



Taxonomic implications of pollen of some species of the genus *Pterospermum* Schreb. (Malvaceae s.l. subfam. Dombeyoideae)

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Received: October 9, 2020

Accepted: November 29, 2020

ABSTRACT

A palynological study of six species of *Pterospermum* was conducted to investigate variation in pollen micromorphological characteristics. The studied species were *P. diversifolium*, *P. elongatum*, *P. jackianum*, *P. javanicum*, *P. lanceifolium* and *P. subpeltatum*. Pollen was subjected to acetolysis, single-grain techniques and dehydration series, and observed using light and scanning electron microscopy. The results reveal some common characters shared by all the studied species, namely: echinate-granulate exine ornamentation; porate pollen class; and circular amb. Variation was found in the ratio of exine thickness, pollen size, and average spine length, outline, shape, measurements, spine size and the presence of an annulus. These characters proved to be valuable for identifying and differentiating the studied species. This study shows that morphological characteristics of *Pterospermum* pollen have taxonomic value, especially for the identification and differentiation of species of the subfamily Dombeyoideae and even other Malvaceae s.l.

Keywords: Dombeyoideae, Malvaceae, Malaysia, palynology, pollen morphology, *Pterospermum*

Introduction

Pterospermum which is also known as *bayur* or *banyur* in Malaysia is a medium-sized tree with stout, rounded buttresses and pale, pink-brown smooth to shallowly fissured bark surface (Ashton 1988). There are about 20 species of *Pterospermum* that can be found in tropical and subtropical regions of Asia including Thailand, India, Myanmar, Taiwan, China, Indo-China and Malesia (Wilkie & Berhaman 2011). Reportedly, there are only eight species of *Pterospermum* in Malaysia with six of them are available in Peninsular Malaysia, namely *Pterospermum acerifolium*,

Pterospermum diversifolium, *Pterospermum jackianum*, *Pterospermum javanicum*, *Pterospermum lanceifolium* and *Pterospermum pecteniforme*. (Turner 1995). Member of this genus can be found on fertile clay-rich soils of well-drained alluvium, riverbanks, gullies and damp hillsides. The timber from this genus has equivalent strength properties to the lighter red *meranti* but is less durable in ground contact. It is also said to be fit for pulping through drastic treatment bleaching to produce silky appearance papers (Ashton 1988). In addition, the timber of *Pterospermum* species is usually used in construction and production of furniture (Wilkie & Berhaman 2011).

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The circumscription of Malvaceae has been very much debated as the traditional Malvaceae *sensu stricto* comprises of a very homogenous and cladistically monophyletic group (Perveen & Qaiser 2009). Recently, Malvaceae *sensu lato* has been defined according to some closely related families which are Malvaceae, Tiliaceae, Sterculiaceae and Bombacaceae. These families are merged into an expanded family Malvaceae *s.l.* by Judd & Manchester (1997), Bayer *et al.* (1999) and Bayer & Kubitzki (2003), to be further divided into nine subfamilies of Byttnerioideae, Grewioideae, Helicteroideae, Sterculioideae, Brownlowioideae, Dombeyoideae, Malvoideae, Bombacoideae and Tilioideae (Perveen & Qaiser 2009). Former genera in Sterculiaceae are now divided and distributed into four subfamilies: Byttnerioideae, Helicteroideae, Sterculioideae and Dombeyoideae according to recent classification by Bayer *et al.* (1999). Meanwhile, molecular study on Malvales by Bayer *et al.* (1999) suggested that *Pterospermum* belongs to the Malvaceae *s.l.* subfamily; Dombeyoideae. According to Ashton (1988), the identification of *Pterospermum* species can be very perplexing as many species manifest sapling, stipule and leaf characters that are very dissimilar from those of the matured trees. Smaller flowers in younger trees lead to description of immature materials of distinct species.

Studies on pollen morphology of Sterculiaceae (Malvaceae *s.l.*) have been conducted by many researchers such as Wodehouse (1935), Selling (1947), Rao (1950), Erdtman (1952), Ikuse (1956), Chaudhuri & Mallik (1965), Litchfield (1966), Sharma (1967), Cristobal (1968), Melhem *et al.* (1976), Robyns *et al.* (1977), Moore *et al.* (1991), Lakshmi (2003), Saba *et al.* (2004), El Hussein (2006), Perveen & Qaiser (2009) and Hamdy & Shams (2010). Previous study by Perveen & Qaiser (2009) managed to recognize two distinct pollen types in the subfamily Dombeyoideae from Pakistan that are based on the length of polar axis.

Previous report on the pollen grains of *Pterospermum* by Ya (1992) revealed that the pollen is spheroidal, amb circular, 3-porate, and exine spiny. The pollen appears closer to Dombeyoideae but differs from Helicteroideae where the pollen is 3-porate or 3-3-colporate, amb triangular or subcircular, and exine reticulate (Ya 1992). This genus needs to be revised as it has no modern monographic treatment (Wilkie & Berhaman 2011). Due to the taxonomic importance of the Malvaceae family and the need for studies to support the circumscription of the group, which remains uncertain, pollen morphology must be characterized in order to support the taxonomic studies of the group (Abreu *et al.* 2014). Therefore, the objective of this study is to investigate and characterize the pollen morphology of six Malaysian *Pterospermum* species using observations under light microscope (LM) and scanning electron microscope (SEM) to add more taxonomic information of the genus.

Materials and methods

For this study, dried pollen samples from six *Pterospermum* species, namely *P. diversifolium* Blume, *P. elongatum* Korth., *P. jackianum* Wall., *P. javanicum* Jungh., *P. lanceifolium* Roxb. and *P. subpeltatum* C.B. Rob were used. Samples were collected from Herbarium specimens of Forest Research Institute Malaysia (KEP), Malaysia and Royal Botanic Gardens Kew Herbarium (K), United Kingdom based on the availability of flower buds. Only one sample was available for each species except for *P. diversifolium* and *P. subpeltatum*.

The dried materials examined were as follows: *Pterospermum subpeltatum* C.B. Rob. **BORNEO. Sarawak:** Batang Rajang, 08/08/1938, Daud & Tachun SFN 35727 (KEP); **BORNEO. Sabah:** Elapura, Sandakan, 18/03/1948, Anthony A 762 (KEP). *P. jackianum* Wall. **MALAYA. Kedah:** Bedong, Sungai Petani, 08/06/1926, T. Guard, Meh KEP 8976 (KEP). *P. lanceifolium* Roxb. **MALAYA. Kedah:** Kampung Masjid, Changlun, 23/05/1941, Jaamat & Sow KEP 57017 (KEP). *P. javanicum* Jungh. **MALAYA. Selangor:** Sungai Buloh Forest Reserve, 11/04/1918, Hamid FMS 1579 (KEP). *P. elongatum* Korth. **BORNEO. Sabah:** Sandakan, 09/04/1935, Castro 4419 (K). *P. diversifolium* Blume. **BORNEO. Sabah:** Keningau, 23/04/1989, Fidilis K SAN 127667 (KEP); **BORNEO. Sabah:** Tenom, 22/05/1964, Rundi M 43189 (K); **BORNEO. Sabah:** Negeri Bubul Forest Reserve, Sempoerna, 25/09/1947, Harvey A 143 (KEP).

The pollen samples were first treated using acetolysis technique modified from Erdtman (1969) before being viewed under Scanning Electron Microscope (SEM) model Carl Zeiss Supra 55vp and photographed under Light Microscope (LM) using digital camera (Olympus BX43F) mounted on the Olympus microscope DP72 (Olympus Soft Imaging Solutions)/Cell^B Software, under 40x and 60x magnifications. Meanwhile, flower buds were softened in boiling water. Under dissecting microscope, anthers were dissected, crushed and separated. Using 1% Teepol, flowers buds were cleaned and centrifuged for five minutes. Glacial acetic acid was added and centrifuged for another five minutes. Acetolysis mixture were then added into the samples and preheated on the hot plate around 70 °C until the pollen turned dark and the mixture of acetolysis turned to brown. The samples were cleansed after being centrifuged for five minutes using glacial acetic acid. Next, 2.5% KOH solution was added and centrifuged. The pollen samples were then cleansed by using distilled water. The process was repeated two times to ensure total removal of the glacial acetic acid. The pollen samples were then dehydrated in a series of graded alcohol: 35, 50, 70, 85, 95% for 10 minutes each, followed by three times dehydration in 100% alcohol, prior to a critical point drying (CPD) process using drying machines model Polaron CPD 7501 and gold coating process using gold coater model BioRad SC500. For LM technique, the pollens were mounted onto the glass



slides by using glycerine jelly and covered by glass slip. Single-grain technique following Zetter (1989) was also used in this study. At least 30 pollen grains were measured for each species to verify the stability of the data obtained. The analysis was done using unweighted pair group method with arithmetic mean (UPGMA) by MVSP version 3.2 Kovach to see the similarities among the studied species. The characters used for the analysis were polar length, equatorial length, P/E ratio, pollen shape, aperture, amb, exine ornamentation, exine thickness ratio, pollen class, pollen size, spine length, spine width, spine shape, distance between spine and the presence of annulus. The description of pollen morphology followed Faegri & Iversen (1992), Erdtman (1969), Hesse *et al.* (2009), Halbritter *et al.* (2018) and Amirul-Aiman *et al.* (2019).

Results

Findings show some similarities and differences in pollen morphological characteristics of the six *Pterospermum* species studied. All species have porate pollen grains and circular amb. The pollen morphology under SEM observations can be seen in Figure 1 and Figure 2. Meanwhile, the pollen morphology under LM observations is shown in Figure 3. Summary of variation found in this study is given in Table 1. Cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) on the six species of *Pterospermum* studied is shown in Figure 4.

Pollen morphological characteristics under LM and SEM

Pterospermum diversifolium (Figs. 1A, 2A, 3A-B)

Pollen size: large. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** porate. **P/E ratio:** 0.94. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Amb:** circular. **Exine thickness:** 1.36 (2.13 ± 0.38) 2.85 µm. **Exine ornamentation:** tectum echinate-granulate, microgranulate; microspines and baculum present on the exine surface; annulus (thickening of exine) presents clearly around the pore; spines uniformly distributed with indistinct perforated base, slightly rounded tip. **Exine thickness ratio (Exine/E):** 0.04. **Size:** measurements from polar view (P) 52.69 (58.14 ± 0.36) 62.88 µm, measurements from equatorial view (E) 59.63 (62.11 ± 0.28) 65.46 µm, spine length 2.50 (2.91 ± 0.15) 3.30 µm, spine width 1.89 (2.23 ± 0.13) 2.61 µm, distance between spine 2.35 (3.96 ± 0.47) 5.98 µm.

Pterospermum elongatum (Figs. 1B, 2B, 3C-D)

Pollen size: large. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** porate. **P/E ratio:** 1.01. **Shape:** prolate-spheroidal. **Aperture:** triporate. **Amb:** circular. **Exine thickness:** 1.32 (1.86 ± 0.35) 2.67 µm. **Exine ornamentation:** tectum echinate-granulate, microgranulate and microspines present; annulus

(thickening of exine) presents clearly around the pore; spines uniformly distributed with indistinct perforated base, slightly sharp tip. **Exine thickness ratio (Exine/E):** 0.03. **Size:** measurements from polar view (P) 58.24 (65.24 ± 0.57) 70.13 µm, measurements from equatorial view (E) 61.65 (64.74 ± 0.29) 67.86 µm, spine length 1.36 (2.00 ± 0.24) 2.90 µm, spine width 1.12 (1.47 ± 0.15) 1.81 µm, distance between spine 4.12 (7.22 ± 0.84) 11.47 µm.

Pterospermum jackianum (Figs. 1C, 2C, 3E-F)

Pollen size: medium-large. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** porate. **P/E ratio:** 0.97. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Amb:** circular. **Exine thickness:** 0.93 (2.53 ± 0.61) 3.27 µm. **Exine ornamentation:** tectum echinate-granulate-perforate, microgranulate; annulus (thickening of exine) presents clearly around the pore; spines uniformly distributed with indistinct perforated base, slightly rounded tip. **Exine thickness ratio (Exine/E):** 0.04. **Size:** measurements from polar view (P) 44.85 (54.15 ± 0.44) 60.45 µm, measurements from equatorial view (E) 49.74 (55.88 ± 0.41) 62.18 µm, spine length 1.76 (2.33 ± 0.17) 2.82 µm, spine width 1.43 (1.87 ± 0.18) 2.27 µm, distance between spine 3.67 (4.72 ± 0.42) 7.01 µm.

Pterospermum javanicum (Figs. 1D, 2D, 3G-H)

Pollen size: medium. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** porate. **P/E ratio:** 1.19. **Shape:** subprolate. **Aperture:** triporate. **Amb:** Convex triangular - circular. **Exine thickness:** 0.55 (0.93 ± 0.33) 1.53 µm. **Exine ornamentation:** tectum echinate-granulate, microgranulate; annulus (thickening of exine) presents clearly around the pore; spines uniformly distributed with indistinct perforated base, slightly oval tip. **Exine thickness ratio (Exine/E):** 0.02. **Size:** measurements from polar view (P) 37.02 (43.03 ± 0.61) 47.42 µm, measurements from equatorial view (E) 29.57 (36.28 ± 0.58) 42.79 µm, spine length 1.06 (1.82 ± 0.26) 2.36 µm, spine width 0.88 (1.04 ± 0.11) 1.36 µm, distance between spine 1.44 (2.61 ± 0.38) 3.95 µm.

Pterospermum lanceifolium (Figs. 1E, 2E, 3I-J)

Pollen size: medium. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** inaperturate. **P/E ratio:** 1.15. **Shape:** subprolate. **Aperture:** triporate. **Amb:** circular-spheroidal. **Exine thickness:** 0.55 (0.83 ± 0.38) 1.37 µm. **Exine ornamentation:** tectum echinate-granulate, microgranulate; annulus (thickening of exine) presents clearly around the pore; spines scarcely distributed with indistinct perforated base, slightly slender with almond shape tip. **Exine thickness ratio (Exine/E):** 0.02. **Size:** measurements from polar view (P) 38.76 (43.86 ± 0.39) 47.5 µm, measurements from equatorial view (E) 34.94 (38.22 ± 0.22) 40.01 µm, spine length 3.13 (4.50 ± 0.33) 5.73 µm, spine width 2.26 (2.63 ± 0.15) 3.12 µm, distance between spine 3.82 (5.28 ± 0.45) 7.40 µm.



Pterospermum subpeltatum (Figs. 1F, 2F, 3K-L)

Pollen size: large. **Polarity:** isopolar. **Dispersal unit:** monad. **Class:** porate. **P/E ratio:** 0.98. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Amb:** circular. **Exine thickness:** 1.64 (2.73 ± 0.41) 3.93 µm. **Exine ornamentation:** tectum echinate-granulate, microgranulate, annulus absent, spines uniformly distributed with indistinct perforated base, slightly sharp tip. **Exine thickness ratio (Exine/E):** 0.04. **Size:** measurements from polar view (P) 53.30 (61.52 ± 0.58) 69.55 µm, measurements from equatorial view (E) 56.29

(62.67 ± 0.48) 70.68 µm, spine length 1.43 (2.19 ± 0.33) 3.17 µm, spine width 1.40 (1.58 ± 0.09) 1.74 µm, distance between spine 3.29 (4.41 ± 0.59) 7.52 µm.

The unweighted pair-group method with arithmetic mean (UPGMA) was conducted using the pollen characteristics such as polar length, equatorial length, P/E ratio, pollen shape, aperture, amb, exine ornamentation, exine thickness ratio, pollen class, pollen size, spine length, spine width, spine shape, distance between spine and the presence of annulus. The dendrogram result is shown in Figure 4.

Dichotomous key of Pterospermum species identification is constructed using pollen morphological characteristics observed in this study

- 1a. Monad, triporate, isopolar, medium to large and large size pollen grains 2
- 1b. Monad, triporate, isopolar and medium size pollen grains 5
- 2a. Prolate-spheroidal pollen shape index ***P. elongatum***
- 2b. Oblate-spheroidal pollen shape index 3
- 3a. Absence of annulus around the pore of the pollen ***P. subpeltatum***
- 3b. Presence of annulus around the pore of the pollen 4
- 4a. Tectum echinate-granulate and microgranulate exine ornamentation ***P. diversifolium***
- 4b. Tectum echinate-granulate and perforate exine ornamentation ***P. jackianum***
- 5a. Convex triangular-circular pollen amb, average spine length on pollen less than 3.00 µm ***P. javanicum***
- 5b. Circular-spheroidal pollen amb, average spine length on pollen more than 3.00 µm ***P. lanceifolium***

