

Article

Pollen Morphological Peculiarities of Selected Mimosoideae Taxa of Hainan Island and Their Taxonomic Relevance

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Abstract: Mimosoideae is one of the taxonomically complex subfamilies of Fabaceae. Several studies have reported the pollen morphology of Mimosoideae taxa and their taxonomic relevance, but no such study was found specifically for Hainan Island in southern China. Therefore, the present study was designed to investigate the selected Mimosoideae taxa and explore the new palynological traits to support and strengthen the systematics of Mimosoideae using multiple microscopic techniques. The polar axis, equatorial diameter of the pollen grains, colpus length and width were measured. The smallest pollen grain size was found in *Mimosa pudica* ($7.8 \times 7.75 \mu\text{m}$), while the largest pollen size was found in *Albizia lebback* ($87.54 \times 77.97 \mu\text{m}$). Similarly, significant variation was found in the exine and colpus surface patterns. The subfamily Mimosoideae is considered eurypalynous because of the variation in pollen traits. In addition, variation was also found in the quantitative traits. Comparatively, the pollen features were found to be helpful at the genus and species levels, as well as in the correct identification and discrimination of the taxa. Hence, this study gives a detailed account of the pollen morphologies of certain selected taxa of Mimosoideae collected from different geographical regions on Hainan Island. The pollen morphological traits were proven to have significant taxonomic potential and can be used as additional tools for the correct identification and discrimination of Mimosoideae taxa. These results will provide the basis for further systematic studies.

Keywords: Mimosoideae taxa; pollen morphology; taxonomic relevance; light microscopy; scanning electron microscopy



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1. Introduction

Fabaceae is one of the most diverse families, being found in most ecosystems around the globe. It has been divided into three subfamilies—Papilionoideae, Caesalpinioideae and Mimosoideae [1]. In terms of modern Fabaceae classifications, the Legume Phylogeny Working Group (LPWG 2017) [2] recognized six subfamilies, whereby the traditional subfamily Mimosoideae was presented as a clade nested in the Caesalpinioideae family with a new level of discrimination. Hence, our study is based on the classifications made by Lewis et al. [1]. Furthermore, the recent classifications, which only propose changes at the subfamily level and have not yet solved the taxonomic problem of the tribes in the Mimosoid clad, do not affect the interpretation of our results. Mimosoideae is a subfamily of Fabaceae, although is sometimes treated as a separate family [3]. Fabaceae contains about 18,000 species belonging to 697 genera [4]. Fabaceae is the second largest family among the angiosperms after Asteraceae [5] and contains three subfamilies, i.e., Mimosoideae, Papilionoideae and Caesalpinioideae [6]. Mimosoideae comprises 3100 species and 58 genera around the world [7]. About 79 genera are distributed worldwide, including in tropical, subtropical, arid and semiarid regions, as well as in rocky soil [8]. The species has numerous filaments and stamens and a freely downwards-extending base [9]. The group was

given the rank of a family by Hutchinson [10], which is rarely used by taxonomists. The Mimosoideae family is broadly dispersed due to its high reproductive rates, making the plants accomplish in producing a lot of viable seeds [11].

The Mimosoideae taxa are used for multiple purposes. For example, in the dry season, they can be widely used as animal foods when there are no pastures, and some taxa are used by local communities for medicinal purposes, firewood and fencing stacks [12]. Before European colonization, the root and bark of *Mimosa* species are used by the indigenous peoples of Northern Brazil to prepare a hallucinogenic drink [13]. The legumes are used as crops, forages and green manures. These Leguminosae plants are also used to synthesize a large range of natural products, including dyes, flavorings and poisons [14]. The medicinal uses of the Fabaceae family in Bangladesh have been studied previously [15]. The usefulness of edible and medicinally important Fabaceae taxa was reported in Argentina [16]. This family is of great importance in urban and indigenous communities all over the world. *Acacia mangium* and *A. auriculiformis* are multipurpose species and are widely used for fuelwood, timber, tanning, ornamental horticulture and agroforestry [17,18]. Similarly, *Mimosa pudica* is used to treat various ailments, i.e., inflammation, vaginal and uterine complaints, asthma and fatigue [19,20]. *Albizia lebbek* has been used to treat diarrhea, anxiety, allergies, skin diseases and asthma [21].

Light and scanning electron microscopy plays a crucial role in the determination of the fine details of plant materials. For example, the pollen traits are important in the classification of various plants groups, and taxonomists mostly depend on these traits to define species boundaries for various taxonomic ranks [22–33]. This microscopy has a wide range of applications in other fields of diverse biological sciences [34,35]. The pollen morphological features are significant in the evolutionary and phylogenetic studies of various plants groups [36].

