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Pollen morphological study in some *Bauhinia* L. species and their phylogenetic indications

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Abstract
Thirteen *Bauhinia* species and two varieties have been subjected in this investigation. Pollen morphological characters studied and photographed using both light and scanning electron microscopes. Identification keys, as well as clustering analysis of the studied taxa according to the pollen characters have been done. This study reveals that the pollen grains of *Bauhinia* species have variable pollen morphological features, with a very wide range of characters such as different dispersal, polarity, symmetry, shape, variable exine ornamentation, different type and number of apertures. Postulation of the evolutionary line within the studied species has been mentioned. These variations confirm the eurypalynous status of the genus.

Keywords: *Bauhinia*- classification- palynology- phylogeny- taxono

Introduction
Genus *Bauhinia* is one of the largest genera belonging to subfamily Caesalpinoideae tribe Bauhinieae (Cercideae), subtribe Bauhiniinae as given by the Legume Phylogeny Working Group [1]. This subfamily belongs to the most diverse family, Leguminosae which has more than 727 cosmopolitan genus and 19,500 species with high economic importance [2]. This subfamily is subdivided into four tribes: Cercideae, Detarieae, Cassieae, and Caesalpinieae [2]. Members of this tribe characterize by their leaf morphological characters. Wunderlin et al. [3] recog- nised two subtribes: Cercidinae (including the genera *Cercis* L., *Adenolobus* (Harvey ex Benth. & Hook.) Torre & Hillc., and *Griffonia* Baill.) and Bauhiniinae (including the genera *Brenierea* Humbert and *Bauhinia* L.). The division of the species under the genus *Bauhinia* has been faced with many controversial opinions [2, 3, 4, 5, 6, 7 & 8]. Duarte-Almeida *et al.* [9] divided the whole *Bauhinia* species into four subgenera: *Barklya* (1 species), *Bauhinia* (140 species), *Elayuna* (6 species), and *Phanera* (150 species). The latter subgenus characterizes by tendril-bearing species, while the three former taxa comprise tree or shrubby species. All the previous classification of the genus relayed on the differences in the morphological characters and to limited extent in the volatile oils [9]. In fact, the taxonomic history of *Bauhinia s.l.* is complex. It has been subjected to large number of taxonomic studies in which it has been recognized either as a single large genus comprising 300 to 350 species e.g. [4], or as several distinct genera e.g. [2 & 5].
Palynological studies of the leguminous family recognized different types of dispersals; monads, dyads, tetrads and polyads; with tricolpate or tricolporate apertures and either reticulate or foveolate exine [10, 11]. The pollen grains of tribe Cercideae has long been known to be diverse and variable. Banks *et al.* [12] described the pollen grains of this tribe by being isopolar, tectate, tricolporate, and released in monads. Surface ornamentation may be micro-reticulate or perforate, and psilate to rugulate. Aperture membranes are granular to coarsely granular. The pollen grains of the *Bauhinia* species have the same characteristic features of the leguminous plants. Studies on the pollen grains of the species belonging to this genus done by [11] mentioned that their pollens shed as monads with variations in exine sculptures. Study done by [13] recognized two types of apertures; colpate and porate; within the pollen grains of *Bauhinia* species. [12] Recognized five pollen structures within the Cercideae clade that is restricted to *Bauhinia s.s.* These include striate ornamentation, having more than three apertures per grain, apertures that are indistinct, and colpate apertures. These variations in the pollen characters can be used as good taxonomical ones in the classification of the genus. Accordingly this investigation carried out to study the pollen grain characters, within thirteen cultivated *Bauhinia* species and one form cultivated in the Egyptian roads and gardens. Meanwhile, this study traces the evolutions within the pollen grains of the studied species, using both Light and Scanning electron microscopes.

Materials and Methods
Mature unopened flower buds were taken from the herbarium specimens of fourteen taxa; thirteen species and two varieties; listed in table 1, opened carefully under the stereomicroscope and the anthers inserted in glass test tubes for acetolysis. The acetolysis procedure was according to [14] for light microscope investigation. Thirty

pollen grain; from each species; have been examined, measured, and photographed using OPTICA (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software. For SEM investigation unacetolysed pollen grains have been sputtered onto cleaned, labeled stubs, coated with 30 nm Gold in a Polaron JFC-1100 coating unit, examined and photographed using JEOL-JSM.I T200 Series Scanning Electron Microscope allocated in the electron microscope unit, Faculty of Science, Alexandria University, Egypt. The stubs were examined carefully and at least ten pollen grains, in each taxon were examined and photographed to ensure the variability in each taxon. The terminology used here are according to [15].
The studied pollen characters subjected to PAST program v.3. to estimate the relationship between the characters as well as between the studied taxa.

Table 1: Studied species, information of the herbarium sheets, source of materials, confirmation of nomenclature and synonyms.

No	Taxa	Collectors & date of collection	Source of materials	Confirmation of nomenclature	Synonyms
1	*B. acuminata L.*	Riham Mahdy 5/7/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia",IPNI	*B. linnaei* Ali *B. acuminata* Vell.
2	*B. blakeana* Dunn.(hybrid)	Riham Mahdy 5/12/2020	Giza:Mazhar botanical garden	The national flowers of Hong Kong. Lau *et al.* (2005).	No
3	*B. forficata* J.H.F.	Riham Mahdy 13/9/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	*B. candicans* Benth. *B. breviloba* Benth. *B. forficata subsp. forficata Basionym Pauletia forficata* (Link) A. Schmitz
4	*B. galpinii* N.E. Br.	Riham Mahdy and Al-Shaarawy 12/7/2020	Al-Abeed Agriculture Farm	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	*B.galpinii var. galpinii Perlebia galpinii* (N.E.Br.) A.Schmitz
5	*B.glabra* Jack	Riham Mahdy 12/3/2020	Giza:Mazhar botanical garden	Catalogue of life check list, The NY Bot. Gard.	*B.heterophylla* Kunth *Schnella glabra* (Jacq.) Dugand
6	*B. grandidieri* Baill	Riham Mahdy 5/7/2020	Giza:Mazhar Bot.Gard.	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	No
7	*B. grevei* Drake	Riham Mahdy 5/7/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	No
8	*B.madagascariensis* Desv.	Riham Mahdy 5/7/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	*B.commersonii* Scott-Elliot

