

Original Research Article

A Palynological Review for Some Species of Family Boraginaceae Juss. From the Egyptian Flora

ABSTRACT

Boraginaceae Juss. is a large cosmopolitan family with nearly 2700 species in c. 150 genera, which are widely distributed in the temperate and tropical regions of the Old and New world. It is one of the most confusable families with its taxonomic problems. Now a day's palynology is a distinct and important botanical discipline. For the Egyptian flora, little palynological attention has cared. This aspect is used to examine some taxa of family *Boraginaceae* Juss. That is represented by the two subfamilies *Heliotropioideae* and *Boraginoideae*. LM & SEM techniques are followed. General pollen morphological characters are investigated. High significant results are obtained. Eight pollen subtypes and two subtypes are derived from the main morphological characters. These data are consumed to describe different taxa. Introducing a well-developed identifying constant and valuable features. An identifying key is produced at different taxonomic ranks. A palynological investigation of the *Boraginaceae* is, however, of great taxonomic significance.

Keywords: *Boraginaceae*; europalynous; heteroaperturate; key; endoapertures; bicolpate.

1. INTRODUCTION

Boraginaceae Juss. [1] is a large cosmopolitan family with nearly 2700 species in c. 150 genera, which are widely distributed in the temperate and tropical regions of the Old and New world [2]. In the last two decades, taxonomic limits of genera, tribes, and even subfamilies have dramatically changed according to the macro-morphological characters and molecular data.

Currently, *Boraginaceae* s.l. is classified into six subfamilies: *Codonioideae*, *Wellstedioideae*, *Cordioideae*, *Ehretioideae*, *Heliotropioideae*, and *Boraginoideae*. This classification is based on distribution, habit, as well as floral and fruit characters. Moreover, the subfamily *Boraginoideae* has, recently, been divided into nine tribes basically on the complex characters of the fruit. These are *Trigonotidae*, *Eritricheae*, *Cynoglosseae*, *Trichodesmeae*, *Lithospermeae*, *Boragineae*, *Echieae*, *Echiochileae*, and *Myosotidae* [2].

Today, we have seen that the study of palynology is a distinct and important botanical discipline. The ease by which the pollen grains can be prepared for study and their morphological diversity make pollen morphology a tool of evidence used to test the position of species or genera within larger entities, instead of using pollen data to make a special classification that may disrupt the taxonomic units.

Members of *Boraginaceae* are europalynous, with a great diversity in morphological types, classified under either colporate or heterocolpate main types, and in which many genera or even species can be recognized by their pollen characters [3, 4, 5, 6, 7].

Pollen morphology has been examined and described in regional pollen floras [3, 8, 9, 10, 11]; while others have studied certain tribes [6, 12] or even some taxa within a single genus [5, 7, 13, 14, 15].

For Egypt, little attention to the pollen morphology has been focused. It's only represented by [16]. According to [17], Boraginaceae is represented by 19 genera and 57 species.

In this study, we have selected 22 species covering 11 genera. These selected species are represented by the two subfamilies *Heliotropioideae* and *Boraginoideae* with seven tribes. This account was set up to give a detailed analysis of pollen morphological characteristics of the studied species, to assess the tribe limits, and to correlate pollen morphology with the taxonomy.

2. MATERIAL AND METHODS

2.1. Pollen material:

Pollen grains of 22 taxa (including 2 subspecies, 4 varieties) covering 11 genera within the Boraginaceae were obtained from herbarium specimens deposited at Cairo University Herbarium (CAI) and South Valley University-Qena Herbarium (QNA: a proposed acronym). The selected specimens are represented by two subfamilies *Heliotropioideae* and *Boraginoideae* with seven tribes, arranged corresponding to their placement in [2]. The pollen data for them are presented in Tab. 2-4.

The specimens have been determined by the 2nd author (El Hadidy) and updated according to the contribution sources of [18,17,19], as well as, [20, 21, 22].

Voucher specimens and permanent slides are deposited in South Valley University-Qena Herbarium (QNA). Details of collecting the investigated taxa are reported in Tab. 1.

2.2. Methods

The survey is based on light and scanning electron microscopy to give clear picture of the morphological features of the pollen and to demonstrate the pollen variability.

2.2.1. For Light microscopy (LM):

All samples were taken from mature anthers of the flowering buds. Material was boiled in H₂O for a few minutes, rehydrated in a 10% aqueous solution of KOH on a clean slide, stained with 10% safranin, mounted in glycerin jelly, and sealed with paraffin for LM observations. Permanent slides were examined by means of a light microscopy (Labomed-Labo America, Inc.) at magnification x100. Photographs were obtained using Leica-DM1000 microscope equipped with Leica-EC digital camera. Measurements of pollen grains were based on 10-15 pollen grains for each sample.

2.2.2. For Scanning electron microscopy (SEM):

Unacetolyzed pollen grains were mounted onto metallic stubs previously coated with double-sided self-adhesive tape. Each sample was sputter-coated with gold in JEOL JFC 1100 E ion sputtering device, and examined with JEOL JSM 5400 LV scanning electron microscopy, accelerated by a voltage of 10-15 KV at SEM Unit, Assiut University, Egypt.

2.3. Terminology:

In this study, the terminology of [23] is followed. Colpus length is described as “terminal” when the colpus length represents 0.8-0.9 times of the total polar axis length; “subterminal” when it represents 0.7-0.8 times of the polar axis; “short” when it represents 0.5-0.7 times of the polar axis length; “very short” when it represents less than 0.5 times of the polar axis length. Simple ectocolpi correspond to the pseudoapertures (without endoapertures); while compound ectocolpi correspond to the true apertures (with endoapertures).

