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-suprategillar, 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). Zavialova 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](image)

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 *Scabiosa* to *Lomelosia* Raf. They recorded two types of pollen apertures, either triturate 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...
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. Geral 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).

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. 4. Pollinarium in Orchidaceae**

**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).
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.

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 dyads, 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 Pteridophytes 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.

When we talk about apertures, we have to consider their positions in citokinaisis 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.
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 (lolongate) or along the equatorial axis (llalongate). 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. 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

**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 lolongate 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 Corus 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 Caryophyllacea, Amaranthacea and tribe trifoliaceae. 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 supratactal 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 retuculate (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. grandi, 4-C. sinensis, 5-C. aurantium, 6-C. latifolia (Taia et al., 2020).
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 phytogeny 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, colporate, 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 evolutionary lines. This opinion is in contrary to the view that the first angiosperms had boat-shaped monosulcate pollen with granular or alicate 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 dervive below Degeneria, Galbulimima, Eupomatia, and Annonaceae, which shifted to granular structure. Granular monosulcate pollen was the ancestral in Annonaceae but gave rise to columellar monosulciates 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.
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 touneyi (Onagraceae). Scale bars are 5 um except for Onagraceae which is 50 um.](image)

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 (*×2500*); (b) Wholly Regular Multi-Pattern Type (WRM), images of *M. halliana* ‘Pink Double’ (*×2500*); (c) Partially Regular Single-pattern Type (PRS), images of M. ‘Red Baron’ (*×3000*); (d) Partially Regular Multi-Pattern Type (PRM), images of M. ‘Everest’ (*×3000*); (e) Irregular Type (IR), images of M. ‘Velvet Pillar’ (*×3000*) (Zhang et al., 2017).](image)
References

Ahmad-Khanbeygi, Z., Sheidai, M., Attar, F. (2011). Morphometry and palynological study of the genus Cousinia sect. Cousinia (Asteraceae) in Iran. Iranian Journal of Botany. 17(2): 158–166.

Albert, B., Matamoro-Vidal, A., Prieu, C., Nadot, S., Till-Bottraud, I., Ressayre, A., Gouyon, P.H. (2022). A Review of the Developmental Processes and Selective Pressures Shaping Aperture Pattern in Angiosperms. Plants 11(357). https://doi.org/10.3390

APG IV - Angiosperm Phylogeny Group (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV., Botanical Journal of the Linnean Society 181: 1-20.

Bellonzi, T.K., Vitorete Dutra, F., de Souza, C.N., Rezende, A.A., Gasparino, E.C. (2020). Pollen types of Sapindaceae from Brazilian forest fragments: apertural variation. Acta Botanica Brasilia 34(2): 327-341.

Bessey, C.E. (1915). ‘The Phylogenetic Taxonomy of Flowering Plants’ American, Annals of the Missouri Botanical Garden. 2(1/2): 109-164.

Blackmore, S. and Barnes, S.H. (1995). Garside’s rule and the micropore tetrads of Grevillea rosmarinifolia A. Cunningham and Dryandra polycyephalata Bentham (Proteaceae). Rev. Palaeaeobot Palynol. 85: 111–121.

Blackmore, S. and Crane, P. (1998). The evolution of apertures in the spores and pollen grains of embryophytes. In Owens, S.J. and Rudall, P.J. (eds), Reproductive Biology pp.: 159–182, Royal Botanic Gardens, Kew, Surrey, UK.

Božič, A. and Šiber, A. (2020). Mechanical design of apertures and the infolding of pollen grain. Proc. Natl. Acad. Sci. 117(43):26600–26607. doi: 10.1073/pnas.2011084117

Brown, R. (1810). "On the Proteaceae of Jussieu" Transactions of the Linnean Society of London. 10 (1): 15–226. doi:10.1111/j.1096-3642.1810.tb00013.x.

De Candolle, A.P. and Sprengel, K. (1821). Elements of the philosophy of plants. Edinburgh. Printed for William Blackwood, Edinburgh, and Cadell, Strand, London.532 pp

Chase, M.W. et al. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. Ann. Mo. Bot. Gard. 80: 528–580

Cruz, P. de F., Mendonca, C.B.F.and Esteves, V.G. (2017). Pollen morphology of selected species of Mimosoideae (Leguminosae) occurring in the Atlantic Forest of the state of Rio de Janeiro, Brazil. Braz. J. Bot.41: 197-208

Dajoz, I., Till-Bottraud, I. and Gouyon, P.H. (1991). Evolution of pollen morphology. Science 253 (5051): 66-68.

Da Luz C.F.P., Mak E.S., Terra I.H., Torrado, P.V. and Filho, C.V.M. (2013). Pollen grain morphology of Fabaceae in the Special Protection Area (SPA) Pau-de-Fruta, Diamantina, Minas Gerais, Brazil. Anais da Academia Brasileira de Ciências 85(4):1329-1344

Doyle, J.A. (2005). Early evolution of angiosperm pollen as inferred from molecular and morphological phyletological analyses. Grana 44: 227-251.