9	*B.monandra* Kurz	Riham Mahdy 22/11/2020	Giza: Orman botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	No
10	*B. purpurea* L.	Riham Mahdy 7/3/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	*B.platyphylla* Zipp. ex Span. *B.triandra* Roxb. *B.castrata* Blanco
11	*B. tomentosa* L.	Riham Mahdy 5/7/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	No
12	*B. vahlii* Wight & Arn.	Riham Mahdy 8/3/2020	Giza:Mazhar botanical garden	"ILDIS LegumeWeb entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	*B.racemosa* Vahl *Phanera vahlii* (Wight & Arn.) Benth.
13	*B.variegata* (L.) Benth.	Riham Mahdy 7/5/2020	Giza: Mazhar botanical garden	Plants of the world on line	*Phanera varigata* (L.) Benth.
14	*B.variegata* (L.) form *alba*	Riham Mahdy 8/3/2020	Doki: near Russian Center	"ILDIS Legume Web entry for Bauhinia"; USDA, ARS, National Genetic Resources Program; "The Plant List entry for Bauhinia"	No

Results

The results obtained are summarized in tables 2and 2 cont. and illustrated in Plates 1,2 and 3. The pollen grains of the studied *Bauhinia* species dispersed as monads, except *B.glabra* they dispersed as polyads (Plate 2, Photo 9). The pollen grains are isopolar radial symmetric, except in *B.glabra* and *B.grandidieri* they are heteropolar bilateral symmetric (Plate 1 Photos 7 & 8 and Plate 2 Photos 10 & 12) and apolar in the spheroidal pollen taxa. The pollen of the studied taxa are monomorphic, i.e. all the pollen grains have ± similar characters. In *B.galpinii, B.grevei, B. variegata* and *B.vareigata* var. *alba* have dimorphic pollen grains i.e. heterostyly which recognized within the same anther (table 2, plate 2 photos 5,6 & 17; Plate 3 photos 17 & 18). All the studied taxa have considerably large pollen grains, excceds 50 µm, medium sizes pollen grains, from 30-50 µm, recorded in *B.galpinii*, *B.monandra*, *B.purpurea*, and *B.varigata*. The pollen shapes in all the studied taxa varied from the subprolate to the prolate. Perprolate pollen grains recorded in both *B.galpinii* and *B. madagascariensis* (Plate 1, photos 5 & 6), while spheroidal pollen grains found in *B.forficata, B.grandidieri, B.grevei, B.monandra* and *B.tomentosa* (table 2, Plate 1 photos 10, 11, 12 & 13).

The pollen apertures and exine ornamentation summarized in table 2 cont. and illustrated in the SEM photographs (Plates 2&3). The studied species have colpate, colporate or colporoidate apertures, except *B.glabra* which have inaperturate pollen grains (Plate 2 photo 10). The number of apertures mostly three, but mono-colpate pollens recorded in *B.acuminata*, tetra-colporoidate pollens in *B.tomentosa* and hexa-colpate in *B.forficata*. Hexaporate pollen grains found in *B.grandidieri* only. The colpi are narrow or wide slit, long or short and not united at the poles or united at the poles (syncolpate). In *B.tomentasa* the pollens have four colpi, each two united before the poles making two oval shapes slits in the equatorial view (Plate 3, photo12). The colpi membranes either granulate or smooth in all the studied taxa. Colporoidate pollen grains recognized in *B.blakeana, B.grevei, B.purpurea* and *B.tomentosa* (Plate 1 photos 2 & 3, Plate 1 photos 1 & 2, 16,19 & 20 Plate 2 photos 3 & 20 and Plate 3, photo 11).

The exine thickness varied greatly between the different species, it was very thin, 0.3 µm in *B.glabra* and reaches 4.9 µm in *B.madagascariensis.* All the taxa have tectate exine, except *B.tomentosa* have clavate bacculae (Plate 3 photo13). The tectum are mostly narrowly or widely striate (Plate 2 photos 15,16 & 18), striate reticulate or clavate in *B.acuminata* (Plate 1 photos 1&2 and Plate 2 photos 1&2). *B.galpinii* the exine is foveolate rugate (Plate 2 photo 8), while in both *B.glabra* and *B.grandidieri* the exine is colleculate or colleculate clavate (plate 2 photos 11).

From the most stable pollen characters obtained the studied *Bauhinia* species can be grouped into three pollen types as follows:-

Type 1 apolar pollen grains *B.forficata,* and *B.tomentosa*
This type has pollen grains spheroidal in shape, or slightly subprolate. In *B.forficata* the pollen grains are spheroidal hexacolpate with reticulate clavate exine. The pollen grains of *B.tomentosa* are spheroidal or slightly prolate-spheroidal with tetracolporoidate aperture, each two united before reaching the polles. The exine in the pollen grains of *B.tomentosa* are intectate with the bacullae stand free with enlarged clavate ends.

Type 2 heteropolar pollen grain *B.glabra* and *B.grandidieri*
In both *B.glabra* and *B.grandidieri* the pollen grains are heteropolar bilateral symmetric and inaperturate in the former and hexaporate in the later. In these two species the exine are tectate colleculate.

Type 3 isopolar pollen grains
Subtype 1 medium sized pollen grains (from 30-50 µm)
B.galpinii, B.monandra, B.purpurea, and *B.varigata* (two taxa).
In this subtype the pollen grains are subprolate, prolate or even perprolate, with three apertures varied from the colpate-colporoidate to colporate. In both *B.galpinii* and *B.varigata*, two types of pollen grains recorded in the same anther (dimorphic), they varied in shape and aperture. The exine tectate with foveolate rugate ornamentation in *B.galpinii*, striate reticulate in both *B.monandra* and *B.purpurea* and striate in *B.varigata*.

Subtype 2 large sized pollen grains (over 50 µm)
B.acuminata, B.blakeneana, B.grevei, B.madagascariensis and *B.vahlii*.
In this subtype the pollen grains are subprolate, prolate or even perprolate. They are monomorphic i.e. of the same type, except *B.grevei* the pollen grains are dimorphic. Number of apertures varied, it is one aperture in *B.acuminata* and three in the rest of the species. The apertures are of the colpate type in both *B.acuminata* and *B.grevei* (type one), while in *B.grevei* (type 2) it is colporoidate. In both *B.madagascariensis* and *B.vahlii* the apertures are colporate. The exine is tectate in all the taxa with reticulate and supra-tectal elements in the form of clavae in *B.acuminata* only, widely striate in *B.grevei* striate in *B.blackeana* and *B.madagascariensis* and striate reticulate in *B.vahlii*.