The investigation of Mimosoid pollen primarily focuses on the characteristics observed via light microscopy [37,38]. There have been no taxonomic micro-morphological studies of Mimosoid pollen, although the limited available literature suggests that exine patterns may have more systematic potential than those of the pollen units or apertures [39]. Most of the Mimosaceae taxa are taxonomically complex because of the close macro-morphological features [40]. Hence, palynological studies may help to solve the taxonomic problems, correctly identify species and defining species boundaries at various taxonomic levels. Palynological studies of the Mimosaceae taxa have been sporadically conducted in different geographical regions around the world [37,41]. Some peculiar pollen characteristics, as well as variations in the pollen traits, have long been known. For example, El Ghazali et al. [42] described the intraspecific variation in pollen morphology and identified various pollen types in *Mimosa* taxa. Similarly, geographical variations in pollen morphology of the Mimosaceae taxa have been studied previously in Sudan [43], with reports that exine sculpturing is almost constant. Pollen diversity and its relevance in the systematics of the Mimosaceae taxa of Pakistan have been analyzed to solve taxonomic problems [41,42,44,45]. Variations in the pollen morphology of *Leucaena* and closely related species were illustrated and described by Hughes [46]. Caccavari and Dome [9] analyzed the pollen morphologies of 77 American *Acacia* taxa and suggested that pollen features can be used as distinguishing factors in the generic limitation process. Hughes [46] analyzed variations in pollen morphology of the *Leucaena* species, which helped show relationships and define generic boundaries within Mimosoideae taxa. The pollen morphologies of 21 *Calliandra* taxa were investigated by De Assis-Ribeiro dos Santos and De Oliveira-Romão [47], who concluded that some of the polyad characters were useful in corroborating previous infrageneric classifications of the genus. In China, a study performed on the pollen morphologies of Mimosoideae taxa and varieties using a scanning electron microscope, which highlighted the similarities among the genera, mainly in terms of the exine, aperture and size [48]. The pollen traits, such as the size and shape of the polyads, exine pattern and presence of apertures, may also be of taxonomic relevance and could be explored further [49]. However, no pollen morphological studies specifically

on Hainan Island have been documented yet. Hence, to fill this gap, further palynological studies are needed.

It is hoped that this palynological study will not only add to the systematic information about certain selected Mimosaceae taxa, but will also provide the basis for further phylogenetic studies. The present study aims (1) to provide descriptions of the pollen types of selected taxa of Mimosoideae and outline their taxonomic relevance and (2) to strengthen and support the taxonomy of Mimosoideae and define species boundaries using both light microscopy and scanning electron microscopy.

2. Material and Methods

2.1. Collection and Identification

For the collection of Mimosoideae taxa, various field trips were arranged in the year 2021 to various geographical regions on Hainan Island (Table 1). The island is characterized by a tropical monsoon climate with a wet season ranging from May to October and a dry season ranging from November to April. The annual average precipitation ranges from 2350 to 2651 mm [50–52]. The species were collected and identified with the help of the available literature Flora of China efloras.org World Flora Online.

Table 1. List of Mimosoideae taxa collected from different geographical regions on Hainan Island.

| Species | Locality | Altitude | Sea Level |
|--|--|--------------------------------|-----------|
| <i>Acacia auriculiformis</i> A.Cunn. ex Benth. | Sanya | N: 18.26415 E: 109.52084 | 119 m |
| <i>Acacia confusa</i> Merr. | Wanning, Botanical Garden, Haikou | N: 18.69396 E: 110.23170 | 118 m |
| <i>Acacia mangium</i> Willd. | Sanya, Chang Jiang | N: 19.33905 E: 108.210 | 33 m |
| <i>Albizia julibrissin</i> Durazz. | Wanning | N: 18.69396 E: 110.23170 | 118 m |
| <i>Albizia lebbek</i> (L.) | Haikou | N: 20.062363 E: 110.3186713 | 3 m |
| <i>Calliandra haematocephala</i> Hassk. | Sanya | N: 18.26415 E: 109.52084 | 119 m |
| <i>Entada phaseoloides</i> (Linn.) Merr. | Wanning, Botanical Garden | N: 18.69396 E: 110.23170 | 118 m |
| <i>Leucaena leucocephala</i> (Lam.) | Haikou , Sanya | N: 20.062363 E: 110.3186713 | 3 m |
| <i>Mimosa bimucronata</i> (DC.) Kuntze | Haikou, Wanning | N: 18.69396 E: 110.23170 | 118 m |
| <i>Mimosa diplotricha</i> C. Wright | Beihualing, Haikou | N: 19.00262 E: 109.81523 | 471 m |
| <i>Mimosa pudica</i> Linn. | Haikou, Wanning, Sanya, Chang Jiang | N: 19.33905 E: 108.210 | 33 m |

2.2. Light Microscopy

For the light microscopic study, the filaments were first separated from the flowers and crushed on a glass slide by adding 2–3 drops of acetic acid. The debris was removed through a needle and then a cover slip was placed on it. The prepared slide was then observed under a light microscope equipped with a digital camera. The pollen micrograph was taken and its various taxonomic features were observed. For pollen descriptions, we mostly followed the terminology used by Punt et al. [53].

2.3. Scanning Electron Microscopy

The anthers were separated from the mature flower as performed by Ali et al. [23]. The anthers were then crushed on the glass slide by adding a few drops of acetic acid and transferred into Eppendorf tubes. With the help of a micropipette, pollen samples from the tubes were taken and placed on a metallic stub attached with double-sided sticky tape. The prepared samples were sputtered with platinum for twenty-five minutes in a Leica Mikrosystem made in Austria with a high-vacuum coater (ACE600) and observed under SEM (Thermo Scientific, Model: verios g 4 uc) Manufacturer Seimer Technology was installed in the analytical and testing center of Hainan University, Haikou, China.

