold by sputter coaster (SPI-Module) and tested with (JEOL-JSM 5500 LV) scanning electron microscope at the Regional Center of Mycology and Biotechnology, Al-Zhar University, Cairo, Egypt. The used pollen sculpture patterns were according to Stearn (1992). the pollen terminology in the present study were according to Erdtman (1952) and Punt et al. (2007). For numerical analysis, the recorded palynological characters were coded as binary codes (0, 1) and a dendrogram was constructed using the NTSYS-PC version 2.02 software program (Rohlf, 2002).

TABLE 1. Collection data according to Endress et al. (2014) and A.P.G. (2016).

Sub-family	Species	Source/ locality
Rauvolfioideae	*Acokanthera oblongifolia* (Hochst.) Benth. & Hook.f. ex B.D.Jacks. -- Gen. Pl. [Bentham & Hooker f.] 2(2): 696. 1876 [May 1876]; nom. inval. (IK) = *A. spectabilis* (Sond.) Hook.f	D
	Alstonia scholaris (L.) R. Br. -- Mem. Wern. Nat. Hist. Soc. 1: 75. 1811 [dt. 1809; issued in 1811] (IK) = *Echites scholaris* L.	A
	Carissa carandas L. -- Mant. Pl. 52. 1767 [15-31 Oct 1767] (IK) = *C. carandas* var. congesta (Wight) Bedd.	D
	Carissa macrocarpa (Eckl.) A. DC. -- Prodr. [A. P. de Candolle] 8: 336. 1844 [mid Mar 1844] (IK) = *C. praetermissa* Kupicha	F
	Cascabela thevetia (L.) Lippold. -- Feddes Repert. 91: 52. 1980 (IK) = *Thevetia peruviana* (Pers.) K.Schum.	E
	Catharanthus roseus (L.) G.Don. -- Gen. Hist. 4(1): 95. 1837 (IK) = *Vinca rosea* L.	E
	Cerbera odollam Gaertn. -- Fruct. Sem. Pl. 2: 193. 1791 (IK) = *Odollamia malabarica* Raf.	C
	Plumeria obtusa L. -- Sp. Pl. 1: 210. 1753(IK) = *P. apiculata* Urb.	E
	Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult. -- Syst. Veg., ed. 15 bis [Roemer & Schultes] 4: 427. 1819 (IK) = *T. citrifolia* Lunan	D
Apocynoideae	*Adenium obesum* Roem. & Schult. -- Syst. Veg., ed. 15 bis [Roemer & Schultes] 4: 411. 1819 (IK) = *A*. arabicum Balf. F	F
	Nerium oleander L. -- Sp. Pl. 1: 209. 1753 [1 May 1753] (IK) = *N. carneum* Dum.Cours.	E
	Wrightia coccinea (Roxb. ex Hornem.) Sims. -- Bot. Mag. 53: t. 2696. 1826 (IK) = *Nerium coccineum* Roxb. ex Hornem.	C
Asclepiadoideae	*Asclepias curassavica* L. -- Sp. Pl. 1: 215. 1753 [1 May 1753] (IK) = *A. margaritacea* Hoffmanns. ex Schult.	F
	Calotropis procera W.T. Aiton. -- Hort. Kew., ed. 2 [W.T. Aiton] 2: 78. 1811 (IK) = *C*. persica Gand.	H
	Cynanchum acutum L. -- Sp. Pl. 1: 212. 1753 [1 May 1753] (IK) = *C. acutum* var. *longifolium* (G.Martens) Ledeb.	B
	Gomphocarpus sinaicus Boiss. -- Diagn. Pl. Orient. ser. 1, 11 : 80. 1849 [Mar-Apr 1849] (IK) = *Asclepias sinaica* (Boiss.) Muschl	G
	Huernia andreaeana (Rauh) L.C.Leach. -- J. S. African Bot. 40 (1): 21. 1974 (IK) = *H*. appendiculata Berger.	H
	Solenostemma argel (Delile) Hayne. -- Getreue Darstell. Gew. ix. t. 38.1825. (IK) = *S. acutum* (L.) Wehmer.	A
Periplocoideae	*Cryptostegia grandiflora* Roxb. ex R. Br. -- Bot. Reg. 5 : t. 435. 1820 [1819 publ. 1820] (IK) = *C. grandiflora* Roxb. ex R.Br.	A

A, Agriculture Museum Garden; B, Al-Azhar University; C, Al-Zohriya Garden, Gizzira; Cairo; D, Botanical Garden, Botany Department, Faculty of Science, Ain Shams University, Alabbassia, Cairo; E, Botanical Garden, Botany Department, Al-Zawia University in Libya; F, Orman Botanical Garden, Giza; G, Saint Katherine, South Sinai; H, Western Mountain in Libya.