Clustering analysis
The dendrogram resulted from the correlation between the studied characters (Fig.1) shows that the characters separated into two groups (I and II) at similarity index 5.85 and each group divided into two subgroups. The first group (I) divided into two subgroups (Ia and Ib) at similarity index 12 and gather characters 1,2,3,5 & 18 i.e. pollen dispersal, polarity, symmetry, amb shape and exine ornamentation in sub group Ia. While the characters 9,10,13,14 & 15 i.e. aperture type and number, colpus membrane ornamentation, presence of pores and pore diameter in subgroup Ib (Fig.1).
The second group collects the characters. 4, 8, 12 & 17 i.e. the state of the pollen (monomorphic versus dimorphic), pollen shape, colpus state and presence of tectum grouped together in subgroup IIa. The characters 6, 7, 11 & 16 i.e. polar, equatorial and colpus lengths beside the exine thickness in subgroup IIb
Fig.2 shows the clustering analysis of the studied taxa. At the beginning the three species, *B.acuminata, B.forficata* and *B.tomentosa* are separated at similarity index 0.0, while the rest eleven taxa divided into two groups (IIa and IIb). *B.glabra* and *B.grandidieri* separated at 33.35 similarity index in group IIb, *B.galpinii, B.purpurea, B. variegate* and *B.variegata, alba* separated at similarity index 66.67 in subgroups IIaa. *B.blakeana,B.monandra, B,vahlii, B.grevei* and *B.madagascariensis* gathered in subgroup IIab.

Identification key according to pollen characters
I-Apolar pollen grains
a. Aperture hexacolpate----------*B.forficata*
b. Aperture tetracolporoidate ----------*B.tomentosa*
II-Heteropolar pollen grains
a. Aperture innaperturate----------*B.glabra*
b. Aperture hexaporate----------*B.grandidieri*
III-Isopolar pollen grains
1- Medium polar axis lengths, from 30-50 µm
a. Dimorphic pollen grains
1- Exine foveolate rugate----------*B.galpinii*
2- Exine striate----------*B.variegata* (two taxa)
b. Monomorphic pollen grains
1-Exine striate-reticulate
1. Aperture tricolporate----------*B.monandra*
2. Aperture tricolporoidate----------*B.purpurea*
2- Long polar axis length, over 50 µm

a. Dimorphic pollen grains ----------*B.grevei*
b. Monomorphic pollen grains
1-Exine striate clavate ----------*B.acuminata*
2-Exine striate reticulate ----------*B.vahlii*
3-Exine striate
i-Aperture tricolporoidate ----------*B.blakeana*
ii-Aperture tricolporate----------*B.madgascariensis*

Table 2: Pollen grain characters of the studied *Bauhinia* species

Taxa	Pollen disp.	Pol.	Sym.	State	Amb	PA L.µm	EA L. µm	P/E	Shape
B. acuminata L.	Monads	Iso	Rad.Sym.	Mono.	Ci	55.9-104.9 72.7±12.8	52.7-78.0 62.5±7.7	1.16	Sub-prolate
B. blakeana Dunn.(hybrid)	Monads	Iso	Rad.Sym.	Mono.	CV	50.0-63.6 55.8±4.7	35.4-59.1 41.9±7.4	1.33	Prolate
B. forficata J.H.F.	Monads	Apol	Rad.Sym.	Mono.	Ci	83.3-100.0 93.1±5.6	74.4-102.2 89.1±9.4	1.04	Spheroidal
B. galpinii N.E. Br.	Monads	Iso	Rad.Sym.	Dimor.	CV	33.6-40.9 37.2±2.6	29.1-34.5 31.3±2.2	1.20	Prolate
					CV	41.1-52.3 49.8±4.7	24.7-34.2 27.8±4.5	1.80	Perprolate
B.glabra Jack	Polyads	Hetero	Bilat.Sym.	Mono.	-----	33.2-41.8 37.1±3.3	26.8-34.5 31.8±2.9	1.17	Sub-prolate
B. grandidieri Baill	Monads	Hetero	Bilat.Sym.	Mono.	----	19.1-24.5 20.3±1.6	17.7-20.0 18.9±0.8	1.07	Spheroidal
B. grevei Drake	Monads	Iso	Rad.Sym.	Dimor.	Ci	63.6-77.3 67.4±5.3	58.2-71.6 64.2±7.0	1.05	Spheroidal
					St	52.6-62.3 57.4±4.9	36.6-48.3 46.4±4.8	1.24	Subprolate
B.madagascariensis Desv.	Monads	Iso	Rad.Sym.	Mono.	St	46.4-58.5 55.1±4.0	55.4-73.2 65.3±7.0	0.84	Peroblate
B.monandra Kurz	Monads	Iso	Rad.Sym.	Mono.	St	43.6-59.1 49.5±4.6	38.6-56.8 49.9±6.0	1.00	Sub-prolate
B. purpurea L.	Monads	Iso	Rad.Sym.	Mono.	CV	43.2-53.2 48.8±3.5	32.3-43.2 37.0-4.1	1.32	Prolate
B. tomentosa L.	Monads	Apol	Rad.Sym.	Mono.	CV	65.8-72.6 68.3±3.4	63.9-68.3 67.9±5.6	1.01	Spheroidal
B. vahlii Wight & Arn.	Monads	Iso	Rad.Sym.	Mono.	CV	50.4-62.7 55.4±4.1	33.2-59.5 44.5±7.3	1.24	Sub-prolate
B.variegata (L.) Benth.	Monads	Iso	Rad.Sym.	Dimor.	St	40.0-51.8 46.2±3.5	29.5-41.4 36.2±4.5	1.28	Sub-prolate
					St	43.2-50.2 48.8±3.5	32.1-43.0 37.0-4.1	1.32	Prolate
B.variegata (L.) *alba*	Monads	Iso	Rad.Sym.	Dimor.	St	35.9-47.3 42.2±3.4	24.1-29.5 27.0±2.2	1.56	Prolate
					St	52.4-58.7 54.4±4.1	44.2-52.5 47.5±7.3	1.15	Sub-prolate

Table 2 cont.