Table 1: Details of the collecting information of species studied

Subfamily: Heliotropioideae Gürke [<i>Heliotropium</i> L., Sp. Pl., ed. 1: 130 (1753)].				
Specimen		Collection origin, collector	Geo-region/ Date	Voucher
Section	Taxon			
Chametropium Griseb.	1. <i>Heliotropium supinum</i> L.	Esna city, Qena Governate: south Egypt, A. Osman, s.n.	Nv/2003	QNA
Odnotropium Griseb.	2. <i>H. arbianense</i> Fresen.	South Sinai, Sant Catherine, Gebel Catherine, A. Osman, s.n.	S/2004-05	QNA
Pterotropium (A. DC.) Bunge.	3. <i>H. bacciferum</i> Forssk. subsp. <i>bacciferum</i> var. <i>bacciferum</i> Forssk.	Gebel Elba, Wadi Habib, A. <i>El-Hadidy</i> , s.n.	Ge/1983	CAI
		Cairo-Ismailia desert-road, 46 Km NE of Cairo, <i>El-Bakry</i> , 426.	Dg/ 29-4-1980	CAI
		Suez-road, Km 23, <i>EL-Hadidi</i> , s.n.	Dg/ 4-4-1952	CAI
		Gebel Elba: E. of Hamra Dam, Wadi Ibib, <i>J. Osborn</i> & <i>I. Helmy</i> s.n.	Ge/ 6-3-1967	CAI
	4. <i>H. bacciferum</i> subsp. <i>bacciferum</i> var. <i>erosum</i> (Lehm.) El Hadidy	Giza: N. of Giza Pyramids, as a weed among <i>Ricinus</i> plants (in deserts), <i>G. Täckholm</i> , s.n.	Nv/ 5-11-1926	CAI
	5. <i>H. ramosissimum</i> Lehm. (Sieb. ex A. DC.)	Naqada city, Qena Gov. south Egypt, A. Osman, s.n.	Nv/1998	QNA
		Kharga oasis: near the town, <i>G. Täckholm</i> , s.n.	On/ 16-1-1928	CAI
	6. <i>H. pterocarpum</i> (A.DC.) Hochst. & Steud. ex Bunge	Gebel Elba, Wadi Habib, A. Osman, s.n.	Ge/2004	QNA
		Expedition to Gebel Elba district: Sand dune of Mersa Abu Naam, <i>Täckholm et al</i> 601	Ge/ 25-1-1962	CAI
		Expedition to Gebel Elba district: Wadi Yahameib, <i>Täckholm et al</i> 244C	Ge/ 22-1-1962	CAI
		Expedition to Gebel Elba district: Delta of Wadi Serimtai, <i>Täckholm et al</i> 1592	Ge/ 5-2-1962	CAI
Subfamily: Boraginoideae				
Specimen		Collection origin, collector	Geo-region/ Date	Voucher
Tribe	Taxon			
Eritrichieae Benth. & Hook. f., Gen. Pl. 2(2): 836 (1876).	7. <i>Asperugo procumbens</i> L.	South Sinai, Sant Catherine, Gebel Catherine, A. <i>El-Hadidy</i> , s.n.	S/ 1983	CAI
	8. <i>Lappula spinocarpos</i> (Forssk.) Asch. ex Kuntze	Wadi Hashim, 48 km before Mersa Matrouh, <i>I. El-Garf</i> , s.n.	Mm/1999	CAI
Cynoglosseae DC. in Meisn., Pl. Vasc. Gen., “Comm.” 2: 187 (1840).	9. <i>Paracaryum intermedium</i> (Fresen.) Lipsky	South Sinai, Sant Catherine, Gebel Catherine, A. Osman, s.n.	S/ 2004-05	QNA
		Gebel Mousa, Sinai, <i>Täckholm et al</i> , s.n.	S/ 22-4-1961	CAI
	10. <i>Paracaryum rugulosum</i> (DC.) Boiss.	South Sinai, Sant Catherine, Gebel Catherine, A. Osman, s.n.	S/ 2004-05	QNA
Trichodesmeae Zak. in Serawsch. 4: 5 (1941).	11. <i>Trichodesma africanum</i> (L.) R.	Pharmacy faculty farms, south valley university, Qena	Nv/2011	QNA

		Br. var. <i>africanum</i>	governorate, <i>N. Hussein</i> , s.n.		
		12. <i>Trichodesma africanum</i> L. R. Br. var. <i>heterotrichum</i> Bornm. & Kneucker	East of Cairo, Wadi Dowiaqa, <i>A. El- Hadidy</i> , s.n.	Nv/1983	CAI
			East of Cairo, Wadi Dowiaqa, <i>G. Täckholm</i> s.n.	Nv/3-12-1926	CAI
			Wadi Hammat near Wadi Hôf, <i>V. Täckholm</i> s.n.	Nv/12-2-1952	CAI
Lithospermeae (DC.) Gürke in Engl. & Prantl, Nat. Pflanzenfam. 4(3a): 118 (1894).		13. <i>Trichodesma ehrenbergii</i> Schweinf.	Expedition to Gebel Elba district: Wadi El Shallal, Bir area, <i>Täckholm et al</i> , 519.	Ge/24-1-1962	CAI
		14. <i>Alkanna tinctoria</i> Tausch	Alexandria: Mandara, <i>Hassib</i> , s.n.	Mm/13-8-1929	CAI
		15. <i>Alkanna orientalis</i> (L.) Boiss.	Sinai: Sinai Mountain region, <i>Hassib</i> , s.n.	S/ April 1940	CAI
		16. <i>Arnebia decumbens</i> (Vent.) Coss. & Kralik	Mariut: Ras el Hekma, <i>Botany Dept. Excursion</i> , s.n.	Mm/2-5-1955	CAI
Boragineae Riedl in Rech., Fl., Iran. 48: 231 (1967).	Genus: Anchusa L., Sp. Pl., ed.1, 133 (1753).	17. <i>Arnebia hispidissima</i> (Lehm.) DC.	Gebel Elba, Wadi Habib, <i>A. Osman</i> , s.n.	Ge/2004	QNA
		Subgenus: Anchusa Feinbrun, Fl. Palaest., 3: 82 (1978); D. F. Chamb. in Davis, P. H. [ed.]. Fl. Turkey 6: 390 (1978). 18. <i>A. hybrida</i> Ten.	On the road Alexandria- Burg El-Arab at kilo 17, <i>M. Imam</i> , s.n.	Mm/22-3-1956	CAI
		Subgenus: Buglossoides (Reichenb.) Guşul., Bul. Fac. Şti. Cernăuţi 1: 280 (1927); Riedl in Rech., Fl. Iran. 48: 236 (1967); Feinbrun, Fl. Palaest. 3: 84 (1978). 19. <i>A. aegyptiaca</i> (L.) A. DC.	Mediterranean coast, Wadi Hashim, 25 km West Matrouh, <i>A. Osman</i> , s.n.	Mm/ 2006	QNA
		20. <i>A. milleri</i> Sperng.	South Sinai, Sant Catherine, Gebel Catherine, <i>A. Osman</i> , s.n.	S/2004-05	QNA
			Sinai: Wadi el Eriq, <i>Hassib</i> , s.n.	S/7-4-1940	CAI
		Subgenus: Hormuzakia (Guşul.) Chamb., Notes Roy. Bot. Gard. Edinb. 35(3): 298 (1977); Chamb. In P. H. Davis, Fl. Turkey 6: 401 (1978). 21. <i>A. aggregata</i> Lehm.	Alexandria province, Alexandria, <i>A. Amer</i> , 9740.	Mm/ 1987	CAI
		22. <i>G. hispida</i> (Forssk.) Bunge	Mediterranean coast, Wadi Hashim, 25 km West Matrouh, <i>A. Osman</i> , s.n.	Mm/ 2006	QNA
	Genus: Gastrocotyle Bunge				

			3 km north of Al- Arish, <i>L. Boulos</i> , s.n.	Di/ 1955	CAI
Echieae DC. in Meisner, Pl. Vasc. Gen. "Comm." 2: 281 (1840), A. DC. in DC., Prodr. 10: 4 (1846).	23. <i>Echium rauwolfii</i> Delile	Pharmacy faculty farms, south valley university, Qena governorate, <i>A. Osman</i> , s.n.		Nv/2005	QNA
Echiochileae Långström & Chase, Pl. Syst. Evol. 234: 137-153 (2002).	24. <i>Echiochilon fruticosum</i> Desf.	Burg El- Arab, <i>Botany Dept. Excursion</i> , s.n.		Mm/ 2016	CAI

3. RESULTS AND DISCUSSION

3.1. General description of the pollen grains of Boraginaceae Juss.

Pollen grains are dispersed as monads, minute- or small-sized, occasionally medium; radially symmetrical, and isopolar or subisopolar, rarely bilaterally symmetrical or heteropolar; mostly prolate or subprolate, sometimes prolate-spheroidal or perprolate. Outline rectangular to rectangular-elliptic or elliptic with hemispherical (rounded) or flattened ends, and with convex or parallel equatorial faces, with or without medial constriction at the equator in the equatorial view, rarely ovate-triangular or triangular; circular-lobate to polygonal or triangular in the polar view. Apertures are colporate, colpate or heterocolpate. Apertures are characteristics to the subfamilies and tribes. In Heliotropioideae (Heliotropium), the pollen grains are 6-8-heterocolpate; while 3-9(-10)-colporate in Boraginoideae (e.g. Boragineae, Echieae, Lithospermeae, and Trichodesmeae). In Eritricheae and Cynoglosseae, tricolporate alternate with three pseudocolpi. The length and width of simple (pseudocolpi) and compound colpi are variable, but simple colpi are commonly narrower and longer. Some genera in Eritricheae and Cynoglosseae possess slight to strong medial constriction (e.g. Asperugo, Lappula, Paracaryum); rarely with endocingulus (e.g. Paracaryum). Endoapertures lalongate or lalongate, rarely circular; sometimes operculate (e.g. Heliotropium) or protruded (e.g. Echium); commonly monorate, rarely diorate (e.g. Arnebia, Lappula). The sculpture of the pollen grains either extends over the entire grain or only over a part of it (i.e. sculpture at the apocolpia differs than that on the mesocolpia). Tectum psilate, perforate, rugulate, fossulate, rarely micro-echinate or reticulate to micro-reticulate. These patterns are uniformly present or in integration with others.