Results

In the present study the pollen data of 19 studied species are summarized in Table 2 and the specific structures in Plate I; text Fig. a-p. Pollen grains monad as in 12 studied species (belonging to Rauvolfioideae and Apocynoideae) or pollinarium as in seven studied species (belonging to Asclepiadoideae and Periplocoideae).

For monad pollen grain; shape class; oblate spheroidal & sub-oblate as in *Carissa carandas*, prolate as in *Plumeria obtusa*, prolate spheroidal as in as in four studied species or oblate spheroidal as in the rest six studied species. Pollen size; small in *Alstonia scholaris, Nerium oleander* & *Tabernaemontana divaricata*, small-medium as in *Carissa carandas*, medium as five studied species or large as in *Carissa macrocarpa, Cerbera odollam & Cascabela thevetia*,. Pollen class, colpate as in *Catharanthus roseus* & *Plumeria obtusa*, colpate and colporate as in *Carissa carandas*, colporate as in five studied species or porate as in four studied species. Shape of pollen in polar view; circumaperture as in *Nerium oleander* & *Wrightia coccinea* or angulaperture as in the rest ten studied taxa. Shape of pollen in equatorial view; spheroidal & sub-spheroidal as in *Carissa carandas*, elliptic as in *Catharanthus roseus*, subspheroidal in *Cascabela thevetia*, *Wrightia coccinea* or spheroidal in the rest eight studied species. Polarity; apolar as in *Adenium obesum*, *Carissa macrocarpa*, *Nerium oleander* & *Wrightia coccinea* or isopolar as in the rest eight studied species. Aperture; simple as in six studied species, simple and composite as in *Carissa carandas* or composite as in five studied species. Sculptur; glebulate as in *Alstonia scholaris*, scabrate-foveate as in *Cerbera odollam*, foveolate as in four studied species or psilate as in the rest six studied species. Columellae, distinct in all studied species.

For pollinarium; Pollinium shape; spoon-like (pseudo-pollinarium) as in *Cryptostegia grandiflora*, obovate as in *Calotropis procera*, long ovate as in *Solenostemma argel*, ovate as in *Huernia andreaeana* & *Cynanchum acutum* or oblong-ovate as in *Asclepias curassavica* & *Gomphocarpus sinaicus*. Pollinium colour; creamy as in *Calotropis procera*, brown as in *Solenostemma argel*, yellow as in *Asclepias curassavica* & *Gomphocarpus sinaicus* or brownish yellow as in *Cynanchum acutum, Cryptostegia grandiflora* & *Huernia andreaeana.* Pollinium orientation; erect as in *Cynanchum acutum* or pendulous as in *Asclepias curassavica*, *Gomphocarpus sinaicus*, *Calotropis procera, Huernia andreaeana* & *Solenostemma argel.* Pollinium – translator attachment; apical as in *Huernia andreaeana,* basal in *Cynanchum acutum*, *Asclepias curassavica*, *Gomphocarpus sinaicus*, *Calotropis procera* & *Solenostemma argel* or wanting as in *Cryptostegia grandiflora*. Pollinium surface sculpture; tuberculate as in *Cryptostegia grandiflora*, reticulate as in *Cynanchum acutum & Huernia andreaeana*, colliculate as in *Calotropis procera,* reticulate-foveate as in *Gomphocarpus sinaicus* or psilate in *Asclepias curassavica* & *Solenostemma argel*. Wing; present as in *Huernia andreaeana* or not detected in the rest six studied species.