Taxa	Aperture		Colpus			Pore diameter	Exine	
	Type	No	Length µm	State	Membrane		Th µm	sculpture
B. acuminata L.	Colpate	1	45.4-95.1 64.8±14.4	Nar, Fr	Smooth	----	1.5-2.8 2.1±0.5	Reticulate & clavate
B. blakeana Dunn. (hybrid)	Colporoidate	3	40.9-50.0 46.4±3.3	W, L	Granulate	0.9-1.4 1.2±0.3	1.4-2.3 1.8±0.3	Striate
B. forficata J.H.F.	Colpate	6	50.0-63.6 55.8±4.7	Nar, Fr	Smooth	---	2.2-3.3 2.9±0.6	Reticulate & clavate
B. galpinii N.E. Br.	Colporate	3	27.3-35.4 31.6±2.6	W, Syn	Smooth	1.4-4.5 2.3±1.0	0.5-2.3 1.5±0.6	Foveolate, rugate
	Colpate	3	38.1-47.3 42.8±4.7	Nar, Fr	Smooth	-----	0.5-2.3 1.5±0.6	Foveolate, rugate
B.glabra Jack	Inaperturate	0	----	--	-----	-----	0.3-0.6 0.5±0.2	Colleculate

B. grandidieri Baill	Porate	6	----	---	------	4.4-6.5 3.6±1.0	0.6-1.3 1.1±0.6	Colleculate, Clavate
B. grevei Drake	Colpate	3	47.7-56.8 51.5±4.7	W, Fr	Smooth	----	3.2-5.0 4.2±0.6	W,Striate
	Colporoidate	3	58.2-71.6 64.2±7.0	W, Syn	Smooth	2.6-3.2 2.9±0.6	3.6-4.5 4.0±0.6	W,Striate
B.madagascariensis Desv.	Colporate	3	40.9-62.7 50.4±11.2	W, Fr	Granulate	5.4-7.3 6.7±0.6	3.2-4.9 4.1±0.5	Striate
B.monandra Kurz	Colpate	3	34.1-40.9 38.6±3.2	W, Fr	Granulate	----	1.8-3.6 2.9±0.7	Striate-reticulate
B. purpurea L.	Colporoidate	3	28.6-45.4 39.0-5.1	W, Fr	Granulate	1.4-3.2 1.8±0.9	0.9-2.3 1.6±0.5	Striate-reticulate
B. tomentosa L.	Colporoidate	4	52.7-58.5 55.2±3.0	Nar, Syn	Smooth	1.2-2.8 1.8±0.9	4.0-4.4 4.1±0.3	Baculate
B. vahlii Wight & Arn.	Colporate	3	40.9-45.4 43.2±1.6	W, Fr	Granulate	8.6-9.1 8.9±0.3	1.4-2.7 2.2±0.3	Striate-reticulate
B.variegata (L.) Benth.	Colporate	3	31.8-45.4 38.8±4.0	W, Fr	Granulate	5.0-7.3 6.4±0.8	0.9-2.7 1.9±0.6	Striate
	Colpate	3	24.5-36.8 33.0±3.9	W, Fr	Granulate	**-----**	1.4-2.3 1.7±0.4	Striate
B.variegata (L.) *alba*	Colporate	3	31.8-45.4 38.8±4.0	W, Syn	Granulate	4.6-6.7 6.0±0.8	0.9-2.7 1.9±0.6	Striate
	Colpate	3	24.5-36.8 33.0±3.9	W, Syn	Granulate	-----	1.4-2.3 1.7±0.4	Striate

Table 3: Characters employed in clustering analysis

No	Character	State	Descriptions
1	Pollen dispersa	Binary	1-Monads 2-Polyads.
2	Polarity	Binary	1-Isopolar 2-Apolar.
3	Symmetry	Binary	1-Radiosymmetric 2-Bilateral symmetric.
4	State	Binary	1-Monomorphic 2-Dimorphic.
5	Amb shape	Multistate qualitative ordered (MQO)	1-Straight 2-Circular 3-Convex.
6	Polar axis length	Continuous.	
7	Equatorial axis length	Continuous.	
8	Shape	Multistate qualitative ordered (MQO)	1-Spheroidal 2-Subprolate 3-Prollate 4-Perprolate.
9	Aperture type	Multistate qualitative ordered (MQO)	1-Inaperturate 2-Colpate 3-Colporoidate 4-Colporate 5-Porate.
10	Aperture number	Continuous.	
11	Colpus length	Continuous.	
12	Colpus state	Multistate qualitative ordered (MQO)	1-Narrow free 2-Narrow syncolpate 3-Wide free 4-Wide long 5-Wide syncolpate.
13	Colpus membrane	Binary	1-Smooth 2-Granulate.
14	Pore presency	Binary	1-Absent 2-Present.
15	Pore diameter	Continuous.	
16	Exine thickness	Continuous.	
17	Exine tectum	Binary	1-Absent 2-Present.
18	Exine ornamentation	Multistate qualitative unordered (MQUO)	1-Striate 2-Striate reticulate 3-Wide striate 4-Reticulate clavate 5-Clavate 6-Colleculate clavate 7-Colleculate 8-Foveolate rugate.

Table 4: Characters employed in clustering analysis in each taxa

Char. Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
B.acu	1	1	1	1	2	73	63	2	2	1	65	1	1	1	0	2.1	2	4
B.blac	1	1	1	1	3	56	42	3	3	3	46	4	2	2	1.2	1.8	2	1
B.for	1	2	1	1	2	93	89	1	2	6	56	1	1	1	0	2.9	2	4
B.galp	1	1	1	2	3	43	29	3	4	3	36	5	1	1	0	1.5	2	8
B.glab	2	2	2	1	3	37	32	4	1	0	0	0	2	1	0	0.6	2	7
B.gran	1	2	2	1	3	20	19	1	5	6	0	0	2	2	3.6	1.1	2	6

B.gre	1	1	1	2	1	62	54	3	2	3	55	4	1	2	4.2	4.2	2	3
B.mad	1	1	1	1	1	55	65	4	5	3	50	3	2	2	4.1	4.1	2	1
B.mon	1	1	1	1	1	51	50	2	2	3	39	3	2	2	2.9	2.9	2	2
B.pur	1	1	1	1	3	49	37	3	3	3	39	3	2	2	1.8	1.6	2	2
B.tom	1	2	1	1	3	68	68	1	3	4	55	2	2	2	1.8	4.1	1	5
B.vah	1	1	1	1	3	55	45	2	5	3	40	3	2	2	8.2	2.2	2	2
B.var	1	1	1	2	1	47	37	3	2	3	38	3	2	2	6.4	1.9	2	1
B.alba	1	1	1	2	1	48	37	2	2	3	36	5	2	2	6.0	1.7	2	1