3.2. A synopsis for the pollen grains types:

- **Subfamily Heliotropioideae (Heliotropium)**

TYPE 1: 6-8-Heterocolpate

Subtype a. tri- to tetra-colporate alternate with tri- to tetra-pseudocolpi- perforate.

Species included: *H. bacciferum*, *H. pterocarpum*, *H. ramosissimum* & *H. arbainense*.

Subtype b. tricolporate alternate with tri-pseudocolpi- fossulate.

Species included: *H. supinum*

- **Subfamily Boraginoideae**

TYPE 2: 6-Heterocolpate-Perforate

Tricolporate alternate with tri-pseudocolpi.

Tribes included: Eritricheae (e.g. *Asperugo*, *Lappula*) & Cynoglosseae (e.g. *Paracaryum*).

TYPE 3: Bicolpate-perforate

Species included: *Echiochilon fruticosum* (Tr. Echiochileae).

TYPE 4: 3-Colporate-Micro-reticulate

Species included: *Echium rauwolfii* (Tr. Echieae)

TYPE 5: 3-Colporate-Micro-echinate

Tribe included: Trichodesmeae (e.g. *Trichodesma*)

TYPE 6: 3-Colporate-Psilate

Species included: *Alkanna* (Tr. Lithospermeae)

TYPE 7: 4-9-Colporate-Perforate

Tribes included: Boragineae (e.g. *Anchusa*, *Gastrocotyle*) & Lithospermeae (e.g. *Arnebia decumens*).

TYPE 8: 6-7-Colporate-Micro-echinate

Species included: *Arnebia hispidissima* (Tr. Lithospermeae).

3.3. The Identification key for the genera studied

1.a.	Pollen heteroaperturate	2
b.	Pollen homoaperturate	5
2.a.	Pollen medium-sized; without constriction at the equator; P/E= 1.03-1.9; 6-8-heterocolpate.....	Heliotropium
b.	Pollen minute- to small-sized; with or without constriction at the equator; P/E= 1.26-2.6; 6-heterocolpate.	3
3.a.	Pollen perprolate (P/E= 2.0-2.6); simple & compound colpi linear, subterminal, and diorate; endoapertures subequatorial.	Lappula
b.	Pollen prolate or subprolate to prolate (P/E= 1.26-1.82); simple & compound colpi linear & rhombic, subterminal, short or very short, and monorate; endoapertures equatorial.	4
4.a.	Ectocingulus present; apo-mesocolpia not differently sculptured; simple & compound colpi short and very short.	Paracaryum
b.	Ectocingulus absent; apo-mesocolpia differently sculptured; simple & compound colpi subterminal and very short.	Asperugo
5.a.	Aperture type colpate.	Echiochilon
b.	Aperture type colporate.	6

6.a.	Pollen isopolar; colpi mono- or diorate.	7
b.	Pollen heteropolar; colpi monorate.	10
7.a.	Colpi diorate.	Arnebia
b.	Colpi monorate.	8
8.a.	Aperture type 3-colporate; pollen without an equatorial band; small-sized; endoapertures lalongate; tectum micro-echinate-perforate.	Trichodesma
b.	Aperture type 4-poly-colporate; pollen mostly with an equatorial band; small- to medium-sized; endoapertures lalongate; tectum psilate-perforate.	9
9.a.	Colpi rhombic-shaped, short (extend for 0.5-0.6x of P-axis); pollen small-sized; 8-9-colporate (polyaperturate); equatorial band granulated.....	Gastrocotyle
b.	Colpi fusiform-shaped, very short (extend for 0.3-0.5x of P-axis); pollen medium-sized; 4-7-colporate; equatorial band reticulate or micro-reticulate	Anchusa
10.a.	Shape prolate rarely subprolate (P/E= 1.2-1.6); colpi terminal or subterminal; tectum psilate.....	Alkanna
b.	Shape subprolate or prolate spheroidal (P/E= 1.1-1.3); colpi terminal; tectum microreticulate to perforate	Echium

This work data reveals 8 types of pollen grains and 2 subtypes, creating artificial keys to facilitate the differentiation between 24 taxa studied in variable taxonomic ranks. Pollen general morphological characters: the size, shape, polarity, apertures types & features, colpi characters, endoapertures and tectum characters are down here spotlighted to discuss their taxonomic implications.

1. Size: the size of pollen grains greatly variable ranges from minute to medium-sized pollen grains; polar axis diameter ranges from 6.0-36.0 μm and the equatorial diameter ranges from 4.0-28.0 μm ; much smaller pollen grains are found in *Paracaryum intermedium* (P= 6.0-10.0 μm), then in *Asperugo procumbens* whereas P= 8.0-10.0 μm . The largest pollen size is found in *Anchusa undulata* subsp. *hybrida* (P= 32.0-36.0 μm), and in *Heliotropium supinum* in which P= 30.0-34.0 μm .

2. Shape & polarity:

2.1. Shape: a considerable variation in pollen grains shapes results. The original shapes prolate and spheroidal are detected and their sub-forms; which are integrated into the term subspheroidal including: prolate spheroidal, subprolate, perprolate and oblate spheroidal. The lowest P/E ratio average is 0.93 (oblate-spheroidal to spheroidal) in *Trichodesma eherenbergii*, the highest P/E ratio average is 2.2 (perprolate) in *Lappula spinocarpos*.

2.2. Polarity: major pollen grains of Boraginaceae are isopolar, heteropolar. Subisopolar grains occasionally appear. Heteropolar grains in *Echium rauwolfii*, *Alkanna tuberculata* and *A. orientalis* due to that one pole is narrower than the other. Some species of *Echium* also are found to be heteropolar by [24]. Subisopolar grains present in three taxa of *Heliotropium*: *H. bacciferum* subsp. *bacciferum*; var. *bacciferum* and var. *erosum*, and *H. ramosissimum* due to a

connecting bridge appears between some colpi margins. Otherwise, taxa are isopolar and radially symmetric, appreciably that only *Echichilon fruticosum* is isopolar and bilaterally symmetric.

3. Apertures types & characters: pollen grains of Boraginaceae mainly found to be either homoaperturate grains or heteroaperturate ones. It is characterized also by large variation of aperture morphology [25]. Homoaperturate pollen group includes the tribes: Boragineae, Echieae, Echiochileae, Lithospermeae and Trichodesmeae of the subfamily Boraginoideae; heteroaperturate pollen group includes the subfamily: Heliotropioideae and two tribes of the Boraginoideae; Cynoglosseae and Eritrichieae. Homoaperturate colpate grain is only in *Echichilon fruticosum* (bicolpate). Those of the colpate aperture type vary by the different number of apertures ranging from 3 to poly-colpate, Echieae, Trichodesmeae and *Alkanna sp.* (Lithospermeae) are 3-colpate, Boragineae and *Arnebia sp.* (Lithospermeae) are 4 to poly (8-9)-colpate. The tribes Cynoglosseae and Eritrichieae and the subfamily Heliotropioideae are heteroaperturate grains; all taxa found in this group are 6-heteroaperturate except in *H. ramosissimum*; 8-heteroaperturate and in *H. bacciferum* subsp. *bacciferum* are 6-8-heteroaperturate. Here we agree with [26] who observed the presence of heteroapertures in Boraginaceae.

