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PRELIMINARY STUDY OF THE POLLEN MORPHOLOGY OF MALAYSIAN ZINGIBERACEAE (TRIBE ALPINIEAE) AND THE TAXONOMIC RELATIONSHIP

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ABSTRACT
MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Preliminary study of the pollen morphology of Malaysian Zingiberaceae (tribe Alpinieae) and the taxonomic relationship. *Reinwardtia* 22(2): 91–102. — The family Zingiberaceae forms an important herbaceous layer in the tropical forests of Malaysia. From a phylogenetic point of view, certain genera of the problematic tribe Alpinieae are non-monophyletic and in need of more taxonomic evidence to support the classification. This study demonstrates how the palynological data could correlate with the proposed phylogenetic data, using representatives from the Malaysian species. The pollen morphology of 21 species from the tribe Alpinieae was investigated. Parameters including polarity, symmetry, shape, size, apertures, exine ornamentation, size of spine, type of spine apex, spine density, and distance between spine were analysed. The results demonstrated that the studied species were conveniently divided into two major groups based on the exine sculpturing of the spheroidal pollens, either psilate as in *Etlingera* and *Hornstedtia*, or echinate as in *Alpinia, Conamomum, Meistera, Plagiostachys, Sundamomum*, and *Sulettaria.* Hence, as far as the study is concerned, the main sculpturing is considered useful to generally distinguish the genera in the tribe.

Key words: Diversity, Johor, systematics, Sarawak, taxonomy, wild gingers.

ABSTRAK
MOHAMAD, S., MEEKIONG, K & SEDEK, A. S. 2023. Penelitian awal morfologi serbuk sari Zingiberaceae Malaysia (puak Alpinieae) dan hubungan taksonominya. *Reinwardtia* 22(2): 91–102. — Suku Zingiberaceae membentuk lapisan herba penting di hutan tropis Malaysia. Dari sudut pandang filogenetik, marga tertentu dari puak Alpinieae yang bermasalah adalah non-monofiletik dan membutuhkan lebih banyak bukti taksonomi untuk mendukung klasifikasi. Studi ini menunjukkan data palinologi dapat berkorelasi dengan data filogenetik yang diusulkan, menggunakan perwakilan dari jenis Malaysia. Morfologi polen dari 21 jenis dari puak Alpinieae telah diamati. Parameter yang dianalisis termasuk polaritas, simetri, bentuk, ukuran, bukaan, perhiasan eksin, ukuran duri, tipe ujung duri, kerapatan duri, dan jarak antar duri. Hasilnya menunjukkan bahwa jenis yang diteliti dengan mudah dibagi menjadi dua kelompok besar berdasarkan perhiasan eksin serbuk sari yang membulat, baik *psilate* seperti pada *Etlingera* dan *Hornstedtia*, atau *echinate* seperti pada marga *Alpinia, Canamomum, Meistera, Plagiostachys, Sundamomum*, dan *Sulettaria*. Oleh karena itu, perhiasan utama dianggap berguna untuk membedakan marga dalam puak secara umum.

Kata kunci: Jahe liar, Johor, keragaman, Sarawak, sistematika, taksonomi.

INTRODUCTION

Zingiberaceae is the largest family among the eight families in the monophyletic order, Zingiberales and has at least 60 genera and 1,900 species (POWO, 2022; Banaticla-Hilario & Altamirano, 2023). The species occur in the tropics and subtropics, of which the Asian tropics hold the highest diversity and number of taxa (Lamb *et al.,* 2013; POWO, 2022). Most Zingiberaceae are evergreen plants that form the important herbaceous layer of the tropical rainforest, especially in forest gaps where light is not scarce. Within Zingiberaceae, diverse genera such as *Alpinia* and *Amomum* are often dominant in humid tropical lowlands, while *Etlingera, Hornstedtia,* and *Plagiostachys* can predominate in habitats that provide more sunlight, such as disturbed areas or secondary forests (Lamb *et al.*, 2013). In Malaysian Borneo, as botanical exploration progresses, more than 250 named taxa in Zingiberaceae have been reported and subfamily Alpinioideae encompassed the highest number of species (De Boer *et al.*, 2018; Mohamad & Meekiong, 2019). On top of that, several Alpinieae genera are highly distributed or endemic to Sarawak, in particular *Epiamomum* (all six species, 100%), *Sundamomum* (10 species out of 16.63%), and *Plagiostachys* (13 taxa out of 33.38%) (POWO, 2020; WCVP, 2020; Mohamad *et al.*, 2020; Mohamad & Meekiong, 2020). Of *Etlingera* in Borneo, 32 taxa out of the known 42 (80%) occur in Sarawak, 30 taxa in Sabah (70%), 25 taxa (65%) in Kalimantan, and 13 taxa (33%) in Brunei (Poulsen, 2006). It is difficult to compare the species richness of Borneo with that of other islands, as only a relatively small number of detailed studies have been carried out (Poulsen, 2006). By 1999, Peninsular Malaysia had documented a lower count of Zingiberaceae species, with a total of 160 species across 18 genera, as reported by Larsen *et al.* (1999). In 2001, Khaw conducted a revision of *Etlingera* in Peninsular Malaysia, which initially comprised 12 species (Khaw, 2001). Subsequently, in the same year, Lim's report added two more species to the list, totaling 14 species (Lim, 2001).

A new classification of Zingiberaceae was proposed by Kress *et al.* (2002) based on the DNA sequence data who recognised four subfamilies and six tribes, namely Siphonochiloideae (tribe Siphonochileae), Tamijioideae (tribe Tamijieae), Alpinioideae which comprises most of the former tribes Alpinieae (tribe Alpinieae and tribe Riedelieae), and Zingiberoideae which includes the former tribes Hedychieae and Globbeae (tribe Zingibereae and tribe Globbeae). The subfamily Alpinioideae is diagnosed by the plane of distichous leaves perpendicular to the rhizome and the absence or reduction of the two lateral staminodes. The taxonomic status of the tribe Alpinieae still needs further clarification, especially for the non-monophyletic genera, including *Alpinia* which consists of different clades (Kress *et al.*, 2005), *Plagiostachys* (nested deep within *Zerumbet* clade IV in *Alpinia*) (Kress *et al.*, 2005; Julius *et al.*, 2008), and *Hornstedtia* (two species are nested within the *Amomum* clade and *H. leonurus* is nested within the *Etlingera* clade) (Pedersen, 2004; De Boer *et al.*, 2018). In the current development, species that were formerly placed under *Elettaria* have been moved to a new genus, *Sulettaria* based on works by Poulsen *et al.* (2019). In the long-term, the species in these genera may have to be transferred to another genus, but for the time being, they are ideally classified based on morphological characters.

The pollen morphology in Zingiberaceae have been investigated in certain genera and several characteristics, including shape, size, symmetry, and polar aperture of the pollen grains, were discovered as beneficial in identifying the species of the particular genus (Larsen *et al.*, 1998; Saensouk *et al.*, 2009; Chen & Chia, 2011; Saensouk *et al.*, 2015) and for classification at the generic and sectional levels (Theilade *et al.*, 1993). In 1990, Mangaly & Nayar, who studied the South Indian Zingiberaceae, reported that the shape of pollen grains ranged from spheroidal, subspheroidal, ovoid, and ellipsoid, whilst the sculpturing of the exine can be echinate, regulate, scabrate, striate, psilate, or verrucate. In addition, they proposed that the thin layer of exine does not provide mechanical strength for the pollen grain, thus most of the taxa have low resistance to acetolysis treatment (Mangaly & Nayar, 1990). Previously, the pollen of Zingiberaceae was regarded as exineless and inapertu-rate (Dahlgren *et al.*, 1985), but this was proved untrue by Mangaly & Nayar (1990) who disco-vered that among the 21 taxa studied, an exine was only absent in *Kaempferia galanga.* All other taxa portrayed a distinct exine layer covering the pollen grain, as found in other genera in the Zingiberales order*: Heliconia* (Heliconiaceae), *Strelitzia* (Strelit-ziaceae) and *Tapeinochilos* (Costaceae). In contrast, the intine consisted of different layers that are well developed and lamellated (Mangaly & Nayar, 1990).

Of the tribe Alpinieae, Mangaly & Nayar (1990) recorded that the pollen shapes of the seven studied species were either spheroidal or subspheroidal, while the exine sculpture was spinulosed in most of the tribe, *viz. Alpinia calcarata, A. galanga, A. zerumbet, Amomum hypoleucum, A. pterocarpum*, and verrucate as in *Elettaria cardamomum*. In contrast, the pollen shapes of *Zingiber* (tribe Zingibereae) were either spheroidal with rugulate ornamentation or ellipsoidal and striate, which closely followed the subgeneric boundaries. Among the different taxa studied, the well developed exine only occurred in *Zingiber.* The presence or absence of ornamentation, however, clarified that there is no correlation to the thickness of the exine layer (Mangaly & Nayar, 1990). Variation in pollen morphology was shown as following the taxonomic borders, even though the tribes encompassed more than one pollen type.

Theilade *et al.* (1993), who explored the pollen structure and morphology of 18 *Zingiber* species from Peninsular Malaysia, Thailand, and Myanmar reported two groups of pollen structure, particularly spherical with cerebroid or reticulate sculpturing in section *Zingiber* and section *Dymezewiczia,* and ellipsoidal with a spiro-striate surface for section *Cryptanthium.* The pollen morphology of *Zingiber* offered a more useful criterion than the ambiguous inflorescence habit for the classification into sections. The present subdivision of the genus is based on the inflorescence habit, whereby the inflorescences of sections *Zingiber* and *Cryptanthium* are radical, and apical for section *Dymezewiczia*. Therefore, based on the pollen morphology, she had proposed that the section *Dymezewiczia* should be included in the section *Zingiber*.

In comparison, Kaewsri & Paisooksantivatana (2007), who investigated 14 taxa of *Amomum* from Thailand, have revealed that the pollen grains are spherical to subspherical, inaperturate, and exine sculptures of psilate and echinate. The exine sculpturing was proven to be significant in dividing the genus into two groups. Additionally, the palynology study of *Curcuma* (tribe Zingibereae) by Saensouk *et al.* (2015) on 14 taxa has described that the pollen shapes ranged from subspheroidal, prolate spheroidal, spheroidal, subprolate, and prolate. The various shapes of pollen grains were proven to be beneficial as a supplementary character to distinguish the species. However, the taxonomic groups based on palynological characters were found to be incoherent with those based on morphological characters. Later, Kajornjit *et al.* (2018) revealed that the size, shape, exine sculpturing, and type of spine apex of 22 taxa of Thai *Globba* could be used to distinguish groups in the genus. However, pollen morphology has low taxonomic value since all taxa are quite similar in all aspects. Pollen characters alone were quite insignificant in resolving the classification of *Globba* as they can not be used to identify all species, but they can provide some information to differentiate some taxa.

Palynology could provide supportive and descriptive information in systematics study. However, most species in Malaysia, especially in the eastern part, have not had their pollen morphology thoroughly studied, and it is a fact that, the complex tribe Alpinieae in the subfamily Alpinioideae encompassed the highest number of species in Sabah and Sarawak. Thus, a study that describes the comparative pollen morphology among the complex genera to provide useful characteristics concerning taxonomy is highly needed.

MATERIALS AND METHODS

Specimens Collection and Identification. Plant specimens were collected and documented from various localities in the states of Sarawak (East Malaysia) and Johor (West Malaysia) as presented in Table 1. The study encompassed a total of three primary vegetation sampling sites, consisting of five totally protected areas, namely Similajau National Park in Bintulu, Niah National Park, and Lambir Hills National Park in Miri, Kubah National Park in Kuching, and Endau Rompin Johor National Park in Johor. Additionally, two forest reserves were included: Fairy Cave Nature Reserve in Bau and Ayer Hitam Utara Forest Reserve in Johor. Furthermore, the research covered five secondary disturbed areas. Twenty-one species of the tribe Alpinieae were retrieved and documented with preference to fertile material. Morphological characters of each collected plant were measured and assessed, especially on floral and reproductive parts, to provide a primary basis for species identification. Specimens were described and verified with type materials through examination of specimens from several herbaria, digital images of types (K, E), protologues, and taxonomic data from online databases (POWO, 2020; BHL, 2020; IPNI, 2020; Newman, 2022; WVCP, 2022) and published materials on related species. Herbarium specimens were deposited at the Herbarium of Forest Department Sarawak (SAR) while the duplicates were kept at the herbaria in Universiti Malaysia Sarawak and Universiti Tun Hussein Onn Malaysia. Figure 1 provides photographs of the studied species in their original habitat.

Pollen Micromorphology Assessment. Pollen grains from the anther of blooming flowers of selected taxa were studied. For each species, about 15–20 pollen grains were measured to ensure consistent and reliable data. Pollens were stored in 70% ethanol. Samples were then dehydrated in an alcohol series of 70%, 80%, 95%, and 100%. Later, the pollen grains in absolute alcohol were dried on aluminium stubs with double-sided carbon tape. Samples were sputter-coated with gold-palladium and examined using an analytical scanning electron microscope (SEM) (JEOL: JSM-6390LA). Parameters of pollen grains that were analysed were polarity, symmetry, shape, size, apertures, exine ornamentation, size of spine, type of spine apex, spine density, and distance between spine. The terminology adopted to describe the pollen morphology was made based on Punt *et al.* (2007), Chen & Xia (2011), and Saensouk *et al.* (2015), while the pollen shape and size classification was made following Erdtman (1969).

Hierarchical Clustering. The key pollen morphology were scored to evaluate the phenetic similarities and interrelationships among the species. Table 1 provides details of the scored key characters. Phenetic similarities of variable characters were enumerated through clustering using PAST3 (PAlentological STatistics) Software Version 3.22. A dendrogram was constructed using the algorithm of the unweighted pair-group method using arithmetic average (UPGMA) based on Bray-Curtis similarity index (Sokal, 1986). The clustering process is estimated by the cophenetic correlation coefficient. The Bray-Curtis index is based on shared similarities divided by total similarities, which can be calculated using the formula below:

Table 1. Scored pollen characters for clustering analysis.

	Pollen characters	Scores
1	Shape	0. Oblate-sphereoidal; 1. Spheroidal; 2. Prolate-spheroidal; 3. Prolate
2	Size	0. Medium; 1. Large
3	Exine sculpturing	0. Echinate-rugulose; 1. Echinate-psilate; 2. Psilate-rugulose; 3. Psilate-reticulate
4	Spine length	0. ≤ 3 µm; 1. ≥ 3 µm; 2. Absent
5	Spine width	0. ≤ 3 µm; 1. ≥ 3 µm; 2. Absent
6	Distance between spine	0. ≤ 3 µm; 1. ≥ 3 µm; 2. Absent
7	Spine apex	0. $\leq$ Blunt; 1. Sharp; 2. Absent

$$BC_{ij} = \Sigma |(n_i - n_j)| / \Sigma (n_i + n_j)$$

In which,
BC_{ij} = Bray-Curtis Dissimilarity of two species i and j
ni = number of characters present in i
nj = number of characters present in j
BC_{ij} Similarity Index = $(1 - BC_{ij}) \times 100$

RESULTS

Pollen Micromorphology

Pollen grains from 21 selected species were classified as monad (single dispersal unit), radial symmetry, apolar, and inaperturate. The pollen shape of Alpinieae species was mainly spheroidal, which was essentially divided into three subgroups: spheroidal, oblate-spheroidal, or prolate-spheroidal. Two main types of exine sculpturing or ornamentation were revealed, in particular, echinate (as shown in *Alpinia* (Figs. 2A–2D and 3A–3D), *Conamomum* (Figs. 2E–2F and 3E–3F), *Meistera* (Figs. 2L and 3L), *Plagiostachys* (Figs. 2M–2N and 3M–3N), *Sundamomum* (Figs. 2O–2P and 3N– 3O), and *Sulettaria* (Figs. 2Q–2R and 3P–3Q) and psilate as in *Etlingera* (Figs. 2G–2J and 3G–3J) and *Hornstedtia* (Figs. 2K and 3K). In detail, the ornamentation can be further defined as echinate with a psilate surface between spine, echinate with a rugulose surface between spine, or psilatereticulate surface.

The pollen sizes were mainly large (50–100 µm) based on the longest axis, except for *A. aquatica* (Figs. 2A and 3A), *A. ligulata* (Figs. 2D and 3D), *S. longipilosa* (Figs. 2Q and 3P), and all species of *Plagiostachys* that displayed mediumsized pollen grains. For echinate pollens, the spine properties, *i.e.*, spine apex (sharp or blunt), size, and distance, were distinguishable between species. The length of the spine varied in each species, from the shortest at approximately 1.96 µm as in *P. glandulosa* (Figs. 2N and 3M), to the longest as in *S. polycarpa* (Figs. 2R and 3Q), at approximately 5 µm. The width of the spine bases was also diverse, with the smallest at 2.02 µm as in *A. aquatica* to a maximum width of 5.12 µm as in *S. polycarpa*. The shortest distance between the spine was displayed by *S. laxesquamosum* (Figs. 2P and 3O), measuring 1.33 µm compared to *C. xanthophlebium* (Figs. 2F and 3F), having the longest distance of 4.20 µm. Table 1 provides details on the pollen morphological characteristics of the studied species. Table 2 displays the studied pollen morphological characteristics in detail. While the SEM micrographs of selected pollen grains were illustrated in Figs. 2 and 3.

Hierarchical Clustering

The dendrogram in Fig. 4 illustrates the phenetic relationship among studied Alpinieae species, utilising scored key pollen characteristics and four main clusters were produced. Exine sculpturing, type of spine apex, as well as spine morphology (distance, width, and length), were essential in determining the final placement of the species, though they were not exactly grouped in accordance with their generic boundaries. Species of *Etlingera* and *Hornstedtia* were closely clustered together (> 95% similarity) based on psilate pollens .

DISCUSSION

Pollen Micromorphology

Alpinia displayed two types of echinulate ornamentation: echinate-psilate for *A. latilabris* (Figs. 2C and 3C), *and A. ligulata* (both from *Zerumbet* clade) and echinate-rugulose for *A. aquatica* (*Zerumbet* clade) and *A. galanga* (Figs. 2B and 3B) (*Galanga* clade). Although *A. aquatica* is classified under the *Zerumbet* clade, the exine sculp-

Fig. 1. Inflorescences and infructescences of the studied taxa in their respective habitats. A. *Alpinia aquatica*. B. *A. galanga*. C. *A. latilabris*. D. *A. ligulata*. E. *Conamomum cylindrostachys*. F. *C. xanthophlebium*. G. *Etlingera coccinea*. H. *E. elatior*. I. *E. inundata*. J. *E. nasuta*. K. *Hornstedtia leonurus*. L. *H. reticulata*. M. *Meistera gyrolophos*. N. *Plagiostachys crocydocalyx*. O. *P. glandulosa*. P. *P. strobilifera* var. *conica*. Q. *Sundamomum laxesquamosum*. R. *S. corrugatum*. S. *Sulettaria longipilosa*. T. *S. polycarpa*. Photos by Salasiah Mohamad.

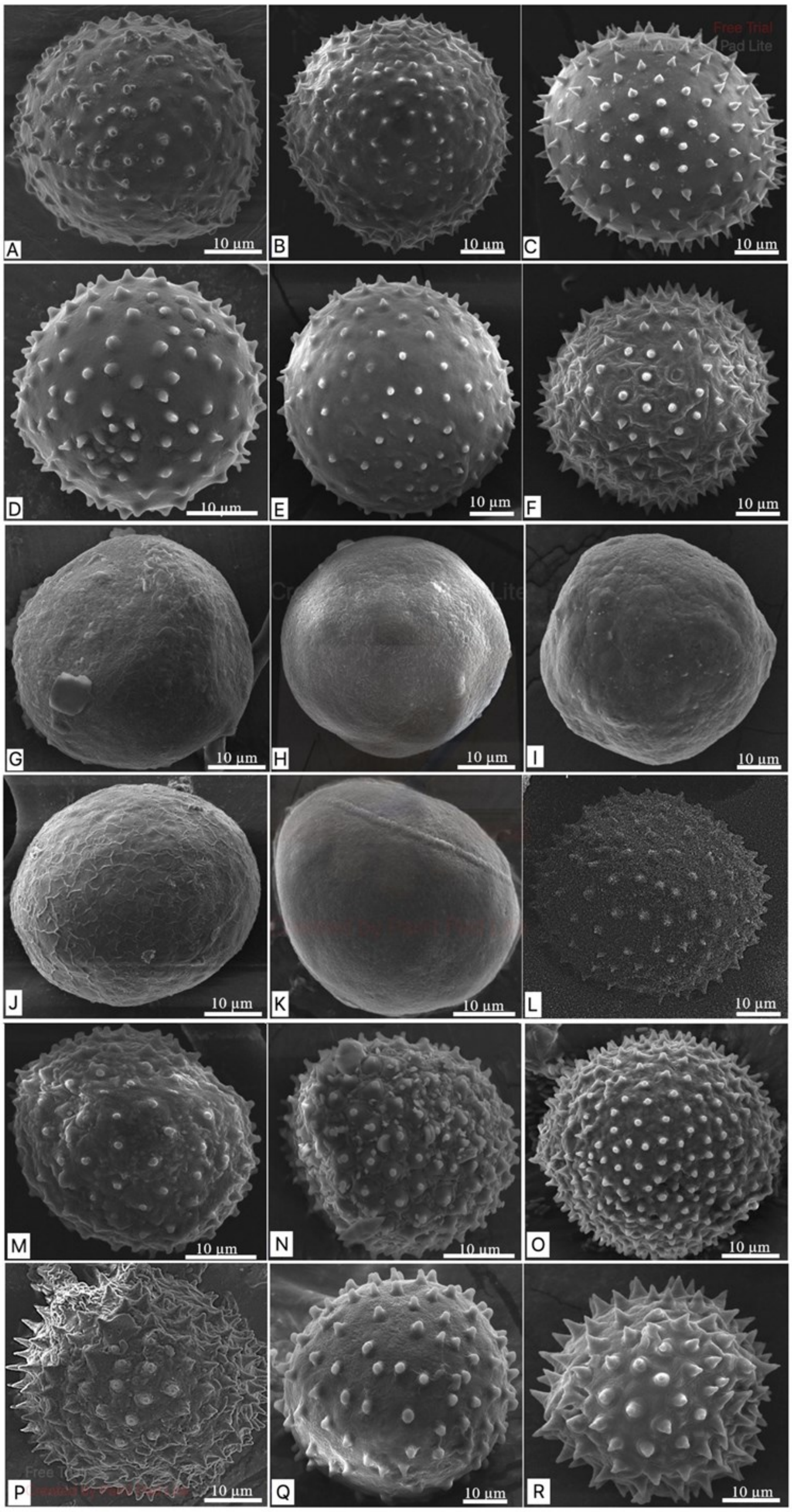

Fig. 2. SEM micrographs of pollen grains of the studied Alpinieae. A. *A. aquatica.* B. *A. galanga.* C. *A. latilabris*. D. *A. ligulata*. E. *C. cylindrostachys*. F. *C. xanthophlebium*. G. *E. coccinea*. H. *E. elatior*. I. *E. inundata*. J. *E. nasuta*. K. *H. reticulata*. L. *M. gyrolophos*. M. *P. strobilifera* var. *conica*. N. *P. glandulosa*. O. *S. corrugatum*. P. *S. laxesquamosum*. Q. *S. longipilosa*. R. *S. polycarpa.* Magnification: B, C, F, H, I, J, L= 1000×. O= 1400×. A, P, R= 1500×. E= 1600×; G= 1700×. D, K, M, N, Q= 2000×.

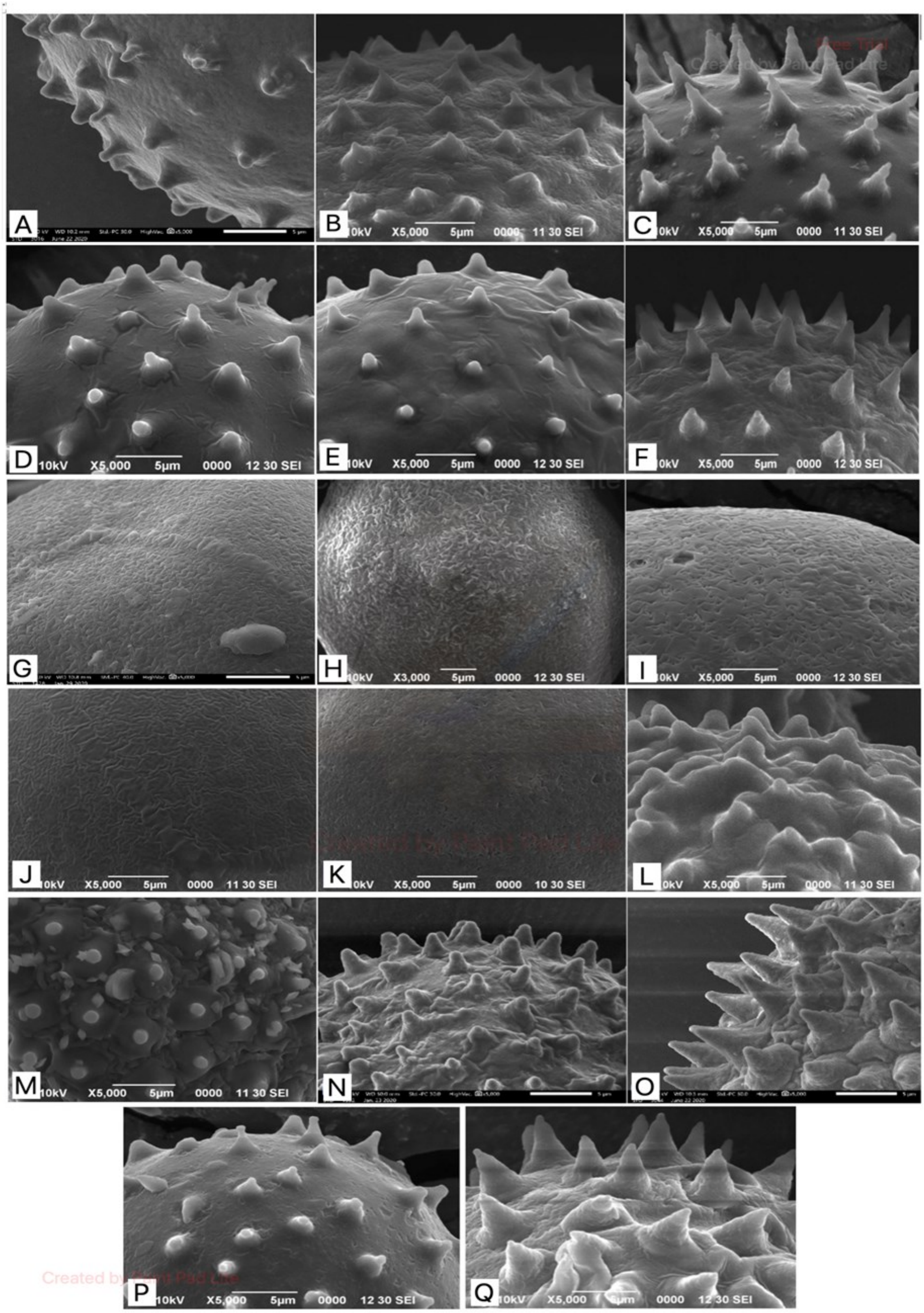

Fig. 3. SEM micrographs close-up of pollen sculpture of the studied Alpinieae. A. *A. aquatica.* B. *A. galanga.* C. *A. latilabris.* D. *A. ligulata.* E. *C. cylindrostachys.* F. *C. xanthophlebium.* G. *E. coccinea.* H. *E. elatior.* I. *E. inundata.* J. *E. nasuta.* K. *H. reticulata.* L. *M. gyrolophos.* M. *P. glandulosa*. N. *S. corrugatum.* O. *S. laxesquamosum*. P. *S. longipilosa.* Q. *S. polycarpa* .

ture and spine distance slightly resemble *A. galanga.* Perhaps their floral morphology, such as smaller flowers, requires similar mechanisms of pollination that decide the characteristics of the pollen. Likewise, two species of *Conamomum* with different labellum sizes unveiled slightly different echinulate sculpturing of either echinate-rugulose or echinate-psilate. However, this presumption relies solely on a limited taxonomic sample, necessitating further testing across various taxa in the respective generic groupings.

Species of *Etlingera, Hornstedtia, Plagiostachys, Sulettaria* and *Sundamomum*, however, recorded uniformly stable and reasonably similar ornamentation. The pollen shape of the studied *Etlingera* seemed to be correlative with their genus groupings based on morphology, whereby the oblatespheroidal pollen was observed in *E. elatior* (Figs. 2H and 3H) (Group A in Sakai & Nagamasu, 2003), while the remaining species (Figs. 2G, 2I, 2J and 3G, 3I, 3J) from Group C'(Sakai & Nagamasu, 2003) exhibited prolate-spheroidal pollen shape. Additionally, three species, including *A. galanga*, *E. elatior*, and *H. leonurus*, that were obtained from two distinct geographical regions (East and West Malaysia), exhibited similar pollen micromorphological characteristics.

Hierarchical Clustering

Although the clustering of pollen morphology was less useful for the entire generic classification, this study still provides information that will be useful in future related research in species delineation since pollen characteristics are helpful in characterising and identifying a specific taxon. Nonetheless, the pollen size, the pollen shape, as well as the spine properties (for echinate pollens), *i.e.*, apex form, distance, width, and length, are considerably functional in species recognition and groupings of some genera, including *Alpinia, Amomum, Conamomum, Meistera, Plagiostachys, Sundamomum,* and *Sulettaria.* Additionally, relying on pollen characters alone is unwise as they cannot be used to identify all species, but they do provide supplementary traits relevant to a particular taxon.

Pollen morphology of the polyphyletic *Alpinia,* unveiled spheroidal, medium- to large-sized, and echinate ornamentation with varying spine characteristics. The current pollen grain results were rather limited in distinguishing the species from two *Alpinia* clades. Cluster analysis of the pollen morphology in Fig. 4 revealed the close relationship between *A. aquatica* and *A. galanga* which probably correlated to their affinity in floral morphology (size of flower and labellum).

Likewise, species of *Hornstedtia* displayed similar pollen morphology, of having spheroidal psilate-reticulate with those of *Etlingera*; in fact, *H. leonurus* was clustered together with some species of *Etlingera* on the basis of molecular phylogenetic analysis. Other comparable data by Lam *et al.* (2010) on Bornean species (*H. havilandii* and *H. tomentosa*) and Acma & Mendez (2018) (on *H. conoidea*) have also recorded similar pollen morphology, thus it may also suggest a stable pollen morphology in members of *Hornstedtia* which are nearly allied to *Etlingera.*

The findings of Furness & Rudall (1999) regarding the widespread occurrence of inaperturate pollen among monocotyledonae were also confirmed through this study. The main pollen ornamentation and shape of Alpinieae species were consistent with previously related reports by Mangaly & Nayar (1990) and Kaewsri & Paisooksantivatana (2007) on *Alpinia* and *Amomum s.l.* species, as well as Lam *et al.* (2010) and Acma & Mendez (2007) on selected *Etlingera* and *Hornstedtia* in Borneo and the Philippines, respectively. Adding to the current data of the family Zingiberaceae, there are about five types of pollen sculpturing reported thus far, *i.e.*, echinate as observed in *Boesenbergia* (Chen & Xia, 2011; Mangaly & Nayar, 1990) and *Globba*, although very rarely, psilate in one species (Kajornjit *et al.*, 2018), verrucose in *Elettaria cardamomum* (Mangaly & Nayar, 1990), reticulate or rugulose in *Curcuma* (Saensouk *et al.*, 2015), and cerebroid and spirostriate in *Zingiber* (Theilade *et al.*, 1993). Other than the main spheroidal shape, *Curcum*a and *Zingiber* appeared to be of ovoid to ellipsoidal shape (Saensouk *et al.*, 2015; Theilade *et al.*, 1993; Mangaly & Nayar, 1990), which was not observed in any Alpinieae species so far. Pollen study on more species, including examination of the exine thickness, would provide more information that may help in defining subgroups or sections of a particular genus, as reported by Theilade *et al.* (1993) on *Zingiber*, Kaewsri & Paisooksantivatana (2007) on *Amomum*, and Kajornjit *et al.* (2018) on *Globba*.

The pollen sculpturing in plants might also be influenced by pollination systems. The pollen sculpture acts as an adhesion enhancer to the pollinators' bodies in the presence of pollen kitt (Grayum, 1986; Pacini & Hesse, 2005). According to the data on gingers pollination guilds by Sakai *et al.* (1999), it was shown that species with echinate pollen sculpturing (*C. cylindrostachys, M. gyrolophos, P. crocydocalyx, P. glandulosa*, and *S. polycarpa*) were associated to Halictic- or Amegilla-guilds, whereas species with psilate ornamentation (*H. leonurus* and *H.reticulata*) were allied to Spiderhunter-guild. Besides the floral characters, *i.e.*, sizes of floral tube, labellum, pistil, and stamen, which played vital roles in the ginger pollination mechanism, the pollen sculpture was also consistent with the pollination guild system. Although the interaction between pollen ornamentation and type of pollinator has been commonly reported in angiosperms, Sannier *et al.* (2009), based on their comparative analysis of other monocots of the Ara-

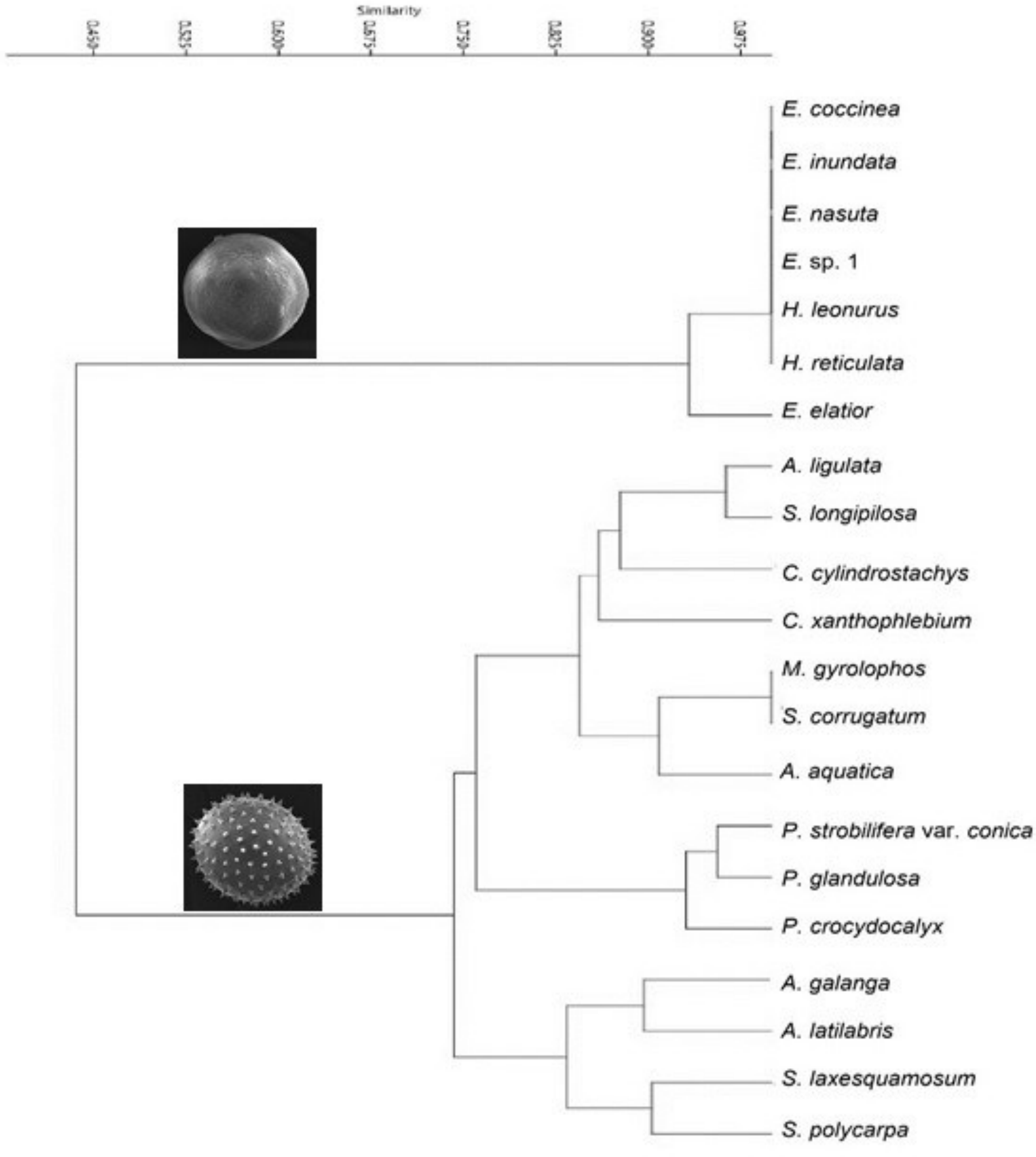

Fig. 4. Dendrogram indicates phenetic relationship of selected Alpinieae species based on pollen characteristics using Bray-Curtis and UPGMA algorithm.

Table 2. Comparison of pollen grain characteristics in the studied Alpinieae.

	Taxa	Localities	PA (µm)	EA (µm)	P/E ratio	Shape	Size	ES	SL (µm)	SW (µm)	SA	DS (µm)
					Alpinia							
1	*A. aquatica*	SNP	48.4	48.1	1.00	SP	M	E-RU	2.12	2.02	B	2.75
2	*A. galanga*	NNP, AHUFR	68.8	68.8	1.00	SP	L	E-RU	2.59	3.43	SH	2.71
3	*A. latilabris*	US	68.9	69.1	1.00	SP	L	E-P	3.60	3.01	SH	3.70
4	*A. ligulata*	FNCR	43.3	41.7	1.04	PS	M	E-P	2.57	2.58	B	3.10
					Conamomum							
5	*C. cylindrostachys*	LHNP	51.0	53.0	0.96	OS	L	E-P	2.20	2.58	B	4.03
6	*C. xanthophlebium*	SNP	58.9	57.5	1.02	PS	L	E-RU	3.14	2.37	SH	4.20
					Etlingera							
7	*E. coccinea*	LHNP	50.9	49.5	1.03	PS	L	P-RE	-	-	-	-
8	*E. elatior*	US,AHUFR	51.1	52.1	0.98	OS	L	P-RE	-	-	-	-
9	*E. inundata*	LHNP	57.7	57.0	1.01	PS	L	P-RE	-	-	-	-
10	*E. nasuta*	KNP	52.2	49.7	1.05	PS	L	P-RE	-	-	-	-
11	*Etlingera* sp. 1	SNP	63.7	58.9	1.08	PS	L	P-RE	-	-	-	-
					Hornstedtia							
12	*H. leonurus*	LHNP, ERJNP	52.3	51.5	1.02	PS	L	P-RE	-	-	-	-
13	*H. reticulata*	KD	59.4	54.9	1.08	PS	L	P-RE	-	-	-	-
					Meistera							
14	*M. gyrolophos*	LHNP	57.7	60.4	0.96	OS	L	E-RU	2.34	2.42	B	2.10
					Plagiostachys							
15	*P. strobilifera* var. *conica*	SNP	42.8	44.0	0.97	OS	M	E-RU	2.03	2.32	B	3.01
16	*P. crocydocalyx*	LHNP	41.0	42.3	0.97	OS	M	E-RU	2.01	2.15	B	2.69
17	*P. glandulosa*	NNP	40.1	41.1	0.99	OS	M	E-RU	1.96	4.75	B	1.69
					Sundamomum							
18	*S. corrugatum*	TB	65.6	64.3	1.02	PS	L	E-RU	2.66	2.23	B	2.07
19	*S. laxesquamosum*	GP	59.5	59.1	1.00	SP	L	E-RU	4.55	5.10	SH	1.33
					Sulettaria							
20	*S. longipilosa*	SNP	40.0	39.2	1.02	PS	M	E-RU	2.55	2.78	B	3.11
21	*S. polycarpa*	TT	54.9	54.9	1.00	SP	L	E-RU	5.00	5.12	SH	1.52

SNP - Similajau National Park Bintulu, NNP - Niah National Park Miri,AHUFR - Ayer Hitam Utara Forest Reserve Johor, ERJNP – Endau Rompin Johor National Park Johor, Fairy Cave Nature Reserve, Bau, LHNP - Lambir Hills National Park Miri, KNP - Kubah National Park Kuching, US - Ulu Sebauh Bintulu, KD – Kidurong Bintulu, TB - Tubau Bintulu, GP - Gunung Podam Bau, TT - Tatau Bintulu, PA - polar axis, EA - equatorial axis, ES - exine sculpture, SL - spine length, SW - spine width, SA - spine apex, DS - distance between the spine, OS - oblate-spheroidal, PS - prolate-spheroidal, SP - spheroidal, L - large, M - medium, E - echinate, P - psilate, RE - reticulate, RU - rugulose, B - blunt, SH – sharp.

ceae and Arecaceae, suggested that pollen ornamentation alone was not the most significant element that determines the pollination system. Other pollen properties, such as pollen kitt or aroma, could also play a role. The relationship between plant and pollinator literally differs between plant taxa that may be affected by geographical area or derived from ancestral characters or as results of adaptative mechanism.

CONCLUSION

Pollen grains of selected Alpinieae species were examined under SEM to facilitate in defining the infrageneric and intergeneric variation. The shape was predominantly spheroidal, either oblate-spheroidal, prolate-spheroidal, to prolate, and the size ranged from medium (< 50 µm) to large (50–100 µm). Hierarchical clustering of the studied species was rather inconsistent with the group boundaries, although it has preliminarily divided the tribe into two major clusters based on pollen ornamentation, *i.e.* psilate and echinate. The main sculpturing is useful to generally distinguish the genera; in particular, the psilate-reticulate pollen is a characteristic of *Etlingera* and *Hornstedtia,* whilst the echinate pollen is common for members of *Alpinia, Conamomum, Meistera, Plagiostachys, Sulettaria,* and *Sundamomum*.

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