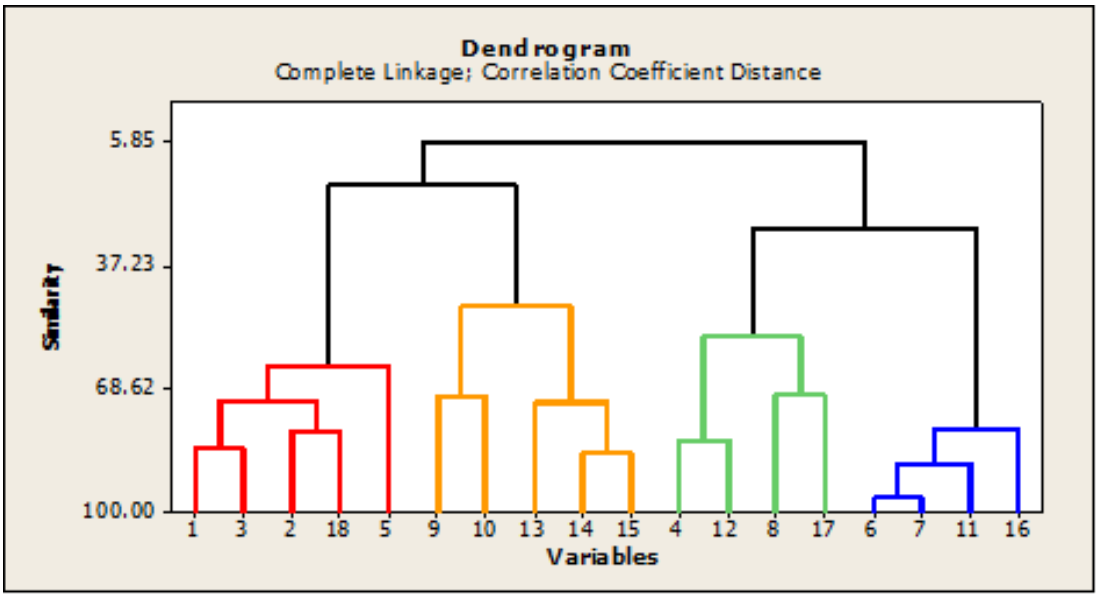


Fig 1: Clustering analysis of the studied characters

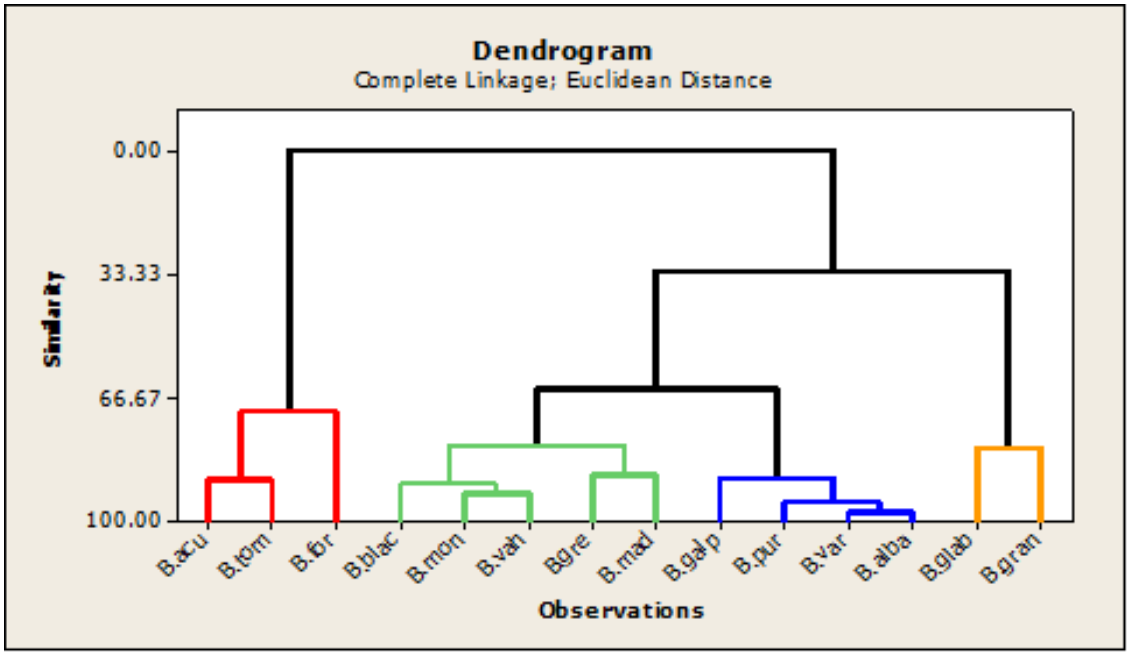


Fig 2: Clustering analysis of the studied taxa

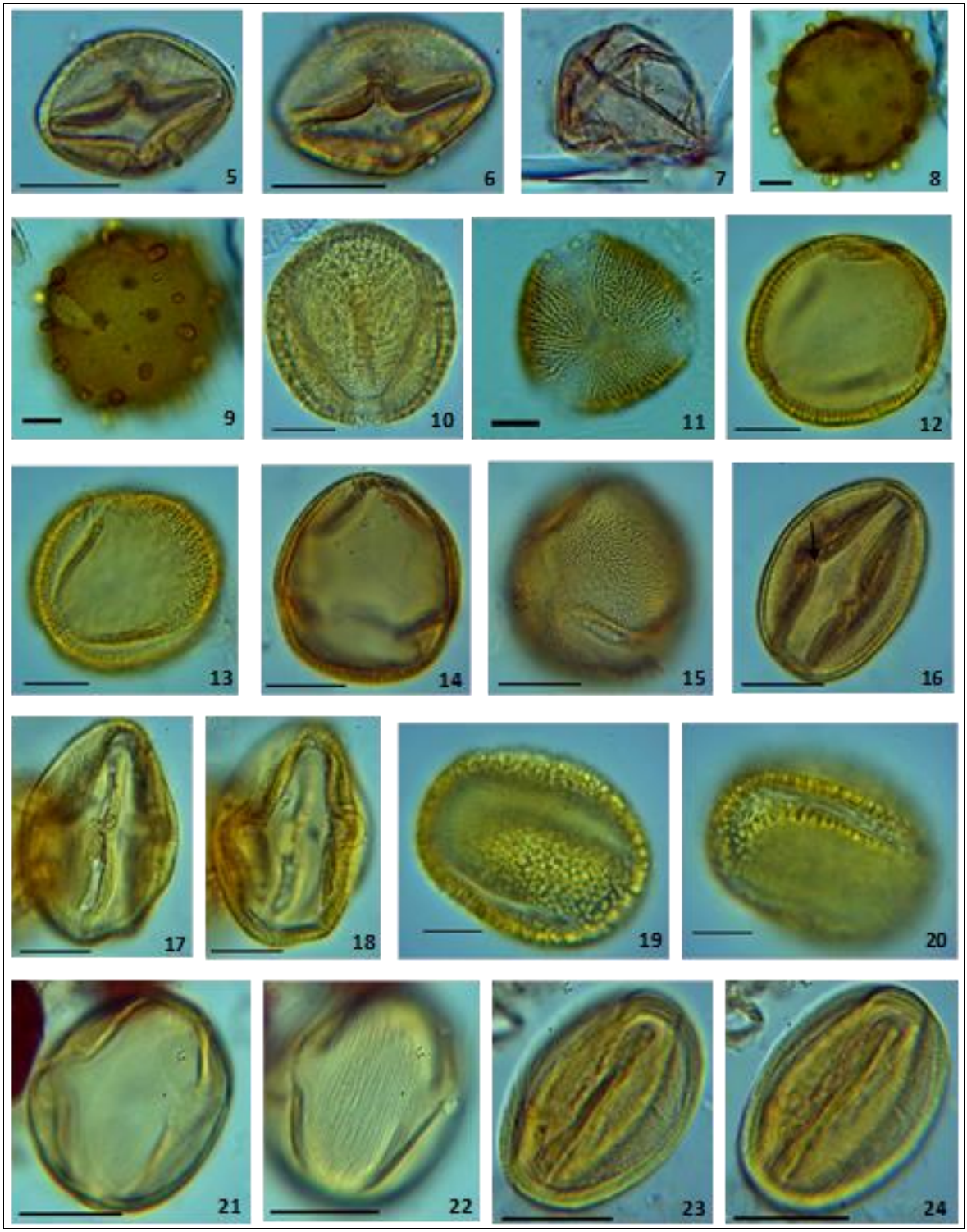
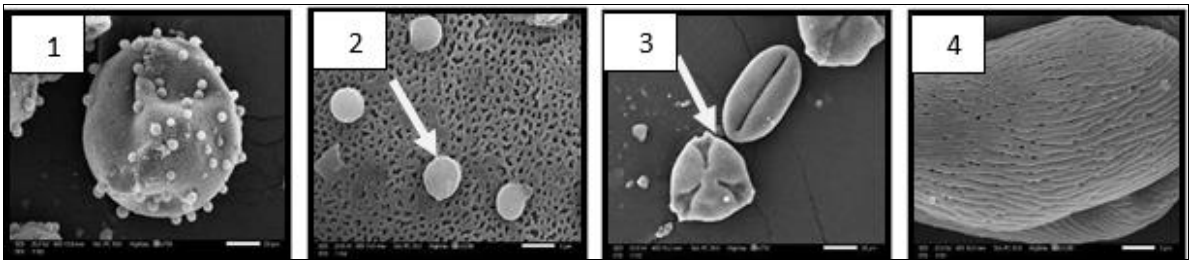


Plate 1: Pollen Grains of Bmthinia taken by light microscope (Scale bar = 20 µm): 1, (high and low focus); 3, 4. B. blakeana (high and low focus), arrows indicate the sma galpinii (high and low focus); 7. B. glabra; 8,9. B. forficata (high and low focus); 10. view); 11.B. gresvi (p olar v iew); 12, 13. B. madagascariensis (high and low focus); 1 (high and low focus); 16. B. putpurea, arrow indicates the pore; 17, 18. B. rabid (hig 20. B. totnentosa (high and low focus); 21, an. B inrigata (high and low focus); 23, 2. alba (high and low focus).



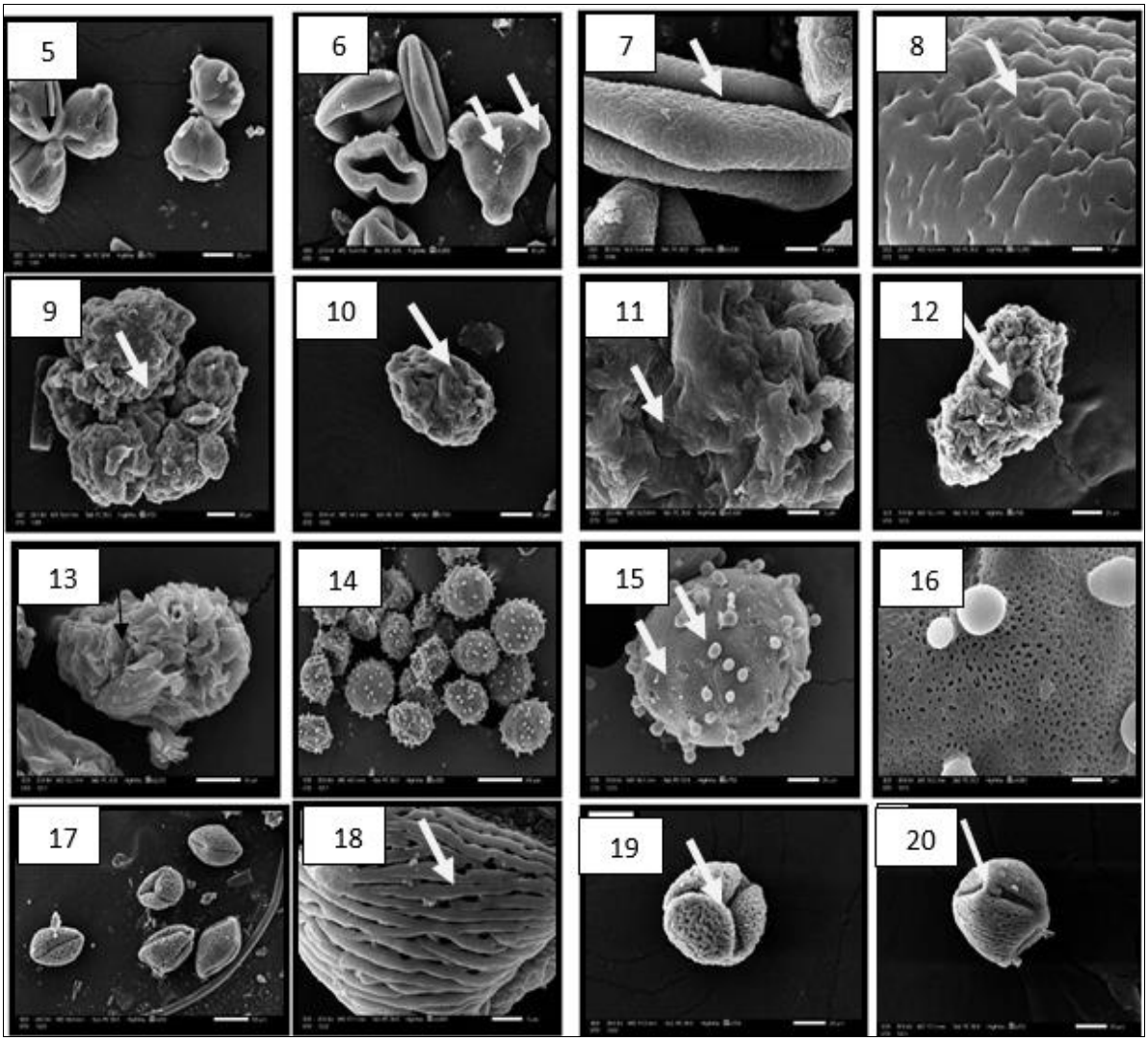
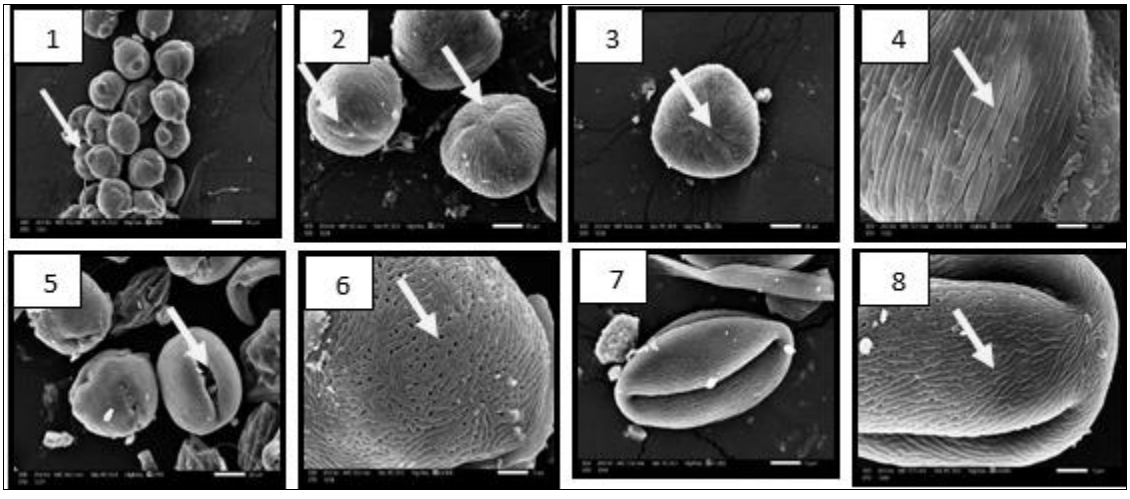