Discussion

In the present study the palynological data were coded as binary codes (0, 1) then subjected for computation using the NTSYS-PC version 2.02 software program (Rohlf, 2002). From the obtained dendrogram (Fig. 1), the species under investigation separated under two series; series I and II. Series I comprises 12 out of the studied species (belonging to Rauvolfioideae and Apocynoideae) at a taxonomic distance 0.81 based on the presence of monad pollen grains. Series II comprises the remaining seven studied species (belonging to Asclepiadoideae and Periplocoideae) at taxonomic distance 0.72 based on the presence of pollinarium. The separation of the studied species into series I & II is in accord with Brown (1811), Cronquist (1981, 1988), Dahlgren (1983), Takhtajan (1987) and Rosatti (1989a, b).

Series I divided into two subseries; subseries A and B. Subseries A divided into two clusters; Cluster 1 and 2. The former cluster includes *Carissa carandas* due to the presence of two pollen class; colpate and colporate with simple and composite apertures. Cluster 2 includes five studied species due to sharing in colporate pollen grain with composite apertures. Subseries B divided into two clusters, 3 and 4. The former cluster includes *Catharanthus roseus* and *Plumeria obtusa* due to sharing in colpate pollen and isopolar. Cluster 4 includes four studied species due to sharing in porate pollen and apolar pollen grain.

TABLE 2. Palynological characters of the studied species of Apocynaceae *s.l.*

A. Monads									
Sp. \ C.	Dimension (µm)	Shape class P\E x100	Pollen size 100P+E\	Pollen class	Shape in polar view	Shape in equatorial view	Polarity	Aperture	Sculpture
Acokanthera oblongifolia	P= 31.5 E= 32	Oblate Spheroidal	Medium	Trizono colporate	Angulaperture	Spheroidal	Isopolar	Composite ectoapertures: colpus slit - like with roundred end	Foveolate
Adenium obesum	P= 31.7 E = 34.7	//	//	Pantoporat 4-6	//	//	Apolar	Simple, ectoapertures: circular leveled.	Psilate
Alstonia scholaris	P= 22 E = 23	//	Small	Trizono colporate	//	//	Isopolar	Composite ectoapertures: colpus fusiform with rounded end	Glebulate
Carissa carandas	P =25 E = 26.5	Oblate spheroidal	Medium	Trizono colporate	//	Spheroidal	//	Composite ectoapertures: colpus -fusiform with tapered end	Foveolate
	P =17.5 E = 22	Sub-oblate	Small	Trizono colpate		Sub spheroidal		Simple ectoapertures: colpus slit-like with rounded end	
Carissa macrocarpa	P=47.2 E = 45.4	Prolate Spheroidal	Large	Pantoporat 6-10	//,	spheroidal	Apolar	Simple ectoapertures:circular and leveled	Psilate
Cascabela thevetia	P= 64 E = 64.6	Oblate Spheroidal	//	Trizono colporate	//	Sub spheroidal	Isopolar	Composite ectoapertures:colpus slit-like with rounded end	Foveolate
Catharanthus roseus	P=41 E = 43.3	//	Medium	Trizono colpate	//	Elliptic	//	Simple ectoapertures: colpus slit-like with tapered end,	//
Cerbera odollam	P=73 E = 69	Prolate Spheroidal	Large	Trizono colporate	//	Spheroidal	//	Composite ectoapertures: colpus slit -like with tapred end	Scabrate-foveate
Nerium oleander	P= 24 E = 24	Oblate Spheroidal	Small	Pantoporat 4-6	Circumaperture	//	Apolar	Simple ectoapertures: circular, sunken	Psilate
Plumeria obtusa	P= 37 E = 20	Prolate	Medium	Trizono colpate	Anglulaperture	//	Isopolar	Simple ectoapertures: colpus slit-like with rounded end	//
Tabernaemontana divaricata	P= 25.6 E = 23	Prolate spheroidal	Small	Trizono colporate	//	//	//	Composite ectoapertures: colpus short	//
Wrightia coccinea	P= 32.5 E = 30	//	Medium	Pantoporat 4-6	Circumaperture	Sub spheroidal	Apolar	Simple ectoapertures: circular, sunken	//

(//): As previous

TABLE 2 Cont.