2.4. Quantitative Analysis

For quantitative analysis, we measured about ten pollen grains in each sample. The polar axis and equatorial diameter of the polyads and colpus length and width were measured using Image J software.

3. Results and Discussion

The Mimosaceae taxa were analyzed using both light and scanning electron microscopic techniques. LM and SEM micrographs are illustrated in Figures 1–5. Details of pollen traits of each species are given below.

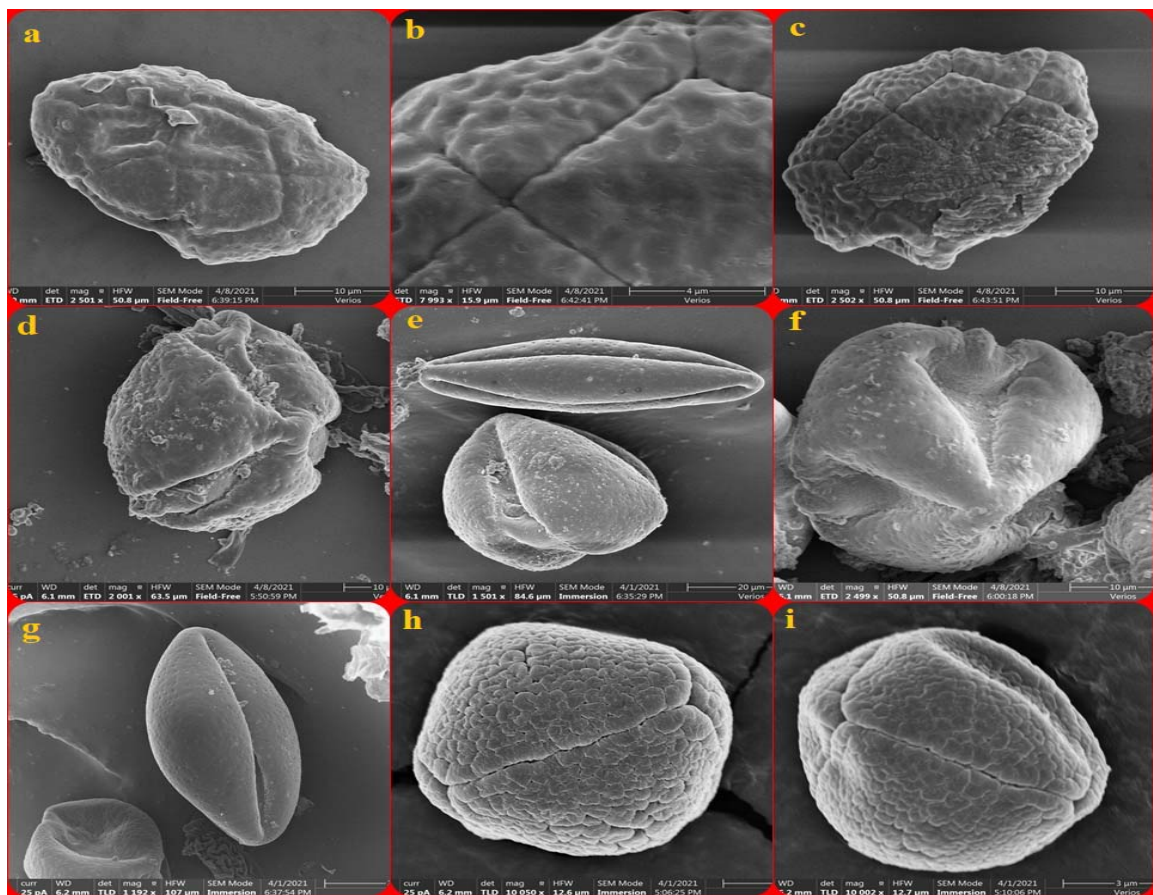


Figure 1. *Acacia confusa* (a–c), showing (a) general view of the pollen and (b) close view of the exine sculpturing. *Entada phaseoloides* (d–g), showing (d) oblique polar view, (e) equatorial view, (f) sunken colpus and (g) zonocolpus. *Mimosa pudica* Wanning (h,i), showing oblique equatorial view. Scale bars: a = 10 μ m; b = 4 μ m; c = 10 μ m; d = 10 μ m; e = 10 μ m; f,g = 10 μ m; h,i = 3 μ m.