3.1. Colpi characters: different shapes of colpi are observed mostly linear and fusiform; rhombic and oblong are also presented. Colpi diameters range from 0.1-5.0 μm . the narrower colpus is linear, with acute ends, 0.1-0.2 μm in diam. in *Lappula spinocarpos*. The widest one is oblong, with obtuse ends, 3.0-5.0 μm in diam. in *Arnebia decumbens*. The length of the colpi is compared relative to the P-axis length. This feature is revealed by describing the colpi as very short, short, subterminal and terminal. This character is somewhat also indicating the colpi length values. Colpi margins appear not thickened or thickened either granulated, perforated or echinate.

3.2. Endoapertures characters: endoaperture shapes mostly found elliptic, rarely circular. Lalongate endoapertures are common, hereafter lalongate ones. A high variation in their sizes, the largest one is lalongate, elliptic and 6.0-9.0 x 3.0-4.0 μm in *Arnebia decumbens*; the smallest one is lalongate, c. 1.0 x 2.0 μm in *Paracaryum intermedium*. Endoapertures present singly for each colpus (monorate) of all taxa studied except in *Lappula spinocarpos* and two species of *Arnebia*: *A. decumbens* and *A. hispidissima* where as two endoapertures for each colpus (diorate).

4. Wall characters: tectum sculpture ornamentations are remarkably different, most of them are psilate perforate. An integration with other shapes presents such as reticulate, granulate, rugulate, and echinate. The simple pattern of sculpture elements occurs in *Alkanna sp.* (psilate), and in *Echium rauwolfii* (micro-reticulate). Otherwise, taxa are characterized by the compound pattern of more than one type of tectal elements. Most taxa studied showed an obvious differentiation between apocolpia and mesocolpia regions' sculpture elements and the rest tectum all over the wall surface. An equatorial band mostly with a distinct sculptured surface is characteristic for taxa of the tribe Boragineae.

CONCLUSION

Thus, the overall results of this peculiar aspect ensured the taxonomic implications of palynology and assessed the eupalynous nature of Boraginaceae. Introducing a well-developed identifying constant and valuable features.

Table 2: Pollen grains characters of the studied species of *Heliotropium* L. (Subfamily: Heliotropioideae):

Sections/Taxa Characters	Sect. Chamotropium	Sect. Odnotropium	Sect. Pterotropium			
	<i>H. supinum</i>	<i>H. arbianense</i>	<i>H. bacciferum</i> subsp. <i>bacciferum</i>		<i>H. ramosissimu m</i>	<i>H. pterocarpum</i>
			var. <i>bacciferum</i>	var. <i>erosum</i>		
P- axis (µm)	30 (31) 34	28 (29) 30	23 (25) 29	16.7 (18.1) 20	21(22)23	23.08 (26.86) 31.25
E-axis (µm)	18 (22) 25	21 (23) 24	20 (24) 28 µm	14.2 (15.5) 16.7	16(18)20	15.38 (16.55) 17.5
P/E (mean)	1.4 (1.5) 1.7	1.2 (1.3) 1.4	1.03 (1.04) 1.2	1.1 (1.2) 1.3	1.1 (1.2) 1.3	1.5 (1.6) 1.9
Pollen shape	Prolate	Subprolate to prolate	Prolate- spheroidal to subprolate	Subprolate or less prolate- spheroidal	Subprolate or prolate- spheroidal	Prolate
Med. Constriction	-	-	-	-	-	-
Equatorial band	-	-	-	-	-	-
Apocolpium diam. (µm)	8-11	7-11	9-12	7-9	3-5	8-10
Aperture type	6- heterocolpate	6- heterocolpate	6(-8)- heterocolpate	6(-8)- heterocolpat e	8- heterocolpate	6- heterocolpate
Colpus characters:						
1. Shape	S: Linear C: Linear	S: Linear C: Linear	S: Linear C: Linear	S: linear C: linear	S: Linear C: Linear	S: Linear C: Linear
2. Length (µm)	S: 23-27 C: 23-27	S: 19-24 C: 19-24	S: 19-20 C: 18-19	S: 15-17 C: 13-16	S: 18-20 C: 15-18	S: 20-29 C: 20-25
3. Relative to P-axis (? x)	S: Terminal, c. 0.8x C: Terminal, c. 0.8x	S: Subterminal, c. 0.7x C: Subterminal, c. 0.7x	S: Terminal, 0.8-0.9x C: Terminal, c. 0.8x	S: Terminal, 0.8-0.9x C: Subterminal	S: Terminal, 0.8-0.9x C: Subterminal,	S: Terminal, 0.8-0.9 x C:Subtermin al, 0.7-0.8x

				, 0.7-0.8x	0.7-0.8x	
4. Diameter (µm)	S: 0.5-1.0 C: 0.5-1.0	S: 0.5-0.75 C: 0.5-0.75	S: 0.5-0.7 C: 1.4-1.6	S: 0.4-0.7 C: 1.0-1.75	S: 0.3-0.5 C: 0.5-1.0	S: 0.3-0.5 C: 0.3-0.6
5. Margin	Not thickened	Not thickened	Not thickened	Not thickened	Not thickened	Not thickened
Endoaperture characters:						
1. Shape	Lalongate (elliptic)	Lalongate (elliptic)	Lolongate (elliptic)	Lolongate (elliptic)	Lolongate (elliptic)	Lolongate, (elliptic)
2. Diameter (µm)	c. 3 x 4	c. 6 x 7	3-4 x 2-2.5	c. 3 x 2	c. 3 x 2	2-3 x 1-2
Wall characters:						
1. Exine thickness (µm)	0.9-1.2	1.3-1.5	1-1.4	0.9-1.4	1-1.4	0.9-1.6
2. Apo-Mesocolpia differentiation	+	-	+	+	+	+
3. Tectum	Perforate-rugulate or fossulate	Psilate-perforate	Perforate-rugulate	Perforate-rugulate	Perforate-rugulate	Perforate-rugulate

Table 3: Pollen grains characters of the studied species in Eritricheae, Cynoglosseae & Trichodesmeae (Subfamily: Boraginoideae).

Tribe/taxa Characters	Tribe: Eritricheae		Tribe: Cynoglosseae		Tr. Trichodesmeae		
	Asperugo procumbens	Lappula spinocarpos	Paracaryum intermedium	P. rugulosum	Trichodesma africanum		T. eherenbergii
					var. africanum	var. heterotrichum	
P- axis (µm)	8 (9) 10	13 (14) 16	6 (8.3) 10	9.78 (10.81) 12.44	20 (22) 23	14 (15) 16	13 (13) 14.2
E-axis (µm)	5 (5.5) 6	5 (6.5) 8	4 (4.6) 5.5	7.78 (8.37) 9.22	17 (18) 18	12 (13) 15	14 (14) 14.4
P/E (mean)	1.5 (1.6) 1.8	2.0 (2.2) 2.6	1.5 (1.8) 1.82	1.26 (1.29) 1.35	1.20 (1.22) 1.24	1.1 (1.2) 1.3	0.92 (0.93) 1
Pollen shape	Prolate	Perprolate	Prolate	Subprolate to prolate	Subprolate	Prolate-spheroidal	Oblate-spheroidal to spheroidal

