



Pollen grain diversity and application in taxonomy and evolution

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Abstract

Background and aims: Pollen grains, the male gametes within the higher plants, have great diversity in morphological characters. In this review, the history of the pollen morphological studies, the applications and importance in plant taxonomy discussed. **Methods.** Literature concerning the importance of studying pollen morphological characters in various ways viz., identification, classification and grouping of plants. **Key Results.** The survey of the recent literature revealed that pollen grain characters and composition have essential role in plant taxonomy, identification and evolution. **Conclusion.** Pollen morphological studies considered an important tool in taxonomical studies and solve many obscure problems in taxonomical confusions when used in precise way.

Key words: Evolution, Harmomegathy, History, Pollen grains, Taxonomy

Brief history

The pollen grains are wonderful realm with fantastic variations, these male gametes in the gymnosperms and angiosperms, are full of mystery discovering. They are very tiny particles look like the dust which cannot be observed by naked eyes. The first observations of pollen grains were by Grew (1682) and afterwards by Malpighi (1901) who recorded very small granules deposited on the petals of the flowers in the mid 17th Century, shortly after the invention of the microscope they seen this dust for the first time. Grew (op.cit.) realized the importance of these dusts in reproduction, after discovering that the stamens bearing them. His observation was very interesting at his time, and led to more examination of the orange or brown dusts on the petals of the flowers to discover an astonishing scene that not all of these dusts are similar and each group of plants have different characters of pollen grains. With the developing of the microscope, scientists became able to make intense examination of these dusts and became easier to realize that these dusts are neither alike nor smooth and have ornamented walls and apertures. In the seventeen sixties onward new observations in pollen grains have been mentioned. Sprengel (1804) found that each plant species has different morphological pollen characters. Sprengel noticed the presence of different apertures either pores or furrows in the wall of the pollen grains or even slits or fissures and distinguished between anemophilous and entomophilous pollen grains (de Candolle & Sprengel 1821). Kolreuter (1806 & 1811), mentioned that intense microscope examinations were not only very imperfect, but also, he discovered that the outer wall of pollen

grains consists of two distinct coats. He reported the presence of spines on the outer coat in some species and made the first trial to classify pollens on the basis of their morphology (Manten, 1966 a & b, 1967 a & b).

Gradually scientists discovered that these dusts are important for pollination and reproduction, and then pollination experiments started in the palm trees by Gleditsch (1751, 1765) for the first time. Linneaus (1750) was the first use of the term pollen grains on these dusts. Later on Robert Brown, in 1810 found that pollen grains of different taxa are different and could be used for systematic studies of seed plants. Brown's observation of minute but significant details was also shown in his publication on the plant family Proteaceae, in which he showed how the study of the morphological characters of the pollen grain could assist in the classification of plants into new genera. Lindley (1836) was from the first use of pollen grain morphology in the classification, organization, natural affinities, and geographical distribution of the whole vegetable kingdom. Lindley work (op.cit.) built the first step in using pollen morphological characters in plant taxonomy and switch the light on this astonishing and wonderful world. More and intense information about the development of the science of palynology and how it progressed is given by Halbritter *et al.* (2018) who gave sequences in the correlation between the invention of microscope and the first notice of the pollen grains. The importance of pollen grain in taxonomy and classification moved and progressed very slowly until Lindley publication (1836). By the first half of the nineteenth century, pollen grain investigations

became from the most important topics between scientists. Wodehouse (1935) mentioned that Purkinje (1830) system of pollen classification deserve more attention. Purkinje (op.cit.) was the first who used pollen grain morphology in plant classification.

Pollen grains and plant taxonomy

Pollen morphological characters used in solving complicated taxonomical problems of interrelationships between various taxa, particularly with reference to the lower ranks of families, subfamilies, tribes, genera and species. Mature pollen grain size, exine sculpturing, and number of pores are the most distinctive features. The study of the symmetry, polarity, shape, size, structure, sculpture and of the apertures of the sporoderm ornamentations can be very useful in taxonomy (Perveen, 2000). Pollen morphological studies gained great interest in solving many taxonomical problems and consider as useful tool in plant identification. The greatest contribution made by a single person was that made by Swede, Gunnar Erdtman, who from the 1950's to the 1970's produced several classic books and papers which remain the basis in this field and required reading till today. Erdman published his books on the taxonomy, descriptions and terminology of the pollen grains. Afterwards pollen morphology

became an integrative tool in most taxonomic works. To use pollen morphology in taxonomical works, researchers must be very accurate and honest in descriptions. The pollens were prepared for light microscope examination by standard method called acetolysis (Erdtman, 1962). Erdtman (1952) illustrated LO-analysis in examining the pollen samples, where 'L' is derived from the Latin word lux meaning light and 'O' is derived from the Latin word obscurities meaning darkness, this analysis is important in investigating the exine stratification by light microscope. In sexine of angiosperm pollen, three regions are encountered- suprattegillar, tegillar and infrategillar. The term tegillum (syn. tectum) denotes the layer of sexine that forms a roof over columella and baculum. The sporoderm can be seen at different focuses with an optical microscope (Fig.1). Zavalova *et al.* (2018) pointed to the importance of using high resolution microscopes in examining the pollen grains as SEM and TEM. Accordingly, Romero *et al.* (2020) developed three convolutional neural network (CNN) classification models: maximum projection (MPM), multislice (MSM), and fused (FM) in an Airyscan optical microscope to increase the resolution power and gives 3D captures in examining the pollen grains. This microscope helps in comparing between pollen taxa and gives precise descriptions.

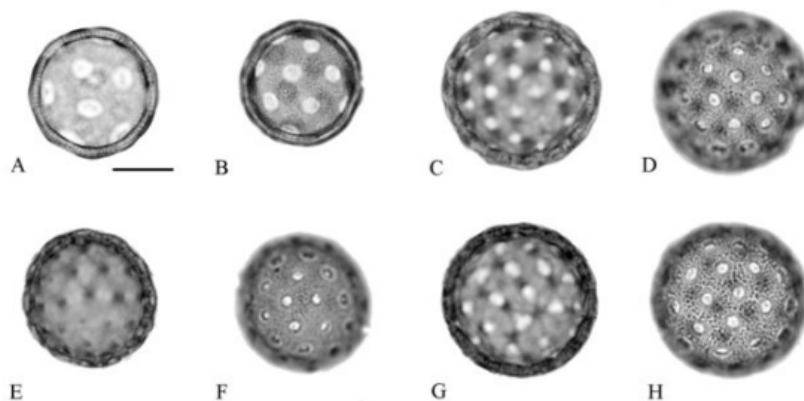


Fig. 1. Pollen grains of *Amaranthus* species under the LM in the two focuses LO analysis after Taia (1996), A, C, E & G the first focus, B, D, F & H the second focus

From the most recent works are that of Mostafa *et al.* (2017) used the pollen characters in solving the taxonomic problems within the Dipsacaceae genera and moved some species of *Scabisoa* to *Lomelosia* Raf. They recorded two types of pollen apertures, either triporate or tetraporate and tricolporate, with different exine ornamentations (Fig.2). Afterwards Tsymbalyuk *et al.* (2021) found that the pollen characters of the genus *Cephalaria* Schrad., family Caprifoliaceae, is similar to those of the genus *Dipsacus* L., which

confirms their inclusion in tribe Dipsaceae. Soares *et al.* (2018) found that pollen characters are useful in solving the confusion between species of the subgenera *Passiflora* and *Decaloba* that can be used for better understanding of the taxonomy of the genus *Passiflora*. Concerning the pollen grains of family Passifloraceae, Richardo & Silverio (2019) found great variability in their reticulate pattern, sporoderm stratification, and aperture characters that facilitate the identification of the three studied subgenera *Passiflora*, *Astrophea*, and

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Decaloba. Ragho (2020) used the pollen morphological characters in the identification of 42 medicinal species growing widely in the field. Concerning family Leguminosae, many palynological works have been done to clarify the relations between the subfamilies, genera and even the species of the same genus. Taia (2004) and Taia *et al.* (2022) studied the relation between the different taxa under tribe trifolieae and the pollen

characteristics of *Bauhinia* species and recorded different pollen characters as well as different dispersal modes which indicate a phylogenetic line within the studied species. Da Luz *et al.* (2013) recorded polyad dispersal mode in *Stryphnodendron adstringens* (Mart.) Coville only which characterize this species within the studied seven genera of the Fabaceae (Fig. 3).