El-Ghazaly, G., Huysmans, S., Smets, E.F. (2001). Pollen development of Rondeletia odorata (Rubiaceae). Am. J. Bot. 88 (1): 14–30.

Erdtman, G. (1952). Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist & Wiksell, Stockholm 539 pp.

Erdtman, G. (1960). Pollen walls and angiosperm phylogeny. Bot. Not. 113: 41–45.

Erdtman, G. (1962). Palynological aspects. Svensk Naturvetensk., 15:219-227

Erdtman G. (1969). Handbook of Palynology – An Introduction to the Study of Pollen Grains and Spores. Munksgaard, Copenhagen 486 pp.

Faegri, K. 1956. Recent trends in palynology. – Bot. Rev. 22: 639 – 664.

Feng-Xia, X. and Kirchoff, B.K. (2008). Pollen morphology and ultrastructure of selected species of Magnoliaceae. Review of Palaeobotany and Palynology 150: 140-153

Furness CA. (2012). Pollen evolution in the Clusioid clade (Malpighiales). International Journal of Plant Sciences. 173(9):1055–1082. doi:10.1086/676714

Furness C and Banks H (2010). Pollen evolution in the early divergent Monocot order Alismatales. International Journal of Plant Sciences. 171 (7): 713–739. doi: 10.1086/654848

Furness, C.A. and Rudall, P.J. (2004). Pollen aperture evolution – a crucial factor for eudicot success? Trends in Plant Science 9 (3): 154-158.

Gleditsch, J. G. (1751). Essai d’une Fécondation artificielle, fait sur l’espèce de Palmier qu’on nomme, Palma dactylifera folio flabelliformi. Histoire de l’Académie Royale des Sciences et Belles Lettres de Berlin année, 1749: 103-108.

Gleditsch, J. G. (1765). Kurze Nachricht von einer künstlichen wohlgegelungenen Befruchtung eines Palmbaumes im Königlichen Kräutergarten zu Berlin. Vermischte Physikalisch–Botanisch–Ökonomische Abh, 1: 94-104.

Grew, N. (1682) The Anatomy of plants, with an idea of a philosophical history of plants, and several other lectures, read before the Royal Society. W. Rawlins, London.561 pp

Grimson, F., Grimm, G.W., Zetter, R. (2018). Pollen Morphology and Palynology of Magnoliaceae. Grana 57 (1–2): 16–116. https://doi.org/10.1080/00173134.2016.1261939

Halbritter, H., Ulrich, S., Grimson, F., Weber, M., Zetter, R., Hesse, M., Buchner, R. Svojtek,
M. and Frosch-Radivo, A. (2018). Illustrated pollen terminology. 2nd ed. Springer. 483 pp.

Hesse, M., Vogel, S. and Halbritter, F. (2000). Thread forming structures in angiosperm anthers: their diverse role in pollination ecology. Plant Systematics and Evolution 222: 281–292.

Johnson S.D., Edwards T.J. (2000). Pollen and pollination. Plant Systematics and Evolution 222(1): 243-269. DOI: 10.1007/BF00984105

Joujeh, R., Zaid, S. and Mona, S. (2019). Pollen morphology of some selected species of the genus Centaurea L. (Asteraceae) from Syria. South African Journal of Botany 125: 196–201

Kant, R. (2019). Contribute significantly in the taxonomy and understanding character evolution in the family Orchidaceae. Taiwania 64(3): 269-279. DOI: 10.6165/tao.2019.64.269

Kölreuter, J.G. (1806). De antherarum pulvere. Nova acta Academiae Scientiarum Imperialis Petropolitanae 15: 359–398.

Kölreuter, J.G. (1811). Dissertationis de antherarum pulvere continuato. Mem Acad Sci Petersbourg 3: 159–199.

Kosenko, V. N. (1999). Contributions to the pollen morphology and taxonomy of the Liliaceae, Grana, 38 (1): 20-30, DOI: 10.1080/001731300750044672.

Kriebel R., Khabbazian, M. and Sytsma, K.J. (2017). A continuous morphological approach to study the evolution of pollen in a phylogenetic context: An example with the order Myrtales. PLoS One.12(12):e0187228.

Kuprianova, I.A. (1967). Apertures of pollen grains and their evolution in angiosperms. Review of Palaeobotany and Palynology. 3(1–4): 73-80.