Median Constriction	Slight	+	Slight	-	-	-	-
Equatorial band	-	-	-	-	-	-	-
Apocolpium diameter (µm)	3-6	6-7	3-5	5-6	4-5	4-5	8-10
Aperture type	6-heterocolpate	6-heterocolpate	6-heterocolpate	6-heterocolpate	3-colpotare	3-colpotare	3-colpotare
<u>Colpus characters:</u>							
1. shape	S: Linear C: Rhombic	S: Linear C: Linear	S: Nearly linear C: Broadly rhombic	S: Rhombic C: Rhombic	Fusiform	Fusiform	Fusiform
2. length (µm)	S: 5-8 C: 3-5	S: 10-13 C: 9-12	S: 4-6 C: 3-4	S: 6-8 C: 4-5	18 (19) 20	11(12) 13	10 (11) 12
3. relative to P-axis (? x)	S: subterminal, c. 0.8x C: very short, c. 0.4x	S: subterminal, c. 0.8x C: subterminal, c. 0.7x	S: short, c. 0.6x C: very short, c. 0.4x	S: short, 0.6-0.7x C: very short, c. 0.4x	Terminal, 0.8-0.9x	Terminal, c. 0.8x	Terminal, c. 0.8x
4. diameter (µm)	S: 0.3-0.5 C: 1-2	S: 0.1-0.2 C: 0.3-0.5	S: 0.2-4 C: 0.6-1	S: 1-1.5 C: 2-2.5	1-2	1-2	0.5-1
5. margin	Thickened	Thickened	Thickened	Thickened	Thickened	Thickened	Thickened
<u>Endoaperture characters:</u>							
1. shape	Lalongate (elliptic)	Lalongate (elliptic)	Lalongate (elliptic)	Lalongate (elliptic)	Lolongate (elliptic)	Lolongate (elliptic)	Lolongate (elliptic), rarely circular
2. diameter (µm)	c. 2 x 3	1-2 x 2-2.5	c. 1 x 2	1-1.5 x 2-3	6-7 x 3-4	5-7 x 3-4	4-5 x 3-5
<u>Wall characters:</u>							
1. Exine thickness (µm)	0.3-0.4	0.4-0.5	0.6-0.7	0.64-0.72	1-1.2	0.98-1	1-1.6

2. Apo-mesocolpia differentiation	+	+	-	-	-	-	-
3. Tectum	Psilate-perforate	Psilate-perforate	Psilate-Perforate	Psilate-Perforate	Micro-echinate-perforate	Micro-echinate-perforate	Micro-echinate-perforate

Table 4: Pollen grain characters of Tribe: Boragineae:

Taxa Characters	Tr. Boragineae				
	Anchusa				Gastrocotyle
	Subg. Anchusa	Subg. Buglossoides		Subg. Hormuzakia	
	<i>A. undulata</i> subsp. <i>hybrida</i>	<i>A. aegyptiaca</i>	<i>A. milleri</i>	<i>A. aggregata</i>	<i>G. hispida</i>
P- axis (µm)	32 (34) 36	26 (27.30) 28.33	24 (26) 27	23 (26) 29	20 (22) 23
E-axis (µm)	23 (24) 25	16 (18.25) 20	17 (19) 21	18 (22) 24	19 (22) 25
P/E (mean)	1.39 (1.42) 1.44	1.33 (1.5) 1.70	1.28 (1.36) 1.4	1.1 (1.2) 1.3	0.92 (1) 1.1
Pollen shape	Prolate	Prolate	Prolate to subprolate	Prolate- spheroidal to subprolate	Spheroidal or Prolate- spheroidal
Med. Constriction	-	-	-	-	-
Equatorial band	+	+	+	-	+
Apocolpium diam. (µm)	15-20	15-17	13-16	11-14	4-5
Aperture type	4-colporate	4(-5)- colporate	4-colporate	6-7- colporate	8-9-colporate
Colpus characters:					
1. shape	Fusiform	Fusiform	Fusiform	Fusiform	Rhombic
2. length (µm)	13 (14) 15	10 (12) 13	10 (10.5) 11	9 (12) 14	10 (12) 14
3. relative to P-axis (? x)	Very short 0.4x	Very short 0.3-0.4x	Very short 0.4-0.5x	Very short 0.4x	Short 0.5-0.6x
4. diameter (µm)	0.3-0.7	0.3-0.7	1-2	0.5-1.4	1-1.5
5. margin	Thickened, granulated	Thickened, finely granulated	Not thickened	Thickened, granulated	Thickened, granulated
Endoaperture characters:					
1. shape	Lalongate (elliptic)	Lalongate (elliptic)	Lalongate (elliptic)	Lalongate (elliptic)	Lalongate (elliptic)
2. diameter (µm)	3-5 x 5-8	2-3 x 3-4	2-3 x 4-7	1-2x3-4	c. 3 x 4
Wall characters:					
1. Exine thickness (µm)	0.52-0.89	1-1.61	1.28-1.56	0.75-0.96	0.8-1.0
2. Apo-mesocolpia differentiation	+	+	+	-	+(reduced)
3. Tectum	Psilate-Perforate with micro- reticulate equatorial band	Psilate- Perforate with reticulate equatorial band	Psilate- Perforate with micro- reticulate equatorial band	Psilate- Perforate with micro- reticulate	Psilate- Perforate with granulated equatorial band