	B. Pollinarium											
Sp. \ C	Length of pollinium sac (µm)	Breadth of pollinium sac (µm)	Length of translator sac (µm)	Breadth of translator sac (µm)	Length of Corpusculum sac (µm)	Breadth of Corpusculum sac (µm)	Shape of pollinium sac	Colors of pollinium	Orientation of pollinium	Translator attachment to the pollinium	Surface sculpture	Wing
Asclepias curassavica	933	346.6	493	133	440	266.6	Oblong-ovate	Yellow	Pendulous	Basal	Psilate	_
Calotropis procera	1035	517.6	411	58.8	470	200	Obovate	Creamy	//	//	Colliculate	//
Cryptostegia grandiflora	1666.6	1145.8	833.3	416.6	_	_	Spoon	Brownish yellow	_	_	Tuberculate	//
Cynanchum acutum	311	194.4	100	55.5	255.5	166.6	Ovate	Brownish yellow	Erect	Basal	Reticulate	//
Gomphocarpus sinaicus	847	235.2	294	70.5	258.8	82.3	Oblong-ovate	Yellow	Pendulous	//	Reticulate-foveate	//
Huernia andreaeana	323.5	258.8	100	41	176.4	117.6	Ovate	Brownish yellow	//	Apical	Reticulate	+
Solenostemma argel	909	200	300	90.9	345.4	181.8	Long-ovate	Brown	//	Basal	Psilate	_

Small (10-24µm); Medium (25-49µm); Large (50-99µm); Very short (50-199µm); Short (200-399µm); Long (400 -599µm); Very long up 600µm; C: Character; Sp.: Species; +: Present; -: Absent.

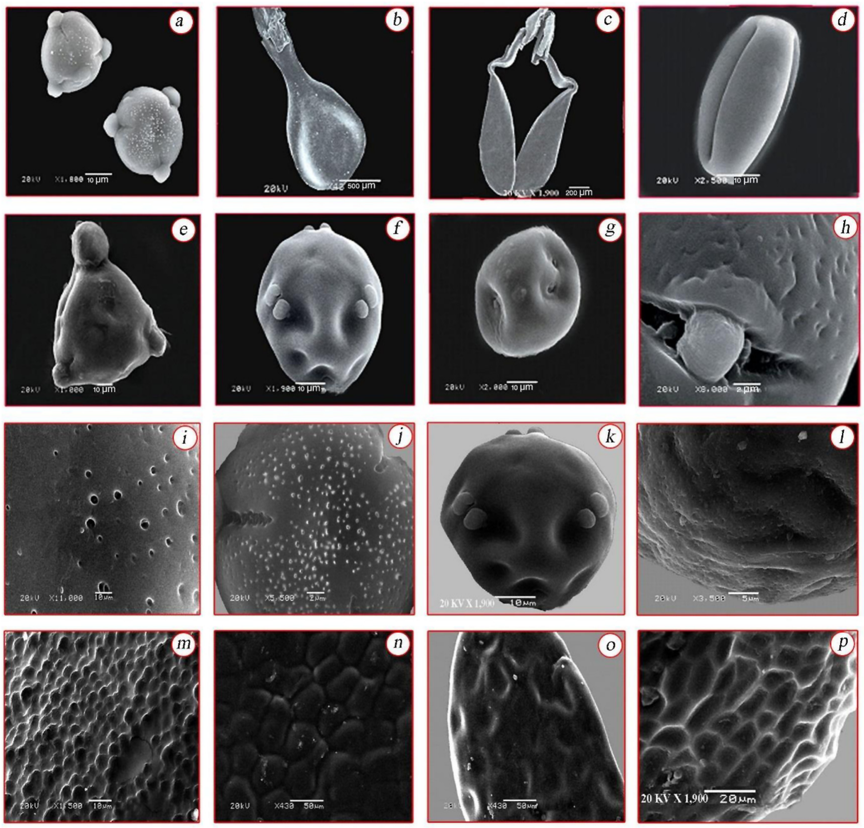

Plate I. (a-p) SEM photomicrograph of pollen morphology [a. Monad pollen in *Alstoniascholaris*; b. Pseudo-pollinium in *Cryptostegia grandiflora*; c. True pollinarium in *Asclepiascurassavica*; d. Colpate pollen in *Plumeriaobtusa*; e. Composite aperture in *Cascabelathevetia*f. Porate pollen in *Carissa macrocarpa*; g. Simple aperture in *Adeniumobesum*; h. Composite aperture & isopolar in *Acokantheraoblongifolia*; i. Foveolate sculpture in *Catharanthusroseus*; j. Glebulate in *Alstoniascholaris*; k. Psilate in *Carissa macrocarpa*; l. Scabrate-foveate in *Cerberaodollam*; m. Tuberculate in *Cryptostegia grandiflora*; n. Colliculate in *Calotropisprocera*; o. Reticulate-foveate in *Gomphocarpussinaicus*; p. Reticulate in *Huerniaandreaeana*].