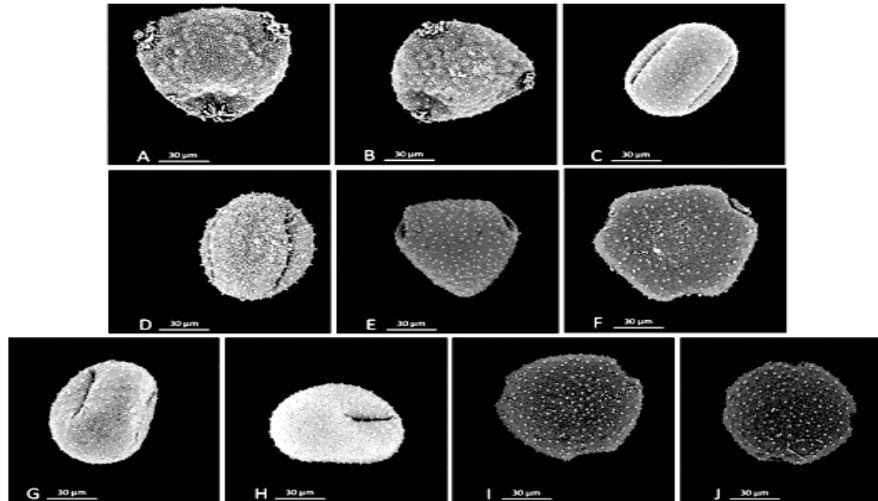


Fig. 2. Scanning electron microscope photographs of pollen grains. A: *Cephalaria kotschyi*, B: *C. procera*, C: *Pterocephalus plumosus*, D: *P. canus*, E: *Dipsacus strigosus*, F: *Scapiosa caucasica*, G: *S. amoena*, H: *S. koelzii*, I: *S. rotata* (triporate), J: *S. rotata* (tetraporate) (after Mostafa *et al.*, 2017)

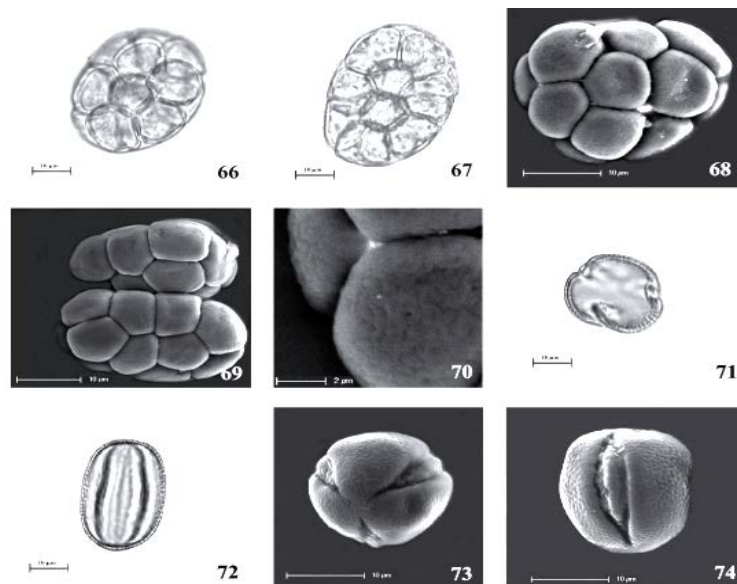


Fig. 3. 66-74- Light and scanning electron micrographs of pollen grains of *Stryphnodendron adstringens* and *Zornia diphylla*. 66-70. *Stryphnodendron adstringens* (Mart.) Coville. 66. Front view of the polyad, optical section, LM. 67. Side view of the polyad, optical section, LM. 68. Side view of the polyad, SEM. 69. Gerar view in two polyads, SEM. 70. Detail of ornamentation, SEM. 71-74. *Zornia diphylla* (L.) Pers. 71. Polar view, optical section, LM. 72. Equatorial view, optical section, LM. 73. Polar view, SEM. 74. Equatorial view, SEM. Scale bar = 2 µm (70); 10 µm (66, 67, 68, 69, 71, 72, 73, 74) (after Da Luz *et al.*, 2013).

From the most interesting things in studying the pollen grains, is their dispersal which characterizes some families, subfamilies, genera or even species. The pollen grains shed from the anther in various ways. It may disperse as separate pollens (monads), two attached ones (dyads), four (tetrads) or in groups called Pollinia. Meanwhile, the type and size of pollinators carrying the pollens affect the distances and efficiency of their dispersal (Wessinger, 2021). The Pollen dispersal unit (PDU) is first used by Pacini (1997) to indicate the status of the pollen grains at shedding from the anthers. Pollens dispersed in groups are united by means of viscous liquid derived from the tapetal layers degeneration, by viscin threads or they are all have common wall (Pacini and Franchi 1996, 1999 a & b; Hesse *et al.*, 2000, Pacini & Hesse, 2005). Within the different taxa which dispersed

their pollen grains in pollinia, Pacini & Franchi (1998 & 2000) recorded 13 PDUs. In Monocots, they recorded several PDUs with the most different one recorded in the family Orchidaceae. Johnson and Edwards (2000) found that the pollinia in both the Orchidaceae and Asclepiadaceae allowed the two families to greater diversity in their pollination system, as the single pollinium contains more than a million pollen grains (Fig. 4). Kant (2019) found that the development of the pollinia in one of the species of Orchidaceae; *Calanthe tricarinata* Lindl. contributed significantly in the taxonomy and understanding character evolution in the family. Not only the monocots which disperse their pollens as pollinia, but also many dicots. families and subfamilies characterize by the pollen dispersal unit, pollinia, as in Apocynaceae, Lamiaceae, Euphorbiaceae and Mimosaceae (Swapna, 2018).