Lechowicz, K., Wrońska-Pilaręc, D., Bocianowski, J., Maliński, T. (2020). Pollen morphology of Polish species from the genus Rubus L. (Rosaceae) and its systematic importance. PLoS ONE 15(5): e0221607. https://doi.org/10.1371/journal.pone.0221607

Lindley, J. (1836). A natural system of botany; or, A systematic view of the organization, natural affinities, and geographical distribution of the whole vegetable kingdom: together with the uses of the most important species in medicine, the arts, and rural or domestic economy (2nd edition). Longman, London.526 pp.

Linnaeus, C. (1750). Sponsalia plantarum. Translated by G.Wahlbom. Stockholm. 72 pp.

Maguire, B., Wurdack, J.J. and Huang, Y. (1974). Pollen Grains of Some American Olacaceae, Grana, 14(1): 26-38, DOI: 10.1080/00173137409434771

Malpighi, M. (1901) Die Anatomie der Pflanzen. I und II Theil, London 1675 und 1679. Bearbeitet von M. Möbius. Ostwald’s Klassiker der exakten Wissenschaften Nr. 120, pp. 163.

Manten, A. A. (1966a). Microfossil-like objects in meteorites. Earth-Sci. Rev., 1(4): 337-341.

Manten, A. A. (1966b). Some current trends in palynology. Earth-Sci. Rev., 2(4): 317-343.

Manten, A. A. (1967a). Lennart von Post and the foundation of modern palynology. Rev.Palaeobotan. Palynol., 1(1-4): 11-22.

Manten, A. A. (1967b). The Second International Conference on Palynology, Utrecht (The Netherlands), August 29 - September 3, 1966. Rev. Palaeobotan. Palynol., 1(1-4):37-59.

Mostafa, E.-N., Sedighi, N.-S., Rosa, E. (2017). Pollen characters as taxonomic evidence in some species of Dipsacaceae from Iran. Bangladesh J. Plant Taxon. 24(2): 129–136.

Maurizio, A. and Louveaux, J. (1960). Pollens de plantes mellifères d'Europe I. Pollen Spores, 2 (2) : 159-182

Nair, P.K. (1979). The palynological basis for the triphyletic theory of angiosperms. Grana, 18: 141-144.

Pacini, E. (1997). Tapetum character states: analytical keys for tapetum types and activity. Canadian Journal of Botany 75: 1448–1459.

Pacini, E. (2000). From anther and pollen ripening to pollen presentation. Plant Systematics and Evolution 222: 19–43.

Pacini, E., Franchi, G.G. (1996). Some cytological, ecological and evolutionary aspects of pollination. Acta Societatis Botanicorum Poloniae 65: 11–16.

Pacini, E., Franchi, G.G. (1998). Pollen dispersal unit, gynoeicum and pollination. In: Owens, S.J. and Rudall, P.I. (eds). Reproductive biology. Kew, Royal Botanic Gardens,pp: 183–195.

Pacini, E., Franchi, G.G. (1999a). Pollen grain sporodom and types of dispersal units. Acta Societatis Botanicorum Poloniae 68: 362–366.

Pacini, E. , Franchi, G.G. (1999b). Types of pollen dispersal units and pollen competition. In: Clément, C., Pacini, E.and Audran J.C., (eds.). Anther and pollen. Springer, Berlin, Heidelberg.pp: 1-11

Pacini, E., Franchi, G.G. (2000). Types of pollen dispersal units in Monocots. In: Wilson, K.L and Morrison, D.A., (eds.). Monocots: systematics and evolution. Melbourne: CSIRO. pp: 295–300.

Pacini, E., Hesse, M. (2005). Pollenkitt – its composition, forms and functions. Flora, 200 (5,6): 399–415.

PalDat (2017). PalDat – A Palynological Database, www.paldat.org (accessed 15 May 2018).

Perveen, A. (2000). Pollen characters and their evolutionary significance with special reference to the flora of Karachi. Journal Biology 24: 365-377.

Purkyně, J.E. (1830). De Cellulis antherarum fibrosis nec non de granorum pollinarium formis: Kessinger Publishing.106 pp

Qiu, Y.L., Lee, J., Bernasconi-Quadroni, F. et al. (1999). The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. Nature 402: 404-407 https://doi.org/10.1038/46536
Ragho, K. S. (2020). Role of pollen morphology in taxonomy and detection of adulterations in crude drugs. *J. Plant. Sci. Phytopathol.*, 4: 024-027. DOI: 10.29328/journal.jsp.1001046

Richardo, J. and Silvério, A. (2019). New trends in *Passiflora* L. pollen grains: morphological/aperture aspects and wall layer considerations. *Protosplasma*, 256 (4):923-939. doi: 10.1007/s00709-019-01350-w.

Romero, I.C., Kong, S., Fowites C.C., Jaramillo, C., Urban, M.A., Obbo-Okuenobe F, D'Apolito, C. and Punyasena, S.W. (2020). Improving the taxonomy of fossil pollen using convolutional neural networks and super resolution microscopy. *PNAS* 117(45): 28496-28505.