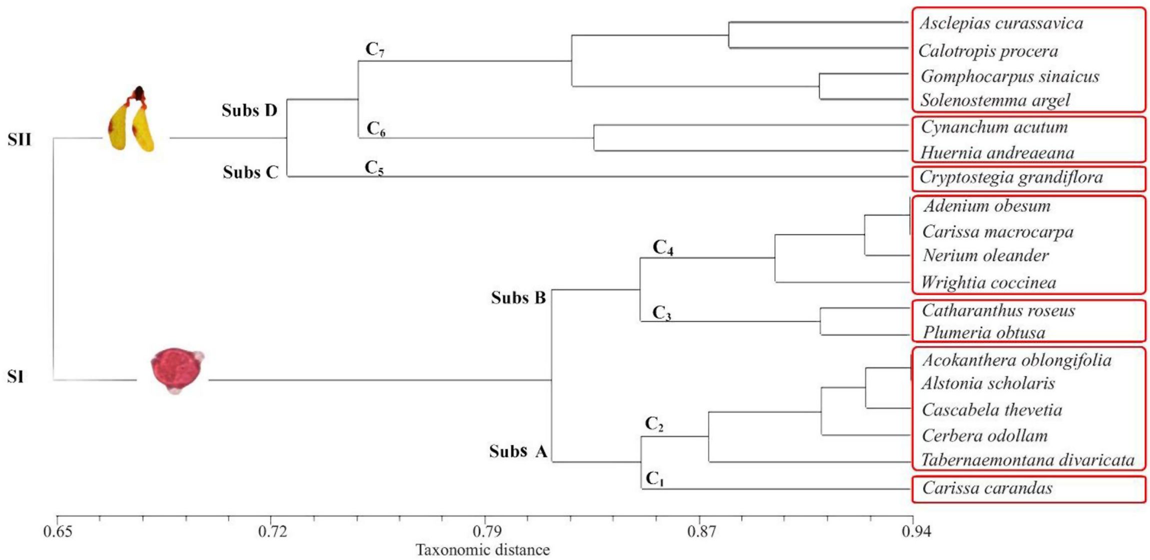

Fig. 1. Dendrogram showing the relationship between the studied species based on pollen criteria [S: Series; Subs: Subseries; C: Cluster].

The separation of series I into two subseries, A & B based on aspect of pollen class indicated that, the pollen class in studied species belonging to Rauvolfioideae show colpate and colporate manner except in *Carissa macrocarpa* (porate pollen class) which gathered with the studied species of Apocynoideae (porate pollen class). This is in accord with Endress & Bruyns (2000), Enderson et al. (2002) and Middleton (2007). Fishbein (2001) stated that Rauvolfioideae is considered the most basal subfamily of Apocynoideae based on pollen class from colpate and colporate vs porate.

Series II divided into two subseries C and D. Subseries C (cluster 5) includes *Cryptostegia grandiflora* (Periplocoideae), due to the presence of pseudo-pollinarium. Subseries D divided into two clusters: 6 & 7 (species with true pollinia). The former cluster includes *Cynanchum acutum* and *Huernia andreaeana* based on small sized pollinia with ovate shape pollinial sac, erect pollinium with basally attached translator (*Cynanchum acutum*) or apical attachment winged pollinarium (*Huernia andreaeana*). Cluster 7 gathered *Asclepias curassavica, Calotropis procera, Gomphocarpus sinaicus* and *Solenostemma argel,* all are sharing the features of pollinarium with pendulous orientation and basal attachment of translator. Pollinial sac shapes varied from ovate as in *Cynanchum acutum*, oblong ovate as in *Asclepias curassavica*, obovate as in *Calotropis procera* or spoon like as in *Cryptostegia grandiflora.* This is in accord with Mo et al. (2010), and Sinha & Mondal (2011), who stated that, the Asclepiadoideae showed great variation in shape of pollinial sac.