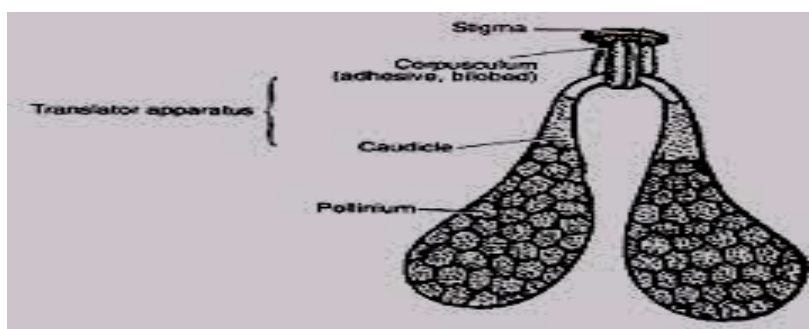


Fig.4. Pollinarium in Orchidaceae

From the most recent taxonomic work concerning the pollen dispersal units is that of Cruz *et al.* (2017) on selected species belonging to subfamily mimosoideae (Leguminosae). They recorded Only polyads dispersal units in tribe Ingeae (*Abarema* Pittier, *Albizia* Durazz., *Calliandra* Benth. and *Inga* Mill.) while two types of pollen dispersal units in the tribe Mimoseae; the bitetrad type (*Mimosa bimucronata*, *Mimosa elliptica*, *Mimosa pellita* and *Piptadenia trisperma*); and the polyad type (*Anadenanthera colubrina*, *Mimosa ceratonia* and

Piptadenia pterosperma). This finding can help in distinguishing the major taxa under this subfamily. Wronska-Pilarek *et al.* (2018) on the *Eruca* species. They found that six out of forty five studied species dispersed their pollens as monads, eight species as tetrads, from 2 to seven species as polyads (Fig.5) while the rest of the studied species are unrecognized. This feature beside other palynological characters assists in the identification and clarification of the subgenera and sections under this genus.

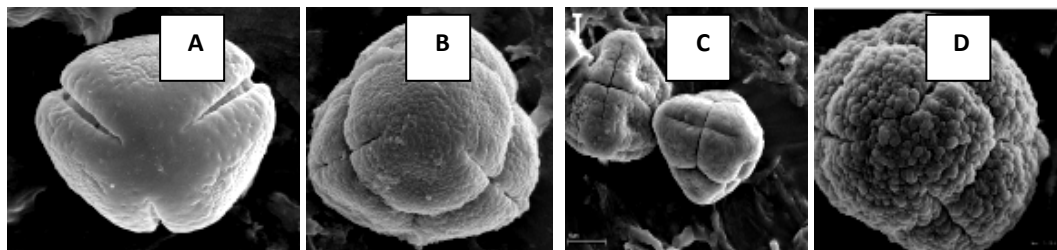


Fig. 5. Pollen dispersal in *Eruca* species A. Monad in *E. fastigiata*, B, C. & D. Tetrads in different *Eruca* species after Wronska-Pilarek *et al.* (2018).

Pollen grain diversity and application in taxonomy and evolution

The second observable characters in the morphology of the pollen grains are their color, polarity and symmetry, as they can be observed easily by the light microscope. The pollen grain color in its fresh natural pollens is usually orange, grade of brown or even yellow. After preservation the color of the pollen grains alters and be of no value in plant taxonomy. Sometimes the color will be characteristic to some taxa as in some members of the Compositae it is violet and blue in some taxa of the Campanulaceae. Joujeh *et al.* (2019) used the

pollen grain color to group the *Centaurea* species into two categories; those with white pollen grains versus species have yellow pollen grains (Fig. 6). Spherical pollen grains are apolar ones i.e., it is difficult to distinguish their two poles, if the two poles are similar the pollens will be isopolar while dissimilar poles make the pollen grains heteropolar (Fig. 7). Symmetry of the pollens corresponds to the similarity in various parts of pollen in respect to a plane where it will be either bilateral symmetry or radial symmetry.

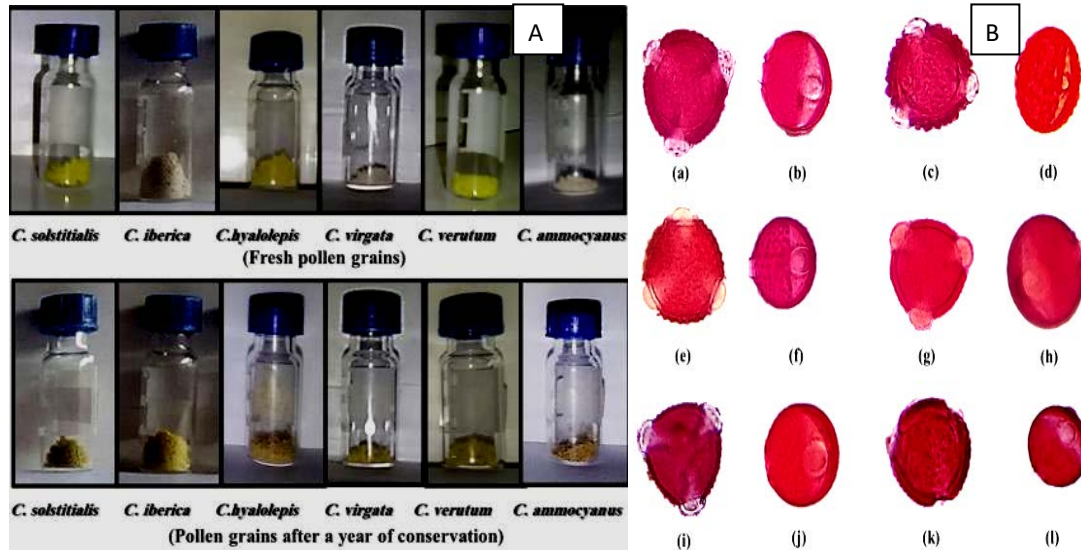


Fig. 6. A color of the pollen grains (fresh & after a year), **B** Shape of the pollen grains by LO investigation; a,b *C.solstitialis*, c,d *C.iberica*, e,f *C.hyalolepis*, g,h *C.virgata*, I,j *C.verutum*, k,l *C.ammocyanus* (After Joujeh *et al.* (2019))

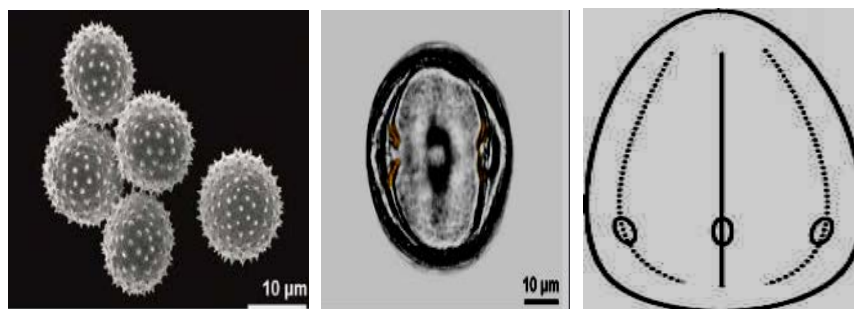


Fig. 7. Apolar symmetric, Isopolar symmetric, Heteropolar asymmetric (After Joujeh *et al.* (2019))

It is worth mentioned that the direction of the colpi is the only thing to recognize the two poles and recognize the polar axis from the equatorial axis of the pollen grains in case of monads. In polyads or in tetrads and even diads, the poles can be distinguished from the pollen attachments, the two attached points are the proximal poles while the two far points are the distal poles. Pantoporate separated pollen grains considered apolar.