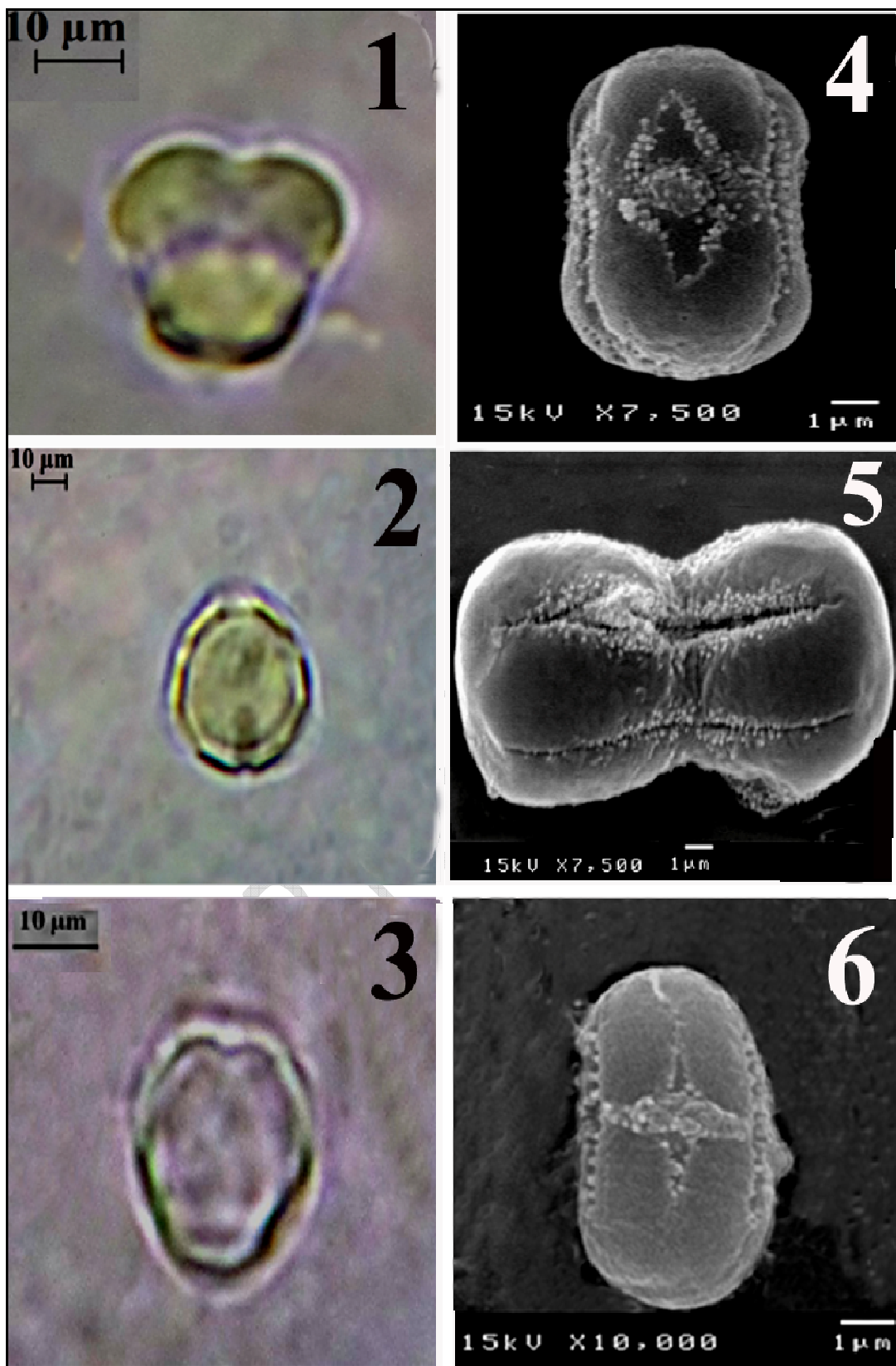


Plate 1. Fig. 1-3: LM-digital images of the pollen polar view for pollen **TYPE 2 (6-heterocolpate-Perforate)**: **1.** *Asperugo*, **2.** *Lappula* & **3.** *P. rugulosum*. **Fig. 4-6:** SEM-micrographs of the equatorial views for: **4.** *Asperugo procumbens*, **5.** *L. spinocarpus* var. *spinocarpus* & **6.** *P. intermedium*.

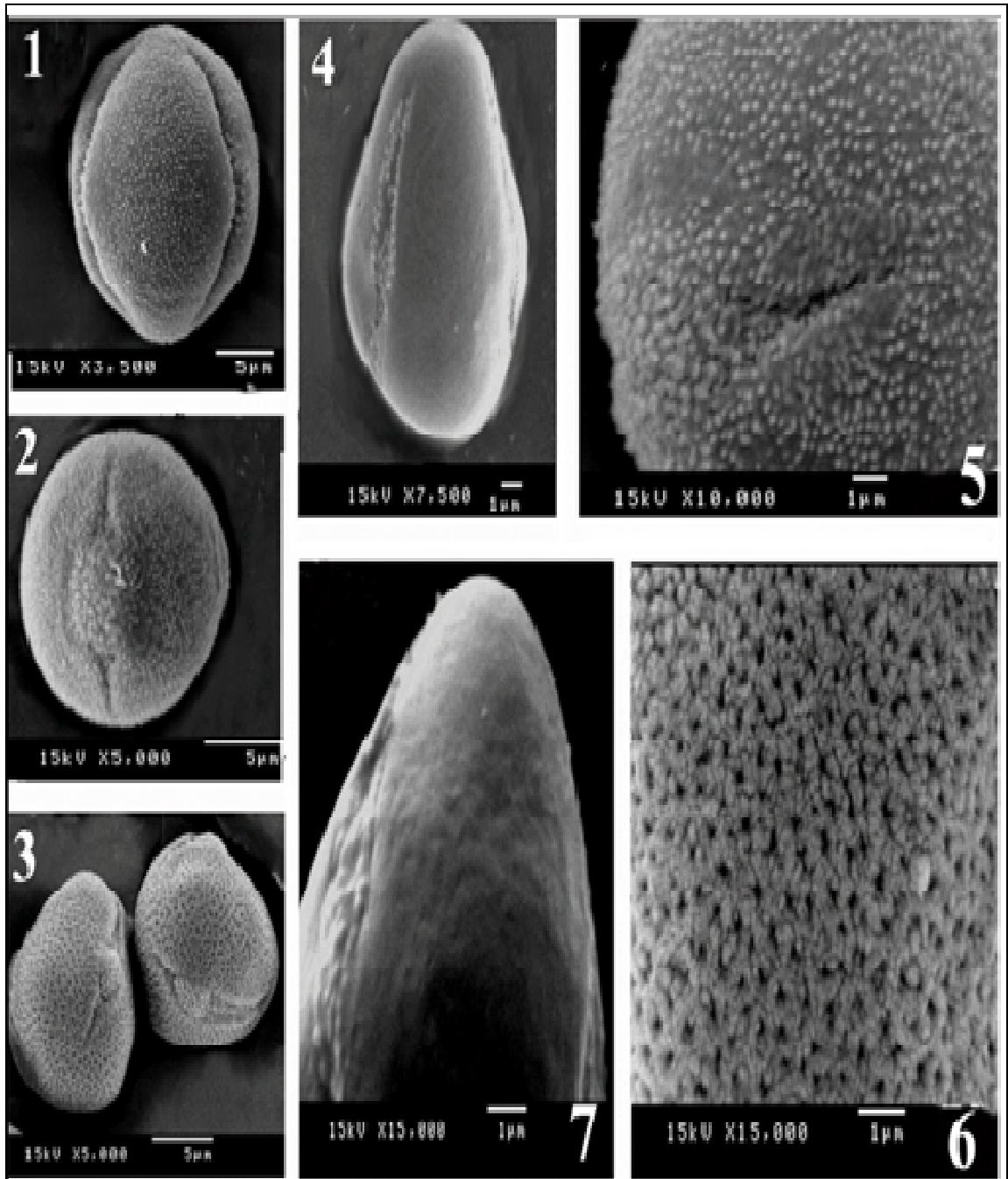


Plate 2. (Fig. 1-7) SEM-micrographs of pollen TYPES 4, 5 & 6 in Echieae, Trichodesmeae & *Alkanna* sp. respectively (equatorial views).

TYPE 4: 3-colporate-Micro-reticulate in **3.** *Echium rauwolfii* & **6.** Tectum Micro-reticulate.

TYPE 5: 3-colporate-Micro-echinate in **1.** *Trichodesma africanum*, **2.** *T. ehrenbergii* & **5.** Tectum Micro-echinate.

TYPE 6: 3-colporate-Psilate in **4.** *Alkanna* sp. & **7.** Tectum Psilate.