The separation of subseries D into cluster 6 & 7 (on the bases of pollinium orientation and translator attachment) is in agreement with Swarupanandan et al. (1996) and Sinha & Mondal (2011). The position of translators whether basal or apical and orientation of pollinia (erect, horizontal or pendulous) are considered diagnostic features for classification of Asclepiadiaceae *s.s.* (Brown, 1811; Swarupanandan et al., 1996; Endress & Bruyns, 2000).

The separation of the studied species of series II into two subseries are in accordance with El-Gazzar et al. (1974), Schill & Jäckel (1978), Arekal & Ramakrishna (1978, 1979, 1980).

From the foregoing pollen criteria in the present study, *Cryptostegia grandiflora* is considered a transition state between the studied species of Rauvolfioideae, Apocynoideae and Asclepiadoideae and this is in accordance with Verhoeven & Venter (2001) and Wyatt & Lipow, (2007). Comparison of the evolutionary trends of the most specific pollen parameters encourage the construction of an evolutionary proposed tentative presentation (Fig. 2) and an artificial key to render identification and separation between the studied species more easily and accurate.

Among taxonomists a great argument has always been arising with respect to the taxonomic character as regard its weight and consistency. For instance, in the present work, the pollen criteria lead to the following diagnostic and evolutionary trends, which can be summarized in the following points through the line of advancement (primitive *vs.* advanced) based on the dicta of evolution (Bessey ,1915).

1. Monads (Rauvolfioideae and Apocynoideae) vs. true pollinia (Asclepiadoideae) through pseudo-pollinarium as in *Cryptostegia grandiflora* (Periplocoideae) (Verhoeven & Venter ,2001; Wyatt & Lipow, 2007).

2. Colpate pollen vs. colporate vs. porate. *Carissa carandas* (colpate and colporate pollen) is considered as intermediate stage between colpate and porate (new observation and record). In *Carissa macrocarpa* (Rauvolfioideae), the porate pollen encourage the gathering of it with the studied species of Apocynoideae (Walker & Doyle, 1975; Moore et al.,1991).

3. Aperturate pollen is from simple vs. composite (Walker & Doyle, 1975; Moore et al., 1991).

4. Isopolar vs. apolar (Walker & Doyle, 1975).

5. Large vs. small sized pollen (Ezcurra, 1993; Ueckermann &Rooyen, 2000).

6. Sculpture of monad pollen is from psilate vs. scarbate-foveate, vs. foveolate vs. glebulate (week diagnostic character) (Walker, 1974; Walker & Doyle,1975; Walker & Skvarla, 1975).

An artificial key of the studied species of Apocynaceae *s.l.* based on pollen characters.

1. Monad	2
+. Pollinarium	13
2. Apolar, porate pollen	3
+. Isopolar, colpate or colporate pollen	6
3. Pollen large sized	*Carissa macrocarpa*
+. Pollen medium or small sized	4
4. Spheroidal pollen	*Nerium oleander*
+. Otherwise	5
5. Oblate spheroidal pollen	*Adenium obesum*
+. Prolate spheroidal pollen	*Wrightia coccinea*
6. Pollen of two types (colpate&colporate)	*Carissa carandas*
+. Pollen one type (colpate or colporate)	7
7. Colpate pollen	8
+. Colporate pollen	10
8. Elliptic shape	*Catharanthus roseus*
+. Spheroidal shape	9
9. Scabrate-foveolate sculpture	*Cerbera odollam*
+. Psilate sculpture	*Plumeria obtusa*
10. Small pollen sized	11
+. Medium or large sized pollen	12
11. Glebulate sculpture	*Alstonia scholaris*
+. silate sculpture	*Tabernaemontana divaricata*
12. Pollen large sized	*Cascabela thevetia*
+. Medium sized	*Acokanthera oblongifolia*
13. Pollinarium winged	*Huernia andreaeana*
+. Pollinarium wingless	14
14. Pollinarium spoon shape & corpusculum absent	*Cryptostegia grandiflora*
+. Pollinarium otherwise & corpusculum present	15
15. Pollinium erect	*Cynanchum acutum*
+. Pollinium pendulous	16
16. Pollinium sculpture tuberculate	*Calotropis procera*
+. Pollinium sculpture otherwise	17
17. Pollinium sac, brown	*Solenostemma argel*
+. Pollinium sac, yellow	18
18. Psilate sculpture	*Asclepias curassavica*
+. Reticulate-foveate sculpture	*Gomphocarpus sinaicus*