Plate 2: Pollen Grains of *Bauhinia* taken by SEM: 1, 2. *B. acuminata* (low and high magnifications, arrow indicates the clavae); 3, 4. *B. blakeana*(low and high magnifications, arrow indicates the pore); 5, 6, 7, 8*B. galpinii*(low and high magnifications, arrows indicate the dimorphic pollens, wide syncolpate and colporate apertures and foveolate rugate exine); 9, 10, 11*B. glabra* (low and high magnifications, arrows indicate the polyad (9), heteropolar, nonaperturate pollens (10) colleculate exine (11); 12, 13. *B. grandidieri* (low and high magnifications, arrows indicate the heteropolar, porate pollens (12), colleculate clavate exine (13); 14, 15, 16. *B. forficata* (low and high magnifications, arrows indicate the thin layers of the colpi and clavate reticulate exine pollens); 17, 18, 19, 20.*B. grevei* (low and high magnifications, arrows indicate the dimorphic shapes, wide striate exine (18), syncolpate aperture (19) small bridged pore (20).



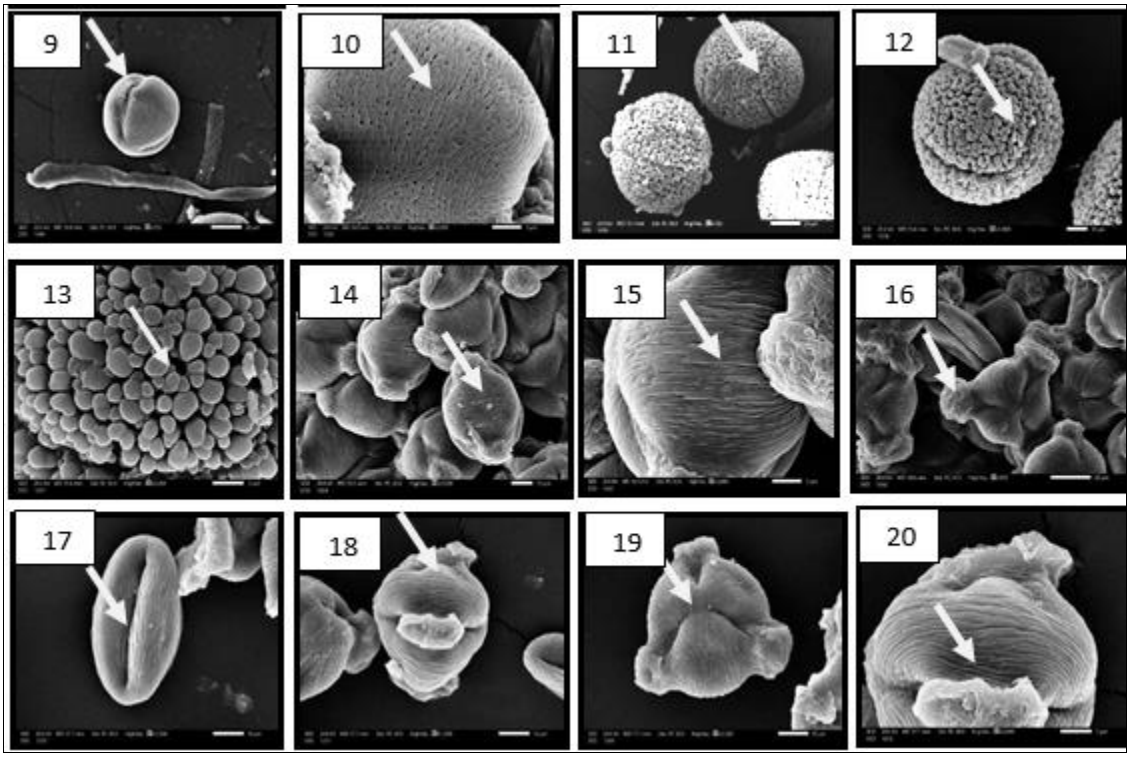


Plate 3: Pollen Grains of *Bauhinia* taken by SEM: 1, 2, 3, 4. *B.madagascariensis* (high low and high magnifications, arrows indicate colporate aperture (1), long free colpi (2, 3) & striate exine (4); 5,6. *B.monandra* (low and high magnifications, arrows indicate colpi (5) and striate reticulate exine (6); 7,8. *B. purpurea* (low and high magnifications, arrows indicate colporoidate aperture (7) and striate reticulate exine (8); 9, 10. *B.vahlii*(low and high magnifications, arrows indicate colporate aperture (9) and striate reticulate exine (10); 11,12, 13. *B.tomentosa* (low and high magnifications, arrows indicate tetracolporate and syncolpate aperture (11 & 12), clavate intectate exine (13); 14, 15, 16. *B.varigata* var. *alba* (low and high magnifications, arrows indicate dimorphic shapes (14), striate exine (15) colporate long free apertures(16); 17, 18, 19, 20. *B.varigata* (low and high magnifications, arrows indicate dimorphic shapes (17 & 18), colporate long free apertures (19), striate exine (20).