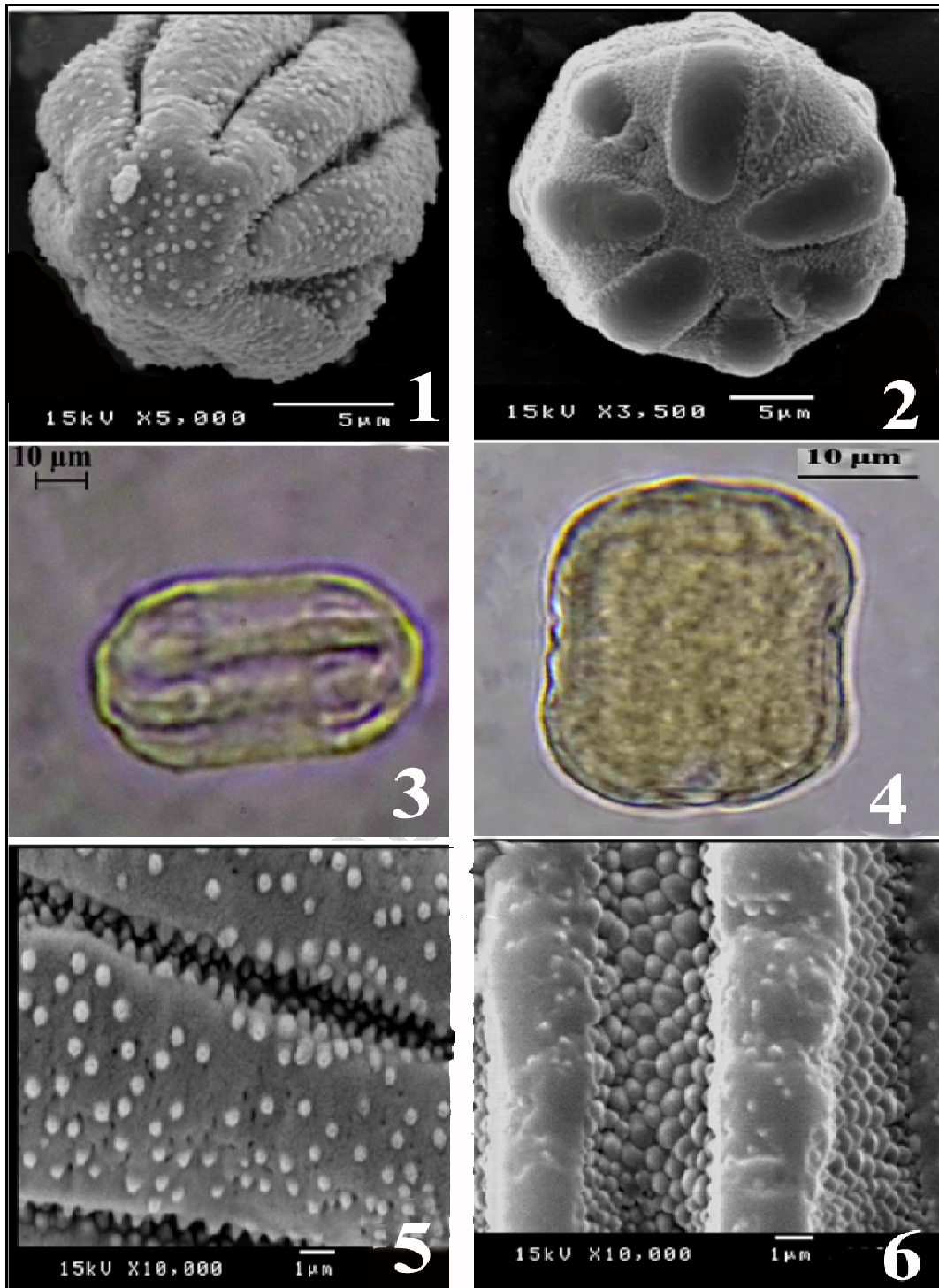


Plate 3. SEM-micrograph & LM-images of polar & equatorial views in *Arnebia* pollen types; **1. & 3.** Pollen **TYPE 8** in *Arn. hispidissima*, **2. & 4.** Pollen **TYPE 7** in *Arn. decumbens*. **5.** SEM-micrograph of tectum in *Arn. hispidissima* and **6.** Tectum of *Arn. decumbens*.

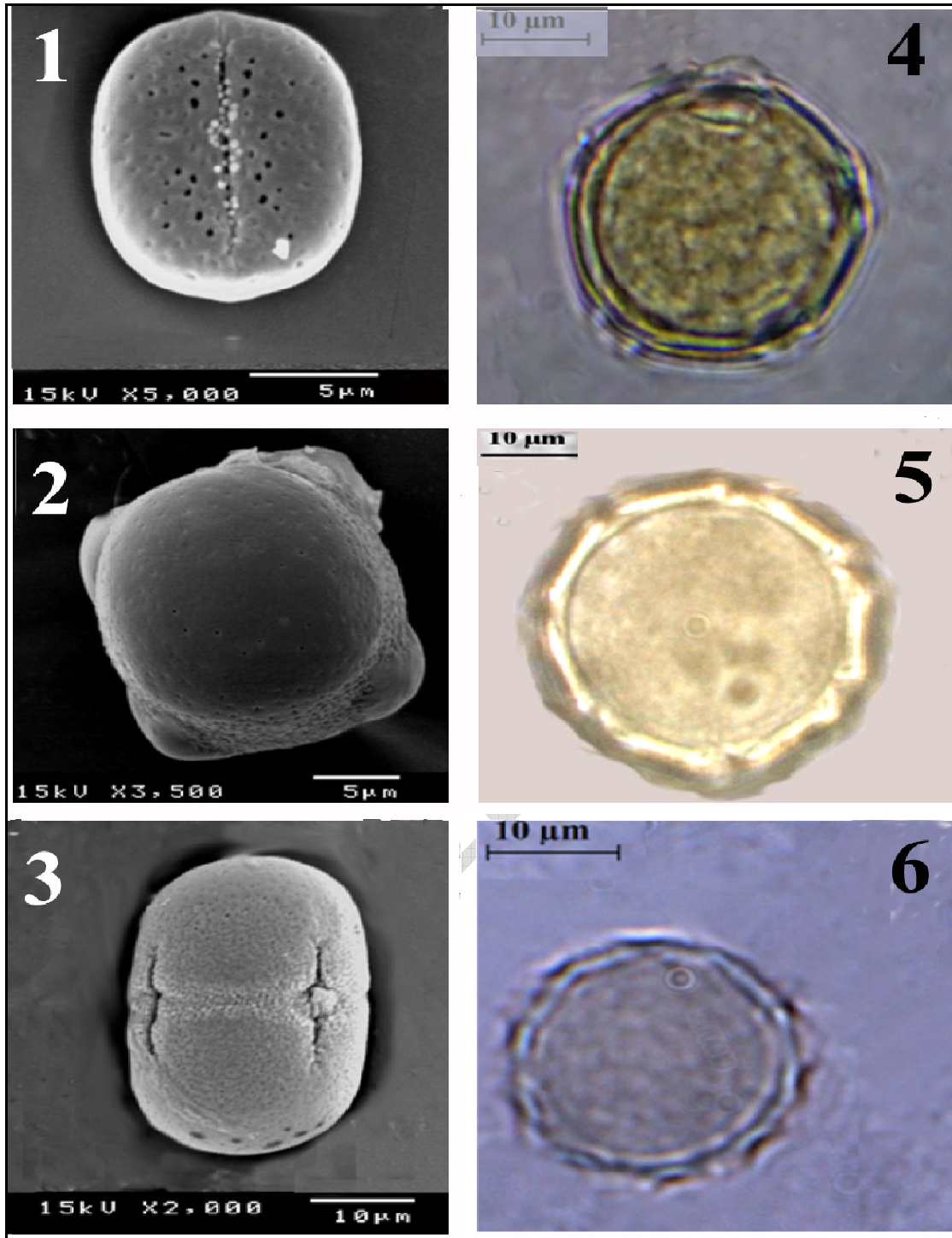


Plate 4. Fig. 1. Echiochilon-TYPE 3 "Bicolpate-Perforate" in *E. fruticosum*. **Fig. (2-6)** Boragineae-TYPE 7 "4-9-colporate-Perforate" in 2. *Anchusa milleri* and 3. *A. undulata* subsp. *hybrida*, 4. *A. aegyptiaca* 5. *A. aggregata* and 6. *Gastrocotyle hispida*.