Pollen grain apertures and their significance in taxa identification

The regions which significantly differ structurally and morphologically from the rest of the pollen wall and where the external exine layer is either absent or very reduced in thickness are termed apertures. These cite are responsible for pollen germination. Types, number, position and shape of apertures consider from the most important feature in pollen morphology and taxonomy. Erdtman (1969) grouped the flowering plants according to their pollen apertures into two categories, those with a single (Polar) pollen aperture ('Monosulcates) and those with three (Equatorial)

pollen apertures ('Tricolpates') (Fig.8). Later and after more than thirty five years, this pattern was partially confirmed using DNA sequencing and molecular phylogenetic reconstruction (Chase *et al.*, 1993, Blackmore & Crane, 1998, Qui *et al.*, 1999, Soltis *et al.*, 1999). Species with tricolpate pollen apertures (or aperture patterns derived from this) form a single major clade, now more commonly known as the eudicots, whereas species with monosulcate or monosulcate derived apertures (monoporate to pantoporate) represent a series of basal angiosperm lineages, including Piperales and monocots. It worth mentioned that the sulcus is similar to the colpus and has more or less rounded ends and usually one in the distal position. In Gymnosperms, the pollens are sulcate, i.e., has a single aperture placed distally. APG systems gathered all the taxa with tricolpate aperture in the largest clade of angiosperms, the Eudicots. Distal monosulcate, the most widespread aperture patterns recorded in monocots and basal angiosperms.

From the most interesting studies in using the pollen aperture in taxonomical grouping is that

of Kosenko (1999) who used the pollen aperture characters of 34 species belonging to 7 genera from family Liliaceae. The results obtained revealed that the genera *Tulipa* and *Lilium* are heterogeneous in both aperture type and exine ornamentation. Pollen of *Tulipa* is monosulcate, 3-aperturate or inaperturate, with a microreticulate-striate, reticulate-implecto-striate, scabrate, perforate-rugulate, perforate-striate exine surface. Pollen of *Lilium* is monosulcate and 3- porate with a macroreticulate exine. The other genera are homogeneous in possessing of single longitudinal aperture (type monosulcate). He concluded that the pattern of exine ornamentation and the structure of the aperture and its membrane are peculiar features for the studied species and genera. Pollen of *Erythronium* and *Tulipa* are occasionally operculate, while in other representatives of the Liliaceae an operculum is lacking. Pollen morphological data support the division of the family into 3 tribes, namely Lloydieae, Lilieae, and Tulipeae.

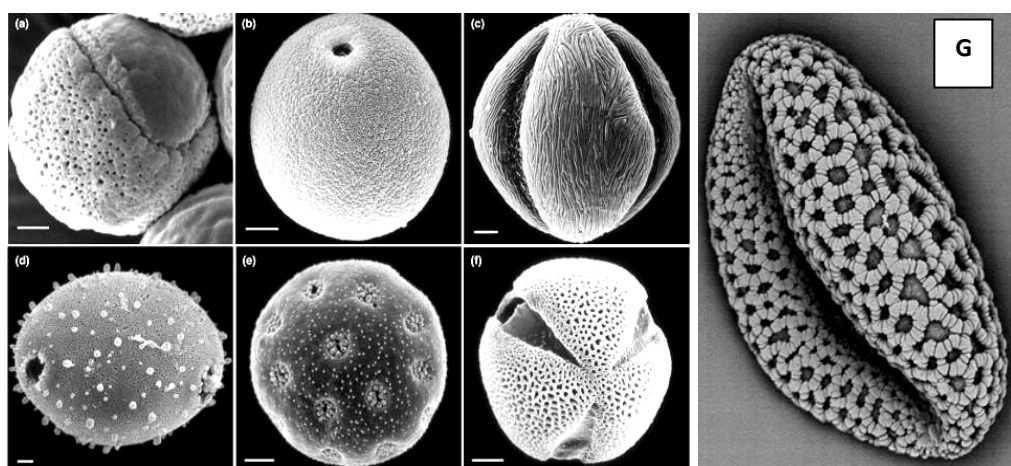


Fig. 8. SEM micrographs of pollen apertures. (a) *Fortuatiabiflora* (hyacinth family), monosulcate pollen. (b) *Poa pratensis* (smooth meadow-grass), with the sulcus reduced to a pore or ulcus. (c) *Acer obtusatum* (maple), tricolpate with elongated and slit-like colpi (equatorial view, two colpi visible). (d) *Adansonia digitata* (baobab), triporate (equatorial view, two pores visible). (e) *Chionothrix latifolia* (amaranth family), with multiple pores. (f) *Verbascum nigrum* (black mullein), tricolporate (polar view), G Sulcate pollen in *Lilium*. Scale bars 5 μm . Photograph (a) courtesy of Paula J. Rudall, photographs (b-f) courtesy of Madeline M. Harley.

When we talk about apertures, we have to consider their positions in cytokinasis and in tetrads. The position of the apertures meets in three or four points in the pollen tetrads called Garside's rule position, while those meeting in pairs at six points in the pollen tetrads called Fischer's rule position (Fig.9). Furness & Rudall (2004) considered these two positions in constructing a phylogenetic prospective within the Angiosperms. They reported two different arrangements of tricolpate (or tricolpate derived) pollen with different

orientations of the colpi in the pollen tetrads; Garside's rule and Fischer's rule; which separates the clade Illiciales from the Eudicots indicating an independent origin for this character. Similarly, pore orientation in the tetrads also follows Garside's rule in some Proteaceae and Oleaceae (Maguire *et al.*, 1974, Blackmore & Barness, 1995). They suggested three to four possible phylogenetic origins for the triaperturate pollen grains in Angiosperms.

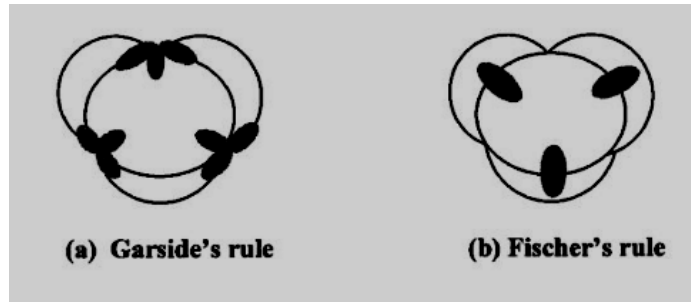


Fig. 9. Tetrahedral tetrad configurations known among angiosperm pollen showing: (a) Garside-type arrangement with apertures meeting in threes at four points on the tetrad. (b) Fischer-type arrangement with apertures meeting in pairs at six points on the tetrad

The apertures are either in the form of circular pores, wide slit (sulcus) or narrow longitudinal slit (colpi). They are simple or compound (colporate or colporoidate), the pores are either elongated along the meridian axis (lalongate) or along the equatorial axis (lalongate). Short colpi described as brevicolpate (*Tordylium*), in some taxa the colpi are very short slit and described as brevissimiaperturate (*Ambrosia*). The edges of the colpi are sometimes surrounded by an ectexine

frame called margo. The margo is a rim in the ectexine with smooth ornamentation and differ than the rest of exine sculpture. The pores when surrounded by a circular ring will be described as annulate. Near the apertures the endexine often enlarges and forms a costa; the pores can also be covered by a kind of operculum or a bridge (Fig.10). Variations in aperture type, position, shape and number are useful characters in solving taxonomic problems.