Discussion

Variations in the pollen morphological characters and ultra structure have been used in solving many taxonomical problems and confusion as well as to distinguish between species and cultivars of fruit trees [16, 17, 18 & 19]. Within genus Bauhinia, the differentiation between the belonging species by morphological characters is confusing and their taxonomic history and classification is long and complicated. Analyses based on morphological characters done [4, 6, 7, 8, 20 and 21] and molecular characters [5, 22 & 23] suggest contrasting relationships within the genus. The pollen grain of *Bauhinia* species have been examined by many investigators [10, 11, 12, 13 and 15] and they found that the *Bauhinia* species have variable pollen morphological characters, with a very wide range of characters such as different shape, variable exine ornamentation, different type and number of apertures; i.e. the genus is eurypalynous. All the previous works restricted to the pollen descriptions and did not gave well clarification the taxonomic relationships between the taxa. Accordingly, this work considers as a trial to understand the relations between the Egyptian road tree *Bauhinia* species on the basis of their palynological characters.

From the obtained results, the pollen grains of the studied species have different characters that can be used in grouping them. The heteropolar pollen grains separate both *B. galpinii* and *B. grandidieri*, while the apolar i.e. circular pollen grains distinguish both *B.forficata* and *B.tomentosa.* The isopolar pollen grains divided into two categories according to their polar axis length. These taxa can be differentiated according to their aperture type and number as well as exine ornamentation. Pollen grains of both *B.grevei and B.variegata* are heterostyly or dimorphic. These characters have been explained by [24] who defined the heterostyly as a genetic polymorphism due to variations in style lengths of the flowers. Pollen size, variation in aperture number and sexine ornamentation are often correlated with heterostyly [25].

The cluster analysis of the studied characters showed that the polar, equatorial and colpi lengths beside the exine thickness are correlated together and the morphism of the pollens, pollen shape i.e. P/E, colpus state (narrow versus wide) and presence as well as the tectate exine are grouped together. All these characters are related to each other and affect the state of the pollen grains. On the other hand the characters; pollen dispersal, polarity, symmetry, amb shape and exine ornamentation are correlated together and all in correlation with the characters aperture type and number, colpus membrane ornamentation, presence of pores and pore diameter. This means that each group of characters came together and affects the whole state of the pollen grains.

The cluster analysis of the studied taxa, according to the studied characters revealed that the three species *B.acuminata, B.forficata* and *B.tomentosa* separated from the rest of the studied species without any similarity. This can be due to the clavate exine ornamentation or clavate supra tectum exine. The rest of the species gave three distinct groups; *B.glabra* and *B.grandidieri* in one subgroup for being the only one with heteropolar exine. The taxa *B.galpinii, B.purpurea, B. variegata* and *B.variegata, alba* in a second subgroup due to the medium length of the polar axis, while the third group gather the species *B.blakeana,B.monandra, B,vahli, B.grevei* and *B.madagascariensis*. These groups acceptable according to the variations in the pollen characters and coordinate with the divisions estimated from the pollen data obtained. These data coordinate with the previous classification of the genus by [20 & 21] in dividing the *Bauhinia* species into two subgenera with five sections. Meanwhile the division of the genus into four subgenera according to their life forms as proposed by [9] can be in partial acceptance with the data obtained from the pollen morphological study. In spite of the great diversity in the morphology of the pollen grains within the studies taxa the separation of the species into several genera as proposed by [26, 27 & 28] is not recommended. The palynological data approved the taxonomical proposal done by [2, 4, 7 & 8] to consider all the *Bauhinia* species as one big genus with several subsection and sections under it.

Phylogenetic postulation

The study of pollen morphology allowed evolutionary biologists to assess phylogenetic relationships among major taxa of Angiosperms, and to understand the fossil record. During this process, pollen grains, which are from the abundant micro- fossils, have mainly studied by discretizing some of its main characteristics such as dispersals, size, shape, aperture and exine stratification and ornamentation. Variations in pollen sizes may be attributed to ecological factors, water availability, harmomegathic effect and dispersal modes. This adaptation considered as phylogenetic trends within the different taxa. According to [29] opinion about aperture phylogeny, the trilete (trichotomocolpate) aperture is the basic type, from which evolved all other apertural types possibly along two main lines namely the tricolpate and the monocolpate lines. He postulated the aperture evolution in the tricolpate line is considered to be in the order (*1*) zonicolpate → zoniporate (*2*) zonicolpate → pancolpate → panporate (*3*) trizonicolpate (by reduction) → inaperturate. In such a scheme, the tricolpate pollen grains are to be considered more primitive aperture types within the Angiosperms.

From the palynological data obtained there are evidences of phylogenetic trends within the studied *Bauhinia* taxa. The monads and small sized pollen grains considered to be primitive compared to those with bigger sizes and the polyads, heteropolar pollen grains recorded in *B.glabra* are advanced than the previous ones. The presence of supra tectal elements found in *B.acuminata* and *B.forficata* considered as advanced character by [25]. Meanwhile they considered the absence of tectum a primitive character, which recorded in *B.tomentosa*. The heteropolar pollen grains with the colliculate exine sculpture found in *B.grandidieri* considered advanced characters. Based on this study, the line of evolution can be traced from the primitive to the advanced as follows:-

*B.tomentosa→B.monandra→B.madagascariensis→B.galpinii→B.purpurea→B. vahlii→B.blakeana→B.variegata→B.grevei→B.acuminata→B.forficata→B. grandidieri →B.glabra***.**

Thus, *B.tomentosa* is the primitive pollen form, while both *B. grandidieri* and *B.glabra* represent the most advanced pollen forms.

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