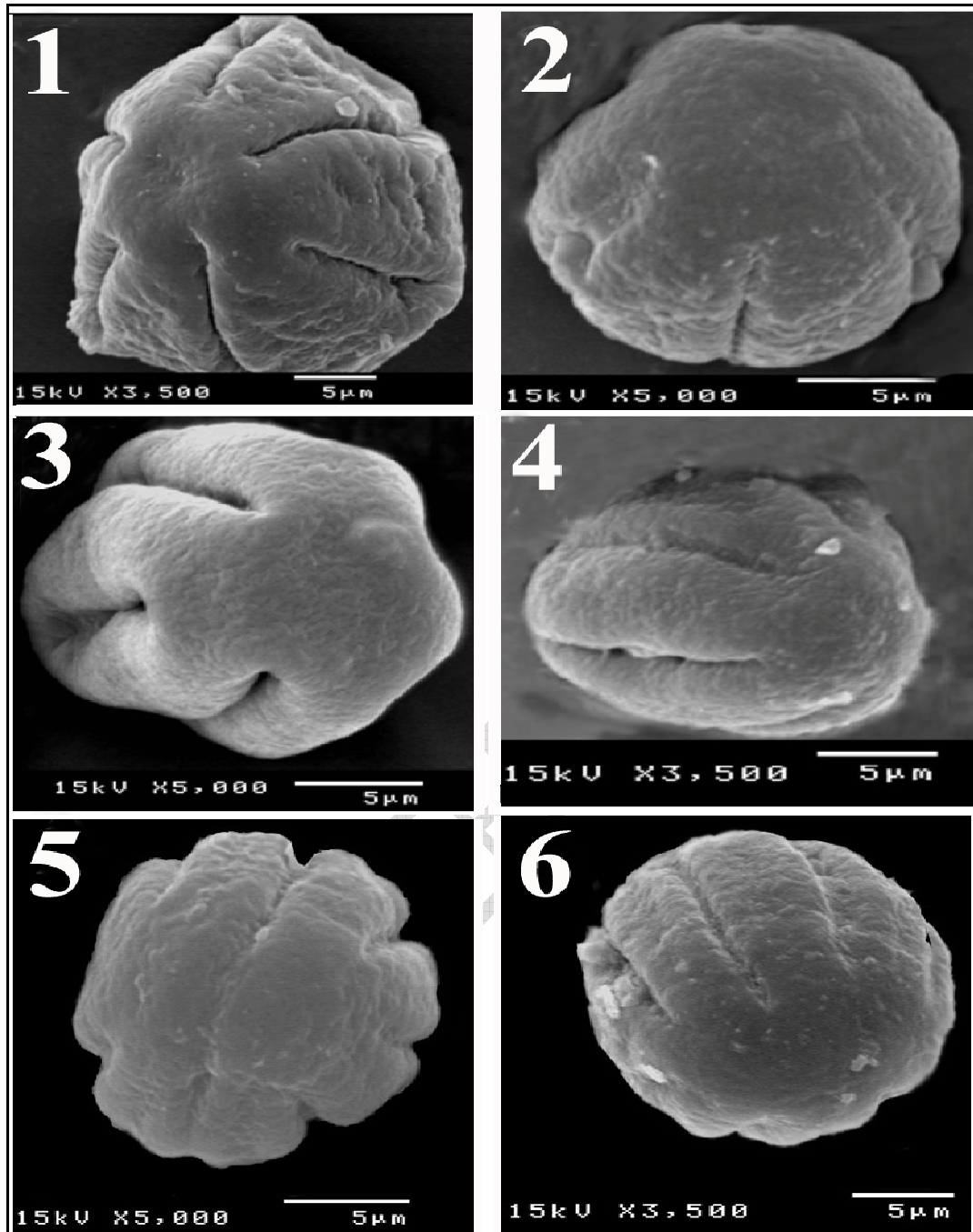


Plate 5. Fig. 1-6: SEM-micrographs of pollen polar views for *Heliotropium* pollen-**TYPE 1 (6-heterocolpate)** in **1.** *H. supinum*, **2.** *H. bacciferum* subsp. *bacciferum* var. *erosum*, **3.** *H. pterocarpum* & **4.** *H. kassasii* (in comparing). **8-heterocolpate** pollen grains in **5.** *H. bacciferum* subsp. *bacciferum* var. *bacciferum* and **6.** *H. ramosissimum*.

REFERENCES

- [1] APG IV. An update of the Angiosperm. Phylogeny Group Classification for the orders and families of flowering plants. Bot J of the Linn Soc. 2016; 181: 1-20.
- [2] Heywood VH, Brummitt RK, Culham A, Seberg O, eds. Flowering Plant Families of the World. Canada, Firefly Books Ltd, 2007.
- [3] Clarke CGS. The Northwest European Pollen Flora: 10. Boraginaceae. Rev of Palaeo & Palyn. 1977; 24(2): 59-101.
- [4] Diez MJ. Contribucion al Atlas Polinico de Andalucia Occidental, I. Boraginaceae. Lagas. 1984; 13: 147-171.
- [5] Diez MJ. A general survey of pollen types in *Anchusa* L. (Boraginaceae) in relation to taxonomy. Acta Bot Gall. 1994; 141(2): 233-242.
- [6] Diez MJ, Valdes B. Pollen morphology of the tribes Eritrichieae and Cynoglosseae (Boraginaceae) in the Iberian Peninsula and its taxonomic significance. Bot J of the Linn Soc. 1991; 101(1): 49-66.
- [7] Hargrove L, Simpson MG. Ultrastructure of heterocolpate pollen in *Cryptantha* (Boraginaceae). Int J of Plant Sci. 2003; 164(1):137-151.
- [8] El Ghazaly G. Pollen morphology of the family Boraginaceae in Qatar. Qat Univ Sci J. 1995; 15(1): 65-75.
- [9] Perveen A, Qureshi US, Qaiser M. Pollen flora of Pakistan- IV. Boraginaceae. Pak J of Bot. 1995; 27(2): 327-360.
- [10] Scheel R, Ybert JP, Barth OM. Pollen morphology of the Boraginaceae from Santa Catarina State (southern Brazil), with comments on the taxonomy of the family. Grana 1996; 35: 138-153.
- [11] Khatamsaz M. Pollen morphology of the Iranian Boraginaceae family and its taxonomic significance. Iran J of Bot. 2001; 9(1): 27-40.
- [12] Bigazzi M, Selvi F. Pollen morphology in the Boragineae (Boraginaceae) in relation to the taxonomy of the tribe. Plant Sys & Evol. 1998; 213:121-151.
- [13] Långström E, Oxelman B. Tribes of Boraginoideae (Boraginaceae) and placement of *Antiphytum*, *Echiochilon*, *Ogastemma* and *Sericostoma*: a phylogenetic analysis based on atpB plastid DNA sequence data. Plant Sys & Evol. 2003; 234: 137-153.
- [14] Al Qura'n S. Pollen characteristics of three *Anchusa* species and the observation of bees as pollen collector in Jordan. Pak J of Entom. 2011; 33(2): 113-117.
- [15] Binzet R. Pollen morphology of some *Onosma* species (Boraginaceae) from Turkey. Pak J of Bot. 2011; 43(2): 731- 741.
- [16] Taia, W.K. and Shiha, M.A. (1999). Palynological investigations in some Egyptian species of Boraginaceae. Journal of Union of Arab Biologists (9B): 385-402.
- [17] El Hadidy A. Boraginaceae. In: Boulos L, ed. Flora of Egypt, vol. 2, (Geraniaceae-Boraginaceae) Egypt, Al Hadara Publications, 2000: 268-309.
- [18] Govaerts R. ed. World Checklist of Seed Plants. Antwerpen, 1995.
- [19] Jarvis C. Order Out of Chaos. London, Linn Soc of London & Nat Hist Mus, 2007.
- [20] (<http://www.theplantlist.org>)
- [21] (<http://gni.globalnames.org>)
- [22] (<http://www.tropicos.org>).
- [23] Punt W, Hoen PP, Blackmore S, Nilson S, Le Thomas A. Glossary of pollen and spore terminology. Rev of Palaeo & Palyn. 2007; 143:1-81.
- [24] Erdtman G, ed. Pollen Morphology and Plant Taxonomy Angiosperms. Stockholm, Almqvist & Wiksell, 1952.

- [25] Willis JC, ed. A Dictionary of the Flowering Plants and Ferns. Cambridge, 1975.
- [26] Iversen J, Troels-Smith J. Pollenmorphologische Definitionen und Typen. Danm. Geol. Unders. 1950; 4,3(8): 1-54.

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