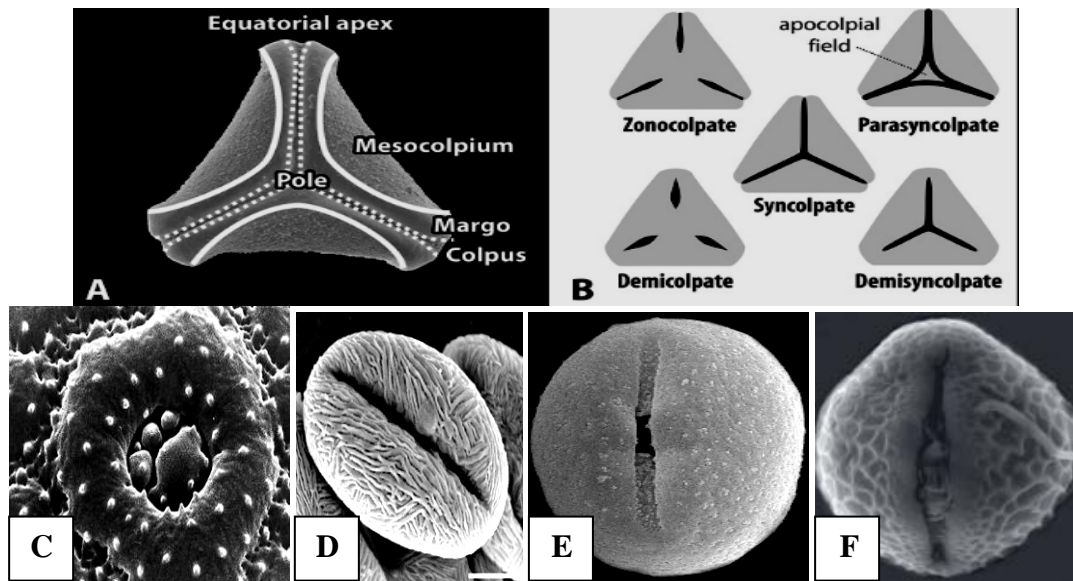


Fig. 10. A. Zonation of a prototypical Loranthaceae pollen grain. B. General aperture types (after Grimson et al., 2018). C-Pore with clear annulus and operculum (lid), *Plantago lanceolata* by Lucia Wick, IPS, D- Colpus without margo in *Centaurium pulchellum*, E- Colporate aperture with faint margo and lalongate pores of *Carica papaya*, F- Colpus with distinct margo in *Galactia martii*

Major error in aperture description (Harmomegathy)

In preparing the pollen grains for light or scanning microscopes, the specimens faced shrinkage in their sizes due to the exposure to chemical and desiccation. Pollen grains require some sort of protective mechanism against external environment in the period before they land on the stigma of a flower. The near-universal protective mechanism

against desiccation in pollen during presentation and dispersal is harmomegathy. This term first used by Wodehouse (1935) a characteristic enfolding of the grain in response to a decreasing cellular volume upon dehydration (Fig.11). From the most interesting works concerning the desiccation effects on the pollen apertures is that of Božič, & Šiber (2020). They found that the exine has soft parts that

enable the pollen grain to fold or expand. These parts affect the aperture shape, type and number and sometimes close the apertures completely. In studying pollen morphology, pollen samples of the same taxa must be examined at different stages and periods to avoid misidentification and fatal

mistakes in their description. The pollen grains changed their color and shape in response to environmental effects. Harmomegathy is one of the serious effects on the pollen size, shape, apertures and even exine ornamentation

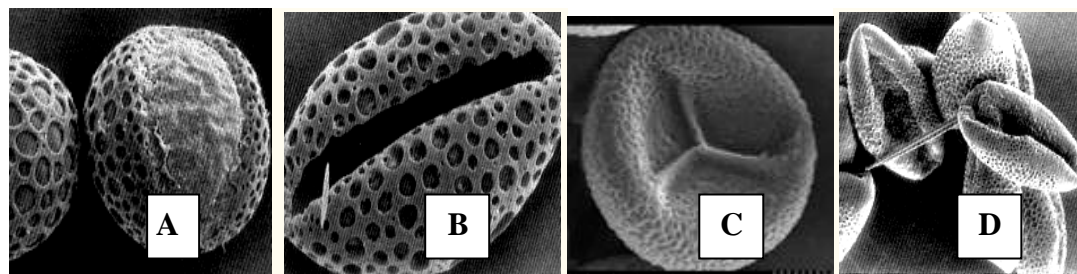


Fig. 11. Sulcus aperture in *Gulroniu cundicum*, A hydrated state, B dehydrated state, C & D Pollen grains in hydrated state i.e., with harmomegathic effect (PalDat, 2017)

Pollen wall structure

The outermost layer of the pollen wall, the exine, is the most important part in the pollen descriptions which carries the most variable characters in use in plant identification and classification. Fig. 12 shows the pollen layers names of the exine according to both Erdman (1969) and Faegri (1956). In spite of that the other pollen wall layers even the cytoplasm have role in the taxa discrimination and must be examined by either high resolution light microscope or TEM in fresh pollen grains without any chemical treatments. The cytoplasm is the cell mass inside the sporoderm is a useful parameter for determining the fresh pollens. Cytoplasm can be smooth and uniform as in *Astragalus* species, or more or less granulous or striate as in *Cistus* species. In *Cornus* species the cytoplasm is vacuolate, while in *Cupressus* it is lacunar with separated portions from the intine (periplasmic

space, Fig.12, 5) because of an abnormal intina thickening. In some species of *Rumex* the cytoplasmic mass varies from pollen to another (Maurizio & Louveaux, 1960).

The intine is the innermost layer encompasses the cytoplasm and made of pectin and cellulose. It is flexible and emerges with the pollen tube in case of fertilization. This layer can be thick in most monocot taxa or thin in some leguminous species (*Lotus*). The intine enlarges under the pores making a triangular area (Fig.12, 4). This character aid in taxonomic discrimination of many related taxa, from which is Feng-Xia and Kirchoff (2008) recorded three layers within the studied taxa of the Magnoliaceae which can be used in the trace of phylogeny within the family. The thickness, structure and surface ornamentation proved to be useful in species discrimination and phylogeny.

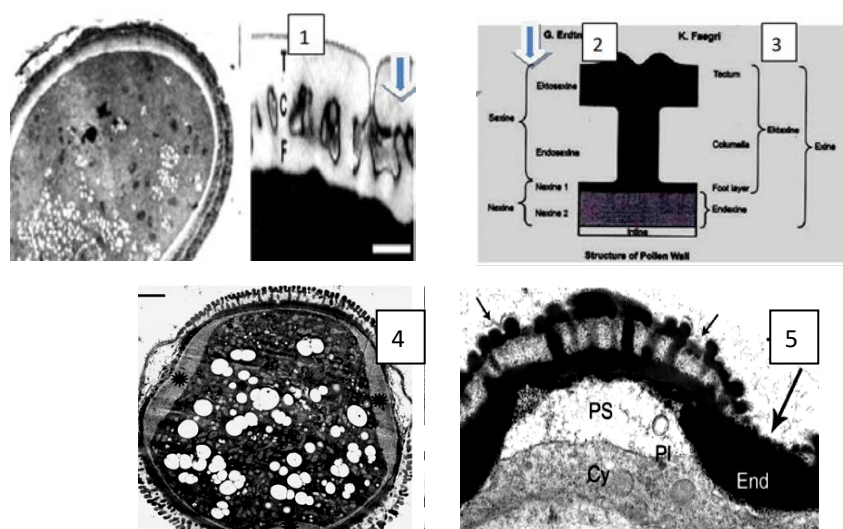


Fig. 12. 1- The pollen wall names after Erdtman and Faegri 2, 3, 4, 5 TEM in the pollen grains, arrows indicate to Exine and Intine, Cy=Cytoplasm, Ps=Periplasmic space, Pl=Periplasmic layer, End=Endexine (El-Ghazaly *et al.*, 2001)

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The most used layer of the pollen grain in taxonomical works is the sexine (tectum, columella and foot layer). The exine thickness, ornamentation and external depositions and sculpturing solved much taxonomical discrimination. Bellonzi et al. (2020) found that apertures variations and exine ornamentation (psilate, rugulate, striate, microreticulate, reticulate) delimit species within the studied Brazilian Sapindaceae pollen grains. Lechowiz et al. (2020) made very interesting study on the pollen grains of the Polish *Rubus* species. They analyzed eleven quantitative and three qualitative pollen characters of 1740 pollen grains representing 58 blackberry species under different subgenera and sections. Their results indicated that the exine ornamentation type with the width, direction of grooves beside striae number and diameter of perforations can be used as peculiar characters in the taxonomy of the genus. They concluded that the variations in the pollen grains don't coordinate with the division of the genus into subgenera and sections and should be treated as auxiliary in taxonomy of the genus.

Exine ornamentation and its role in plant discrimination

The details and ornamentations of the exine surface makes up this layer of great use in plant taxonomy. The different layers of the exine are best visualised with the help of high-magnification/high-resolution microscopy (e.g., electron or confocal), but they can be recognized with light microscopes. The palynological database PalDat (www.paldat.org), provides free access to high-quality SEM and TEM pollen images from a large number of plant taxa, serving as an excellent resource for anyone interested in morphology of pollen walls (PalDat, 2017). Sculpture of the sexine considered from the most interesting things in

studying pollen and spore morphology. Taia (1994, 1996 & 2004) used the pollen wall sculpture in clarifying the relation between members of Caryophyllaceae, Amaranthaceae and tribe trifolieae. These works beside many others used the pollen apertures and exine sculpture in the delineation of the species under the same genus (Taia and Shiha, 1999 & 2001; Taia et al. 2020 & 2021) (Fig. 13). When studying the ornamentation of the pollen grains, it is important to recognize the tectate pollens from the intectate ones, Tectate pollen grains have complete roof which may be smooth (psilate) or with suprategal elements. These elements take several shapes according to their shape, apices and base, as they differ from echinae, clavae, spines, granules or make striations or compacted lines. In most taxa the tectum is incomplete or with small holes or punctae. The holes called luminae which differ in shape, width and symmetry. The walls of the holes are called muri are different, as well, between taxa. The number of luminae/unit area must be counted and the width of the muri must be measured. According to the counts and similarity of luminae and measurements of the muri the exine will be tectate perforate, foveolate, microreticulate or reticulate (Fig.14 & 15). The muri, in some taxa, elevated making ridges as in *Selaginella* and *Lilium* sp. (Fig. 14) or carrying granules or echinulate microstructures (Valdespino, 2017). From the use of pollen exine characters in taxonomical studies is that of Song et al. (2017) in studying 38 taxa belonging to nine genera from tribe Spiraeae (Rosaceae). They recognized four sexine ornamentation types based on the length and direction of the ridges of the sexine, as well as branching of the collumellae, endexine structure and presence of orbicules. They concluded these characters can help in the distinguishing the phylogenetic directions within the studied taxa.

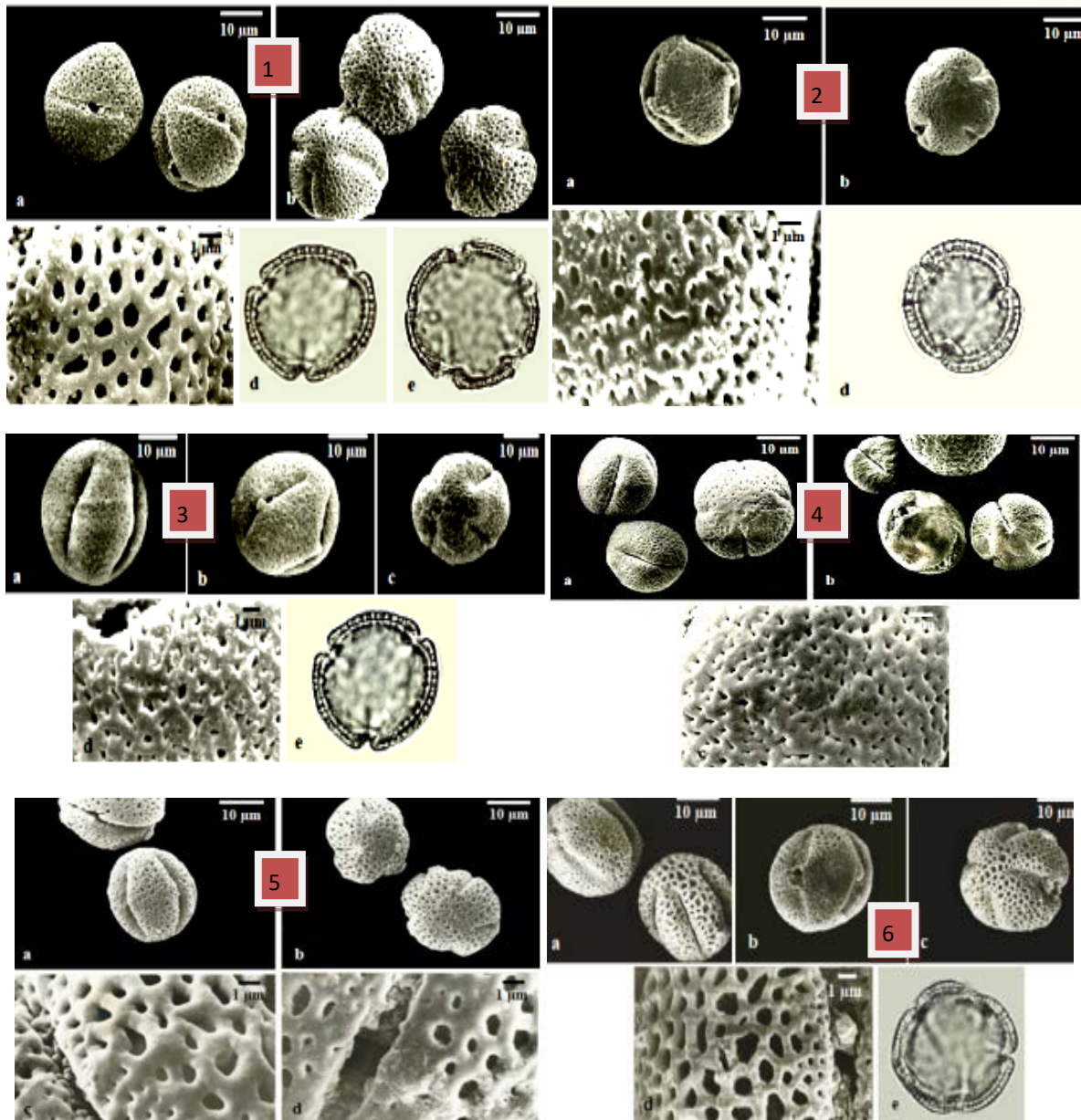


Fig. 13. Different plates showing the variations in pollen wall sculpture and apertures within *Citrus* species. 1-*Fortunella margarita*, 2-*C. reshni*, 3-*C. grandis*, 4-*C. sinensis*, 5-*C. aurantium*, 6-*C. latifolia* (Taia et al., 2020).

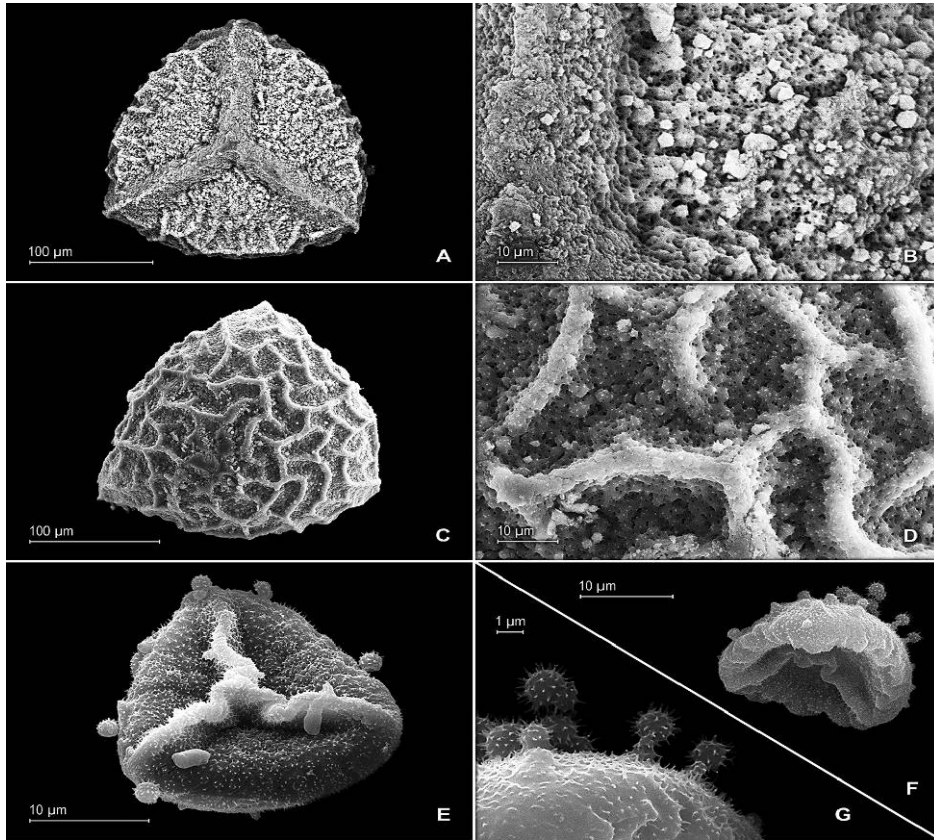


Fig. 14. *Selaginella itheae*, A, B Megaspore proximal face, C, D Megaspore distal face, E, G, F Microspore distal-equatorial-proximal faces, G, F capitates projections and echinulate microstructures (Valdespino, 2017).

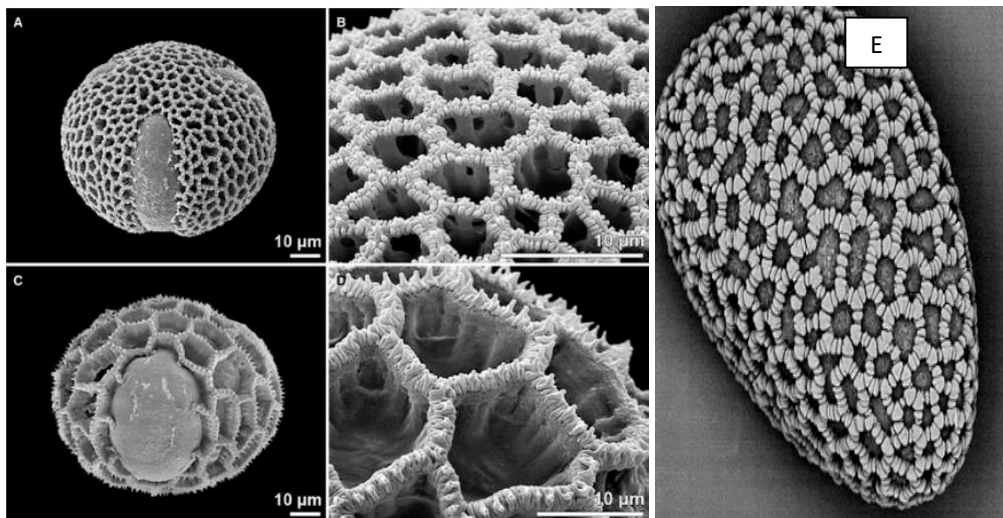


Fig. 15. Pollen dimorphism-different ornamentation. A-D. *Armeria alpina*, Plumbaginaceae. A-B. Morph 1, reticulate. C-D. Morph 2, reticulate (Halbritter *et al.*, 2018), E- reticulate exine in *Lilium* sp.

Evolution

The study of the pollen evolution has been considered from the most peculiar topics in this field. Information given from pollen and spore

fossil records used to unravel the evolutionary history of plant taxa, in both time and space. It is employed also as a benchmark

in phylogenetic studies for estimating differentiation times of different levels of taxa. Pollen grains and fungal spores considered from the ideal sources to understand the evolution of plants on the Earth. Pollen morphology allowed biologists to trace the phylogeny of the major botanical groups as well as to understand the fossil record. The study of pollen morphology has historically allowed evolutionary biologists to assess phylogenetic relationships among Angiosperms. During this process, pollen has mainly been studied by discrediting some of its main characteristics such as size, shape, aperture type and number as well as exine thickness, formation and ornamentation. The evolution and systematic utility of pollen shape and size within seed plants have been studied by quantifying pollen grains with traditional morphometric methods that take into account linear measurements and/or meristic variables (Furness & Banks, 2010; Ahmed- Khanbeygi, 2011 and Furness, 2012). Kuprianova (1967) postulated three basic evolutionary levels in the structure of pollen apertures: (1) Proximal apertures (Bryophyta, Pteridophyta, rarely Gymnospermae, Monocotyledonae, Dicotyledonae). This level corresponds to the Palaeozoic. (2) Distal apertures (Gymnospermae, Monocotyledonae and rarely Dicotyledonae). They are typical of the Mesozoic. (3) Equatorial apertures (Dicotyledonae). These are characteristic of the Palaeozoic. Nair (1979) used the palynological characters in constructing the triphyletic theory of the angiosperm origin. He considered that both the pollen grains and spores have important morphological characters which can be used to trace the angiosperm origin, evolution and diversification. He pointed to the importance of considering the exine structure and ornamentation as well as the apertures type, number and positions as indicators to the angiosperm ancestors. He draws a line of the pollen evolution from the primorphous present in thallophyta (indistinct aperture) to the trimorphous (trilete, monolete or alete) present in the archegoniatae and ending with the polymorphous pollen grains (colpate, colpate, and porate with different numbers and forms) present in the angiosperms. He considered the trimorphous type as ancestor to the angiosperms. He postulated his theory by considering the angiosperms are triphyletic, with three independent roots, the monocotyledons, magnolia root and the ranalean root and each one evolved and diversified separately according to the environmental conditions. This theory coordinate with the principles of Bessey dicta (1915) who considered that the evolution is not always upward, homogenous structures are less advanced, and evolution does not necessarily involve all the plant organ. Bessey draw his dicta as the present angiosperms took different phylogenetic ways and

not all of them result from the same evolutionary line.

Dajoz *et al.* (1991) study considered from the most interesting ones in tracing the evolution of pollen apertures over time; they concluded that the morphology of angiosperm pollen has evolved toward an increasing number of apertures, among other things. From a neo-Darwinian point of view, this means that (i) some polymorphism for aperture number must exist and (ii) there must be some fitness increase associated with increasing the aperture number. They indicated to the presence of different pollen types with different aperture numbers occur in the same species. Doyle (2005) had another conclusion about the evolution of plants according to their pollen characters, which turn out our thinking. He found from the phylogenetic analyses of the molecular and pollen morphological data that the ANITA group (Amborella, Nymphaeales, Illiciales, Trimenia, and Austrobaileya); which considered the base of Angiosperm; has globose monosulcate pollen with more or less columellar structure and a continuous tectum was ancestral and a foveolate-reticulate tectum arose soon after. This opinion is in contrary to the view that the first angiosperms had boat-shaped monosulcate pollen with granular or atectate exine structure. The oldest recognized Cretaceous angiosperm pollen may represent the latter grade of evolution. He thought that structure described as granular evolved independently from columellar within Nymphaeales, Magnoliales, and Laurales. In Magnoliales, columellar Myristicaceae and Magnoliaceae diverge below Degeneria, Galbulimima, Eupomatia, and Annonaceae, which shifted to granular structure. Granular monosulcate pollen was the ancestral in Annonaceae but gave rise to columellar monosulcates and permanent tetrads. In Laurales, reduction and granularization culminated in the fragile exines of Lauraceae. Although absence of a distinctly staining endexine in Magnoliales has been considered evidence that the laminated endexine of gymnosperms was lost before the origin of angiosperms, presence of a thin endexine now appears to be ancestral pollen grains. These results refute the view that granular structure supports a relationship between angiosperms and Gnetales, Bennettitales, and Pentoxylon. Relationships with groups with alveolar exines (e.g., Caytonia, glossopterids) and/or reticulate-columellar Triassic Crinopolles pollen now seem equally likely. Wallace *et al.* (2011) made a review article about the development of the pollen and spore wall to identify the genes involved in the basal lower angiospermous plants. Albert *et al.* (2022) found that the two evolutionary lines; monoaperturate and triaperturate; pollen grains are maintained by selection during the evolution process basing on mutant studies and comparative analyses of early pollen development.

Pollen grain diversity and application in taxonomy and evolution

From the worth mention work in using pollen characters in tracing the evolution within the Myrtales, is that done by Kriebel *et al.* (2017). They used pollen size and shape in tracing the phylogenetic relationship of order Myrtales families; the small families Alzateaceae, Crypteroniaceae, and Penaeaceae (collectively the “CAP clade”), as well as the large families Combretaceae, Lythraceae, Melastomataceae,

Myrtaceae, Onagraceae and Vochysiaceae. They used morphometric and morphospace methods to evaluate pollen change in the order using time-calibrated, supermatrix phylogeny. They test for conservatism, divergence, and morphological convergence of pollen and for correlation between the latitudinal gradient and pollen size and shape (Fig.16).

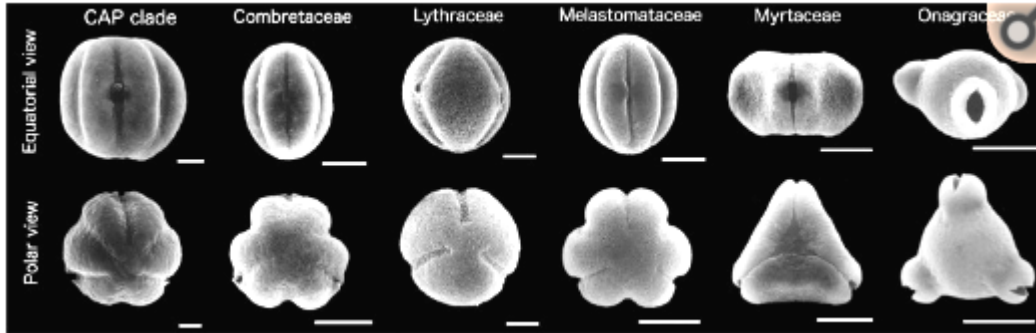


Fig. 16. Examples of pollen grains in Myrtales (Kriebel *et al.* ,2017). Scanning electron micrographs of pollen grains from selected species of Myrtales. Representing the CAP clade is *Saltera sarcocolla*; *Bucida macrostachya* in equatorial view and *Conocarpus erecta* in polar view (Combretaceae); *Heimia salicifolia* (Lythraceae); *Miconia alypifolia* in equatorial view and *Miconia caesia* in polar view for (Melastomaceae); *Tristania conferta* (Myrtaceae); *Calylophus toumeyii* (Onagraceae). Scale bars are 5 μ m except for Onagraceae which is 50 μ m.

The study done by Zhang *et al.* (2017) on 128 germplasms of *malus* species (Crapapple) pollen grains (44 natural species and 84 varieties) based on observations of pollen exine ornamentation characteristics. They extracted three qualitative variables with binary properties (Xi: regularity of pollen exine ornamentation; Yi: scope of ornamentation arrangement regularity; Zi: ornamentation arrangement patterns). Then the matrix data (Xi,Yi,Zi) were converted to decimal data through weight assignment. Their result

indicates that, the exine ornamentation of all three dimensions present the evolutionary trend of regular→irregular, wholly regular→partially regular, and single pattern→multiple patterns (Fig. 17). This study reveals that the exine ornamentation can be of use in tracing the phylogenetic degree within the studied taxa. They found that the evolutionary degree increased significantly along Xi → Yi → Zi. This study gave new considerations in tracing the phylogenetic state of the taxa using pollen grain characters.

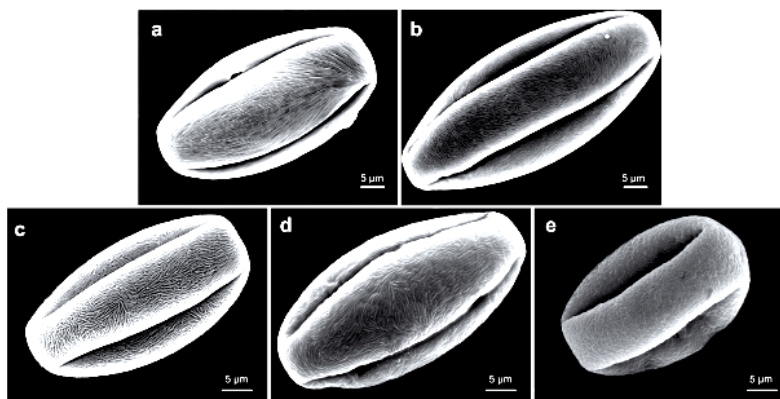


Fig. 17. Representative scanning electron microscopic images of five types of flowering *Malus* pollen exine ornamentation. (a) Wholly Regular Single-pattern Type (WRS), images of *Malus robusta* ($\times 2500$); (b) Wholly Regular Multi-Pattern Type (WRM), images of *M. halliana* ‘Pink Double’ ($\times 2500$); (c) Partially Regular Single-pattern Type (PRS), images of *M. ‘Red Baron’* ($\times 3000$); (d) Partially Regular Multi-Pattern Type (PRM), images of *M. ‘Everest’* ($\times 3000$); (e) Irregular Type (IR), images of *M. ‘Velvet Pillar’* ($\times 3000$) (Zhang *et al.*, 2017).

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