Soares, T.L., Jesus, O.N., Souza, E.H., Rossi, M.L. and Oliveira, E.J. (2018). Comparative pollen morphological analysis in the subgenera *Passiflora* and *Decaloba*. *An. Acad. Bras. Ciênc.* 90 (2 suppl 1):2381-2396.

Soltis, P., Soltis, D. and Chase, M. (1999). Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* 402: 402-404 https://doi.org/10.1038/46528

Song, J.H., Oak, M.K., Roh, H.S., Hong, S.P. (2017). Morphology of pollen and orbicules in the tribe *Trifolieae* (*Leguminosae*). *International Journal of Recent * 17 (2): 91-97.

Swapna, S. (2018). The morphological diversification of pollen grains of three different species belongs to (*Lamiaceae, Asclepiadaceae*, *Euphorbiaceae*). *International Journal of Recent Scientific Research.* 9(2, D): 23973-23975

Taia, W.K. (1996). Palynological studies on the Egyptian species of the Amaranthaceae. *The Conference Proceeding of Assiut Univ.* 13-22.

Taia, W.K. (1994): On the pollen morphology of some Egyptian Caryophyllaceae. *J. King Saud Univ.* 6 (2): 149-165.

Taia, W.K. (1996). Palynological studies on the Egyptian species of the Amaranthaceae. *The Conference Proceeding of Assiut Univ.* 13-22.

Taia, W.K. (2001). Palynological Study within some Egyptian species of Boraginaceae. *J. Union Arab Biol.* Cairo 9 (B), *Botany*: 385-402.

Taia, W.K. and Sheha, M.A. (1999): Palynological investigations in some Egyptian species of Boraginaceae. *J. Union Arab Biol.* Cairo 9 (B), *Botany*: 385-402.

Taia, W.K. and Sheha, M.A. (2001): Palynological study within some *Atriplex* species. *Biosci. Res. Bull.* 17 (2): 91-97.

Taia, W.K., Ibrahim, M.M. and Abdel-Satar, M. (2020). Pollen Morphological Variations among some Cultivated Citrus species and its Related Genera in Egypt. *Jordon Journal of Biological Sciences* 13(4): 499-508.

Taia, W.K., Ibrahim, M.M, Hassan, S.A. and Asker, A.M. (2021). Palynological Study of the genus *Fagonia* L. (*Zygophyllaceae*) in Libya. *Libyan Journal of Science & Technology.* 13 (1): 1-9.

Taia, W.K., Mahdy, R.A. and Bassiouni, E.M. (2022). Pollen morphological study in some Baulinia L. species and their phylogenetic indications. *International Journal of Botany Studies.* 7 (2): 515-526.

Tsymbalyuk, Z.M., Celenk, S., Masyakin S. and Nitsenko, L. (2021). Pollen morphology of some species of the genus *Cephalaria* Schrad. (*Caprifoliaceae*) and its significance for taxonomy. *Microsc Res Tech.* 84(4):682-694. doi: 10.1002/jemt.23627

Valdespino, L.A. (2017). Novel fern- and centipede-like *Selaginella* (*Selaginellaceae*) species and a new combination from South America. *PhytoKeys* 91: 13-38. https://doi.org/10.3897/phytokeys.91.21417

Wallace, S., Fleming, A., Wellman, C.H., Beerling, D.J. (2011). Evolutionary development of the plant spore and pollen wall. *AoB plants*, 2011. doi:10.1093/aobpla/plr027

Wessinger, C.A. (2021). From pollen dispersal to plant diversification: genetic consequences of pollination mode. *New Phytologist*, 229: 3125–3132. doi: 10.1590/0001-3765201720170248.

Wronska-Pilarek D., Szkudlarz P. and Bocianowski J. (2018). Systematic importance of morphological features of pollen grains of species from *Erica* (*Ericaceae*) genus. *Plos one*, 13(10), e0204557. https://doi.org/10.1371/journal.pone.0204557

Wodehouse, R.P. (1928) The phylogenetic value of pollen grain characters. *Ann. Bot.* 42: 891–934.

Wodehouse, R.P. (1935) *Pollen grains. Their structure, identification and significance in science and medicine.* McGraw–Hill, New York. 574 pp.

Zavialova, N.E., Tekleva, M.V., Polevova, S.V. and Bogdanov, A.G. (2018). Electron Microscopy for Morphology of Pollen and Spores. 10th European Palaeobotany & Palynology Conference. (Poster)- DOI: 10.13140/RG.2.2.12909.84968

Zhang, W.X., Zhao, M.M., Fan, J.J., Zhou, T., Chen, Y.X. and Cao, F.L. (2017). Study on relationship between pollen exine ornamentation pattern and germplasm evolution in flowering crabapple. *Sci Rep* 7, 39759. https://doi.org/10.1038/srep3975