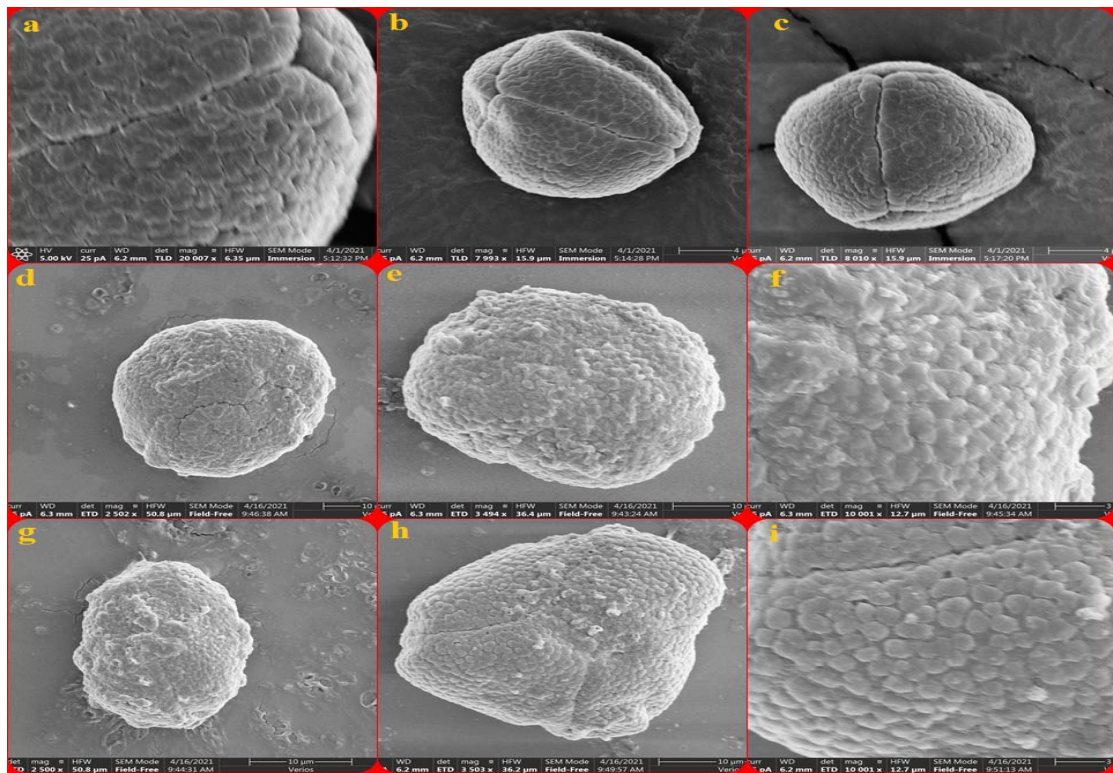


Figure 2. SEM micrographs showing the pollen grains of *Mimosa pudica* Wanning, showing (a) close view of exine sculpturing, (b) the polar area and (c) equatorial view. *Mimosa diplotricha* Beihualing (d–g), showing (d) equatorial view, (e) polar view, (f) close view of the exine and (g) prolate-shaped pollen. *Mimosa diplotricha* Haikou (h,i), showing (h) polar view and (i) close view of the exine sculpturing. Scale bars: a = 1 μm ; b,c = 4 μm ; c = 10 μm ; d,e = 10 μm ; f = 3 μm ; g,h = 10 μm ; i = 3 μm .

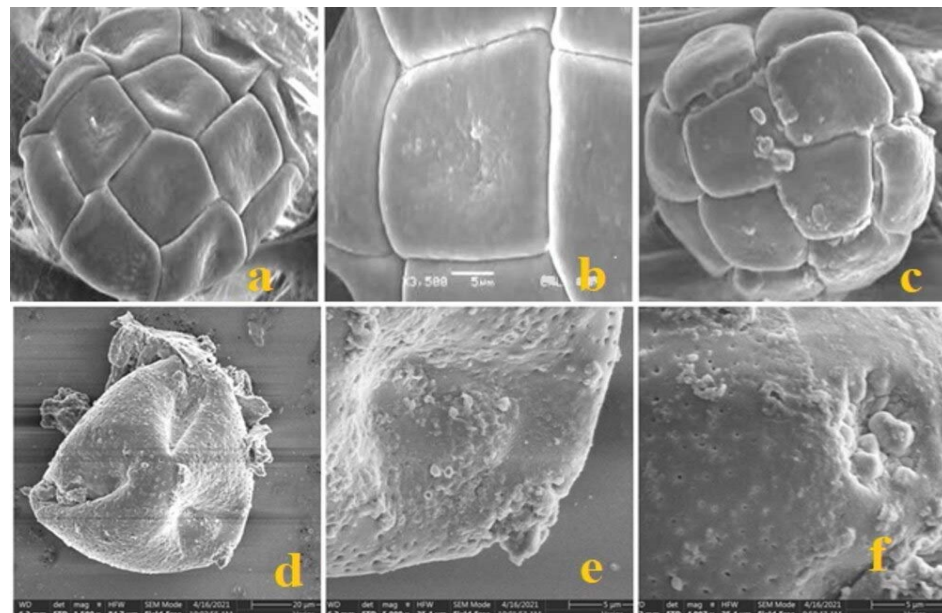


Figure 3. The SEM micrographs of *Albizia lebback* pollen grain (a–c), showing (a) quadrangulate pollen in polar view outline, (b) a close view of an individual grain of the polyad and (c) inaperturate pollen. *Leucaena leucocephala* (d–f), showing a (d) polar view, (e) close view of the colpus surface membrane and (f) the apocolpium region. Scales: (a) 10 μm ; (b) 5 μm ; (c) 10 μm ; (d) 30 μm ; (e–f) 5 μm .

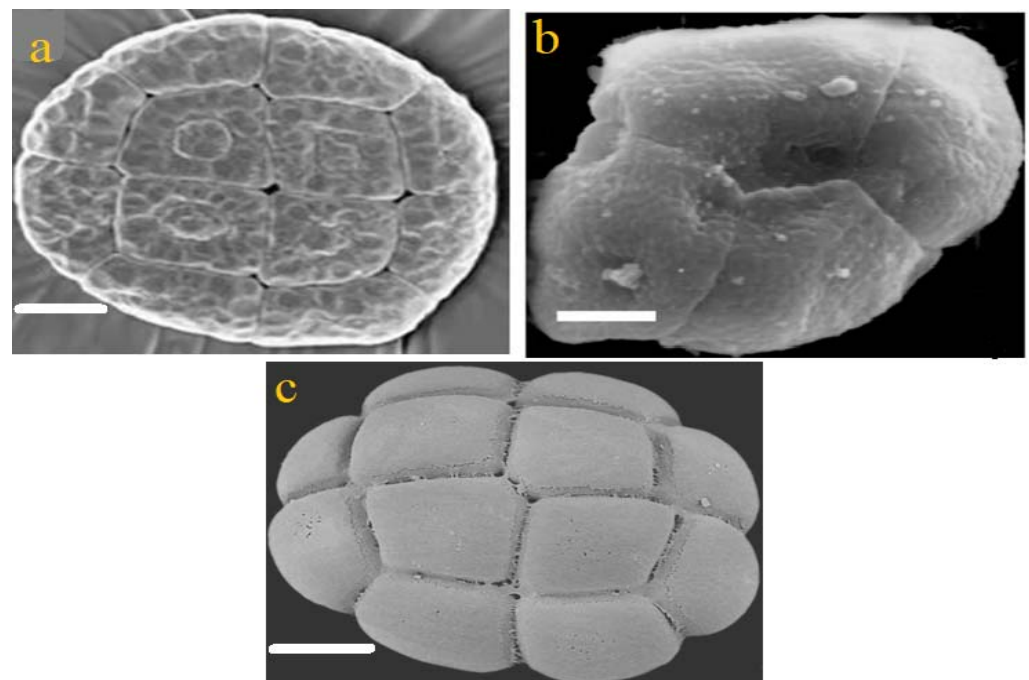


Figure 4. SEM micrographs of the pollen: *Acacia mangium* (a) showing octad polyads; *Mimosa bimucronata* (b) showing bitetrad polyads; *Albizia julibrissin* (c) showing 12-grain polyads. Scale bars: a = 10 μm ; b = 2 μm ; c = 10 μm .

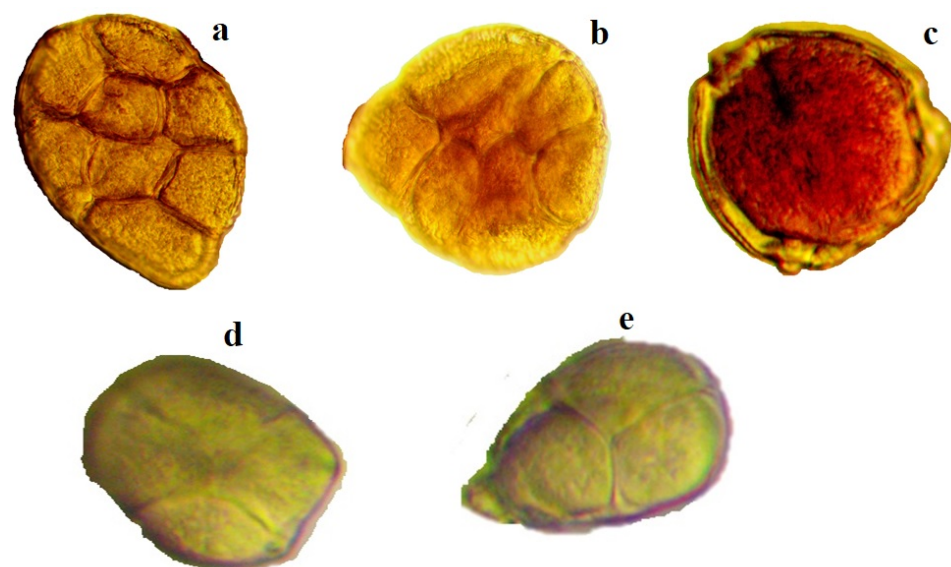


Figure 5. Light micrographs of pollen. *Calliandra haematocephala* (a,b), showing (a) equatorial view and (b) polar view. *Entada phaseoloides*, (c) showing polar view. *Mimosa diplotricha*, (d) showing tetrad-type pollen. *Mimosa pudica* (e), showing triad-type pollen. Scale bar = 50 μm .

3.1. *Acacia auriciformis*

The pollen of *A. auriciformis* was a polyad comprising 16 grains. Eight grains are arranged in the center, which is surrounded by eight periphery cells. Each grain was to be found inaperturate, while the exine sculpturing was found to be psilate. The average diameter of the polyads was noted as 32 μm . Similarly, some other species of *Acacia* have been described previously and the polyad type was also reported to be pollen (16–32-celled), with or without distinct apertures [54–56]. The general description of polyads and the interrelationships of the grains in *Acacia* have also been discussed previously [57].

3.2. *Acacia confusa*

The pollen was shed in polyads (12 grains), the outline in the polar view was rhomboidal, the symmetry was bilateral and the polarity was heteropolar. The aperture condition was noted as inaperturate. Exine sculpturing was found as mega-reticulate and rarely scabrate. The reticula were thick and flattened, having a rarely scabrate structure, while the lumina was filled with a somewhat micro-scabrate structure (Figure 1a–c). The pollen size was noted as $23.19 \times 13.88 \mu\text{m}$, while the individual grain diameter was $5.745 \mu\text{m}$.

3.3. *Acacia mangium*

The pollen of *A. mangium* was polyad of 12 grains (Figure 4a). The four grains were arranged in the center, surrounded by eight periphery grains. The exine sculpturing was found to be megareticulate and heterobrochate. An operculum was observed in the central grains of the polyads. The average diameter of the polyad was noted as $35.61 \mu\text{m}$ (Figure 1a). To the best of our knowledge, no descriptive palynological studies have been reported yet. However, Wang et al. [58] documented only the isolation and characterization of flower-specific transcripts in *Acacia mangium*.

3.4. *Albizia julibrissin*

The pollen type was polyad, comprising 12 grains. The outline in polar view was elliptical. The central 4 grains were arranged in tetragonal form. The exine sculpturing was psilate (Figure 3a–c). The pollen size was noted as $71.81 \times 81.01 \mu\text{m}$, while the individual grain diameter was $25.13 \mu\text{m}$ (Figure 4c). The pollen of *A. julibrissin* was closely related to that of *A. lebback* and *A. procera*. For example, Khan et al. [41] documented the pollen diversity and its implications in Mimosaceous taxa using light and scanning electron microscopic techniques, and reported psilate–poveolate and sparsely psilate–scabrate exine sculpturing in *A. procera* pollen. Hence, these traits were found to be useful in delimiting and defining species boundaries within the genus *Albizia*.

3.5. *Albizia lebback*

The pollen type was polyad (12-grains), while the outline in polar view was spheroidal. The central 4 grains were arranged in tetragonal form. The exine sculpturing was psilate to rugulate (Figure 3a–c). The pollen size was $87.54 \times 77.97 \mu\text{m}$, while the individual grain diameter was $24.35 \mu\text{m}$. However, in comparison with the previous results, the 16-celled polyad-type pollen grains reported by Agashe and Caulton [59] were not corroborated by our findings. Moreover, a minimum length of $28 \mu\text{m}$ and maximum length of $57.4 \mu\text{m}$ and a minimum width of $30.5 \mu\text{m}$ and maximum width of $43.05 \mu\text{m}$ were reported by Parveen and Qaiser [44] in the pollen grains of *A. lebback*. Similarly, the aperture and exine sculpturing of *Albizia* was found to be smooth or almost smooth and different from *Calliandra*, *Cylindrokelupha* and *Zygia*, due to the presence of tubercles [48].

3.6. *Calliandra haematocephala*

Pollen was shed as octad polyads (8 grains), while the outline in the polar view was a pyramid. The two central large-sized grains are in the center and the other grains lie in the periphery. As found in the previous study by Guinet [39], individual pollen grains from the same polyads showed a marked heteromorphic structure, with peripheral grains differing from the central grains in shape, size and less obviously ornamentation. The exine sculpturing was found to be verrucate, aerolate and somewhat reticulate towards the periphery. The pollen tip acute and the base was constricted. The surface of pollen was found to be undulate. According to Van Campo and Guinet [60], the pollen dispersal units of *Calliandra* are bi-tetrads and polyads. Similarly, Chen [61] mentioned that the bi-tetrad pollen of the *Calliandra* is family is rare in Angiosperms. Some other species of this genus also have polyad-type pollen [47]. The pollen grain octads, shed as polyads, were flattened and oval-shaped, while the longest axes ranged from 164 to $173 \mu\text{m}$ and the shortest axes ranged from 82 to $93 \mu\text{m}$. Similarly, the tectate and perforate exine was found

in the Philippines in *Calliandra haematocephala*. However, to clarify the differences in exine sculpturing of the appendix in detail, transmission electron microscopy will be needed.

3.7. *Entada phaseoloides*

The pollen type was monad, the outline in polar view was ovate and was elliptical in equatorial view, the symmetry was bilateral and the polarity was heteropolar. The aperture condition was noted as trizonocolpate. The orientation of the colpus was sunken and wide, having an obtuse tip, while the sculpturing was scabrate to verrucate and sometimes psilate. The exine sculpturing was perforate and rarely scabrate, with ubisch bodies found on its surface. The polar area was found to be very small. The pollen size was $34.61 \times 26.76 \mu\text{m}$. The colpus length was noted as $21.31 \mu\text{m}$ and the width was $6.72 \mu\text{m}$. The polar area was $5.45 \mu\text{m}$ and the meso-colpium diameter was 25.33 . Furthermore, Rao and Lee [62] documented the pollen flora of Singapore and Malaya and observed a tricolporate and subprolate structure, a size of $30 \times 35 \mu\text{m}$ and a faintly reticulate exine pattern of the *Entada spiralis* pollen. Similarly, the shape of *Entada scandens* pollen grains and other pollen morphological features of *Entada phaseoloides* were reported in a previous study [54].

3.8. *Leucaena leucocephala*

The pollen type was monad, the outline in polar view was triangular, the symmetry was bilateral and the polarity was isopolar. The aperture condition was noted as tricolpate. The orientation of the colpus was sunken and wide, having an obtuse tip, while the sculpturing was scabrate to rugulate. The exine sculpturing was perforate and rarely scabrate. The polar area was found to be very small. The pollen size was $21.48 \times 21.35 \mu\text{m}$. The colpus length was $9.27 \mu\text{m}$ and the width was $3.4 \mu\text{m}$. In comparison with a previous study [45], where the exine sculpturing was found to be subpsilate, this was not corroborated by our findings, while the polar axis was $42.42 \mu\text{m}$ and the equatorial diameter was $31.5 \mu\text{m}$. The colpus length was $17.01 \mu\text{m}$ and the width was $8.61 \mu\text{m}$. The mesocolpium area was $23.31 \mu\text{m}$, while the apocolpium was $39.95 \mu\text{m}$. The sexine was thicker than the nexine. Hence, such variation may be due to variation in the pollen acetolysis techniques and the different geographical regions, i.e., the tropical rain forest in Hainan Island and subtropical regions in Pakistan having different altitudes and different environmental filters.

3.9. *Mimosa bimucronata*

The pollen was small in size and shed in polyad bitetrads containing 8 grains, i.e., four pollen grains in two planes (4 + 4). The prolate was spheroidal and tetragonal, the polarity was heteropolar and the symmetry was bilateral. Aperture condition was found as tri- to tetracolporate. The exine sculpturing was found to be rugulate and rarely scabrate (Figure 5b). The pollen size was noted as $16.7 \times 12.6 \mu\text{m}$. Similarly, 14 species of the Mimosoideae family of the Atlantic Forest in Brazil were investigated, and it was reported that pollen grains of *Mimosa bimucronata* are bitetrad with exine sculpturing that is rugulate, which supports our findings. However, bitetrad-type pollen is also found in *Mimosa elliptica* and *Mimosa pellita*. Hence, further palynological studies are needed to explore new palynological traits, which will help to solve the taxonomic problem within the *Mimosa* genus.

3.10. *Mimosa diplotricha*

The first sample of this species was collected from a tropical rainforest in Beihualing. The pollen was shed as tetrad polyads, the shape was tetrahedral, the symmetry was bilateral and the polarity was heteropolar. The aperture condition was noted as inaperturate. The uniplanar tetrad with the proximal sides of two individual units is directly connected, while the other two units are separated. The pollen size was noted as $19.1 \times 22.39 \mu\text{m}$, while the individual grain diameter was $14.28 \mu\text{m}$. The exine sculpturing was predominantly areolate and verrucate, rarely being scabrate and gemmate. Ubisch bodies were found as supra-tectal elements (Figure 2d–g). The second sample was collected from Haikou, and an

additional perforate, scabrate and densely verrucate exine sculpturing was found, while the center of the polyad was observed as concave, which was found to be different from the former species. The pollen size was noted as $22.21 \times 22.11 \mu\text{m}$, while the individual grain diameter was $13.47 \mu\text{m}$ (Figure 2h,i). In conclusion, variation was found in these two populations of *Mimosa diplotricha*. This may have been due to the variations in altitude and environmental factors such as temperature and humidity. The previous study reported that the number of grains per polyad and their shape are useful taxonomic traits in the characterization of *Mimosa* taxa [63]. Hence, our study confirmed that the number of grains per polyad and their shape are valuable taxonomic characteristics for the characterization of Mimosaceae taxa. Additionally, the structure of the polyads may protect the pollen grains from dehydration in dry habitats.

3.11. *Mimosa pudica*

The first sample was collected from Wanning. The pollen was shed as tetrad polyads, the shape was tetrahedral, the symmetry was bilateral and the polarity was heteropolar. The aperture condition was noted as inaperturate. The uniplanar tetrad with the proximal sides of two individual units is directly connected, while the other two units are separated. The exine sculpturing was predominantly areolate and psilate, with various sized verrucae having an isodiametric shape (Figure 1h,i, Figure 2a–c). The pollen size was noted as $7.8 \times 7.75 \mu\text{m}$. The individual grain diameter was $3.85 \mu\text{m}$ and the diameter of each verruca was $0.64 \mu\text{m}$. In comparison with previous studies, the exine sculpturing was characterized by tubercles that were inconsistent with our results, except for the tetrads type of pollen noted by Agashe and Caulton [59]. Inaperturate tetrahedral- and tetrad-type pollen with an average diameter of $9 \mu\text{m}$ in *M. pudica* samples in Singapore has also been reported previously [62].

In Fabaceae, the pollen evolution ranges from simple to tetrads and polyads. The simple grains were confirmed in our study, for example for the pollen from *Entada phaseoloides* and *Leucaena leucocephala*. The pollen morphology of the *Mimosa* genus has been extensively studied. For instance, pollen grains of about 255 species have been described, of which 247 were studied analyzed by light microscopy, 216 by scanning electron microscopy and 19 by transmission electron microscopy [37,41,63–68]. These studies documented detailed descriptions of the pollen morphologies of many *Mimosa* taxa, showing the variation in the different types of pollen within the genus, i.e., polyads, tetrads and octads in various shapes and sizes, with verrucate to microverrucate sculpturing, porate apertures in the subdistal position and the presence of an operculum and annulus [69]. However, in the present study, this was not noted in *Mimosa bimucronata*, where the bitetrad 8-grain polyads were arranged in two planes (4 + 4) with a rugulate exine structure.

Jamwal et al. [70] investigated the characteristic features of *Albizia* pollen, i.e., polyad (16-celled), prolate-spheroidal, tricolporate, isopolar and radially symmetric. Sizes ranged from $57 \mu\text{m} \times 49 \mu\text{m}$. The tectum was previously investigated and shown to be psilate to faveolate. Similarly, Aftab and Perveen [45] conducted a palynological study of cultivated trees in Karachi, Pakistan, and observed 14 celled polyads, which were bilaterally symmetrical, with pollen sizes of $79.59 \mu\text{m} \times 74.235 \mu\text{m}$ and a tectum subsilate for *Albizia lebback*. In comparison with this, 12 grains per polyad were observed in the present study for *A. lebback*, meaning our findings did not corroborate theirs.

In *Acacia*, polymorphism has been found in the grains of each polyad (numbering 4, 8, 12, or 16 grains) in Australian species, while 16 to 32 grains were found in polyads in African species [71]. Five species of Pakistani *Acacia* were studied by Perveen and Qaiser [44], who concluded that morphological differences in pollen of the Mimosoideae species are significant at the tribe and genera levels. Caccavari and Dome [9] analyzed the pollen morphologies of 77 American *Acacia* species and suggested that pollen features can be used as a distinguishing factor for the generic limitation of *Acacia*. Similarly, Rajurkar et al. [72], reported 16 grains per polyad in *Acacia* species and variations in their morphological traits, i.e., size, shape and exine pattern, which were found to be helpful in genera- and species-

level identification. However, in comparison with these findings, 16 grains per polyad were found in the present study for *Acacia auricfoliformis* and 12 grains per polyad were found in *A. confusa* and *A. mangium*. The latter species can be delimited from the former due to the presence of operculum on the central grain surface of the polyad. Hence, our study also confirmed that morphological traits of the pollen of *Acacia* species are useful at the generic and specific levels.

The pollen morphology of *Leuceana leucocephala* species was noted as a perforate and scabrate exine pattern, while pollen units of monad and tricolpate types were found in the present study. In comparison with a previously published study [46], the variation in pollen morphology of the *Leucaena* species was found to be helpful in showing the relationship and defining generic boundaries within Mimosoideae. For instance, they reported perforate and punctate exine sculpturing with pollen units of monad and tricolporate types in *Leucaena leucocephala*. However, psilate to scabrate exine sculpturing in Pakistani *L. leucocephala* was found by Khan et al. [41]. These findings mostly support our study results.

The pollen morphologies of 11 *Calliandra* taxa were investigated by De Assis-Ribeiro dos Santos and De Oliveira-Romão [47], who concluded that some of the polyad characteristics are useful in corroborating previous infrageneric classifications of the genus. Several palynological studies included *Calliandra* taxa. Sorsa [37], gathered data from nine species. Similarly, Guinet and Hernández [73] made a comparison between *Calliandra* and *Zapoteca*, the former of which has eight grains per polyad, while the latter has 16 grains per polyad. Our study corroborated these findings (8-grain polyads) in *Calliandra haematocephala*. Other studies [74–76] recorded the pollen morphology of one or species of the genus.

4. Conclusions

Taxa from the subfamily Mimosoideae collected from different geographical regions on Hainan Island were analyzed using both light microscopy and scanning electron microscopy. Both LM and SEM morphological descriptions of the pollen can be helpful for plant taxonomists to correctly identify and discriminate the Mimosoideae taxa at generic and specific levels. Significant variation was found in both the qualitative and quantitative traits of the pollen, confirming the Mimosoideae species as eurypalynous. The pollen traits were significantly proven to have taxonomic potential that will support and strengthen the systematics of this subfamily. This study will also provide a basis for further phylogenetic and molecular studies to strengthen the systematics of the Mimosoideae subfamily. Heteromorphism was found in the shape of the monads in the pollen association (polyads, bitetrad, octads). Additionally, exines exhibited heteromorphism in the colpus surface membrane ornamentation of the studied taxa. Interspecies variation was present in the exine ultrastructure of the pollen grains of the studied Mimosoideae taxa. Furthermore, palynological, anatomical, molecular and phylogenetic studies are needed to support and strengthen the systematics of the Mimosoideae subfamily.

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