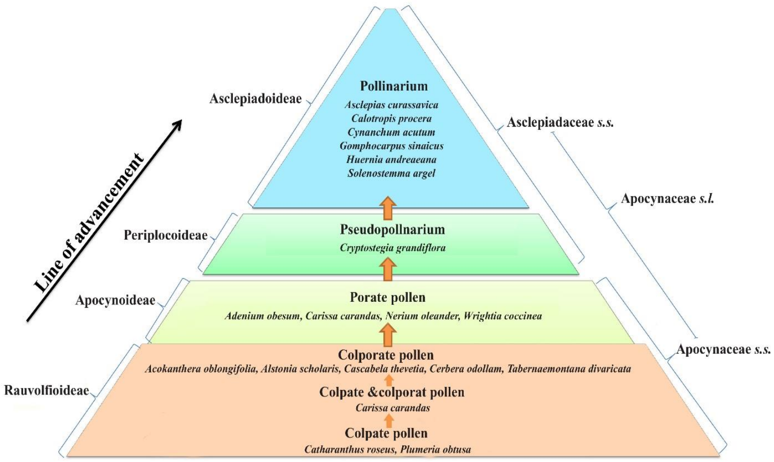

Fig. 2. Proposed schematic presentation illustrating the line of advancement within the studied species based on the pollen class based on the dicta of evolution of Bessey (1915).

Conclusion

In the present study the most specific pollen characters of the studied species reinforced the treatment of Apocynaceae *s.l.* as two distinct families (Apocynaceae and Asclepiadaceae).

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مساهمة دلالات حبوب اللقاح في تقييم العلاقات بين بعض الأنواع من الفصيلة السوسبية بمفهومها الواسع

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تعتبر الفصيلة الدفلية بمفهومها الواسع من الفصائل الجاذبة للإهتمام إستنادا لتنوع اشكال حبوب اللقاح. وقد شملت هذه الدراسة حبوب لقاح لتسعة عشر نوعا (تنتمي لأربعة تحت فصائل وثمانية عشر جنسا). تم تجمع وفحص صفات حبوب اللقاح باستخدام الميكروسكوب الضوئى والالكترونى الماسح. تهدف الدراسة إلى إلقاء الضوء على التباين فى صفات حبوب اللقاح ومدى مساهمتها في الفصل بين الانواع قيد الدراسة و تقييم الوضع التصنيفى للفصيلة الدفلية بمفهومها الواسع. وقد خلصت الدراسة إلى مجموعة من الصفات التشخيصية على سبيل المثال: أشكال، تراكيب وتجمعات حبوب اللقاح، الزركشة، التباين فى الفتحات والثقوب على السطح الخارجي وكذلك إتجاه وطريقة ربط الناقل في البولينيات. وقد أظهرت صفات حبوب اللقاح في نوع *Carissa macrocrpa* التابعة لمجموعة (Rauvolfioideae) ضمها مع الأصناف المدروسة في مجموعة (Apocynoideae) بناءا على طريقة التثقيب على سطح الخارجى. كما أظهر شكل البولينيا في نوع *Cryptostegia grandiflora* حالة ربط بين ألانواع منفردة حبوب اللقاح (Monads) التابعة لتحت الفصيلة (Apocynoideae & Rauvolfioideae) و متجمعة حبوب اللقاح فى شكل بولينيا (Pollinia) التابعة لـ (Asclepiadoideae). تم إخضاع الصفات المستخلصة لبرنامج (NTSY, 2.02) والتحصل على شجرة تصنيفية بالإضافة إلى عمل رسم توضيحى تطورى وتصميم مفتاح اصطناعى لمناقشة العلاقات بين الأنواع المدروسة. وقد خلصت الدراسة إلى إعتبار الفصيلة الدفلية بمفهومها الواسع وحدتين تصنيفيتين منفصلتين (الفصيلة الدفلية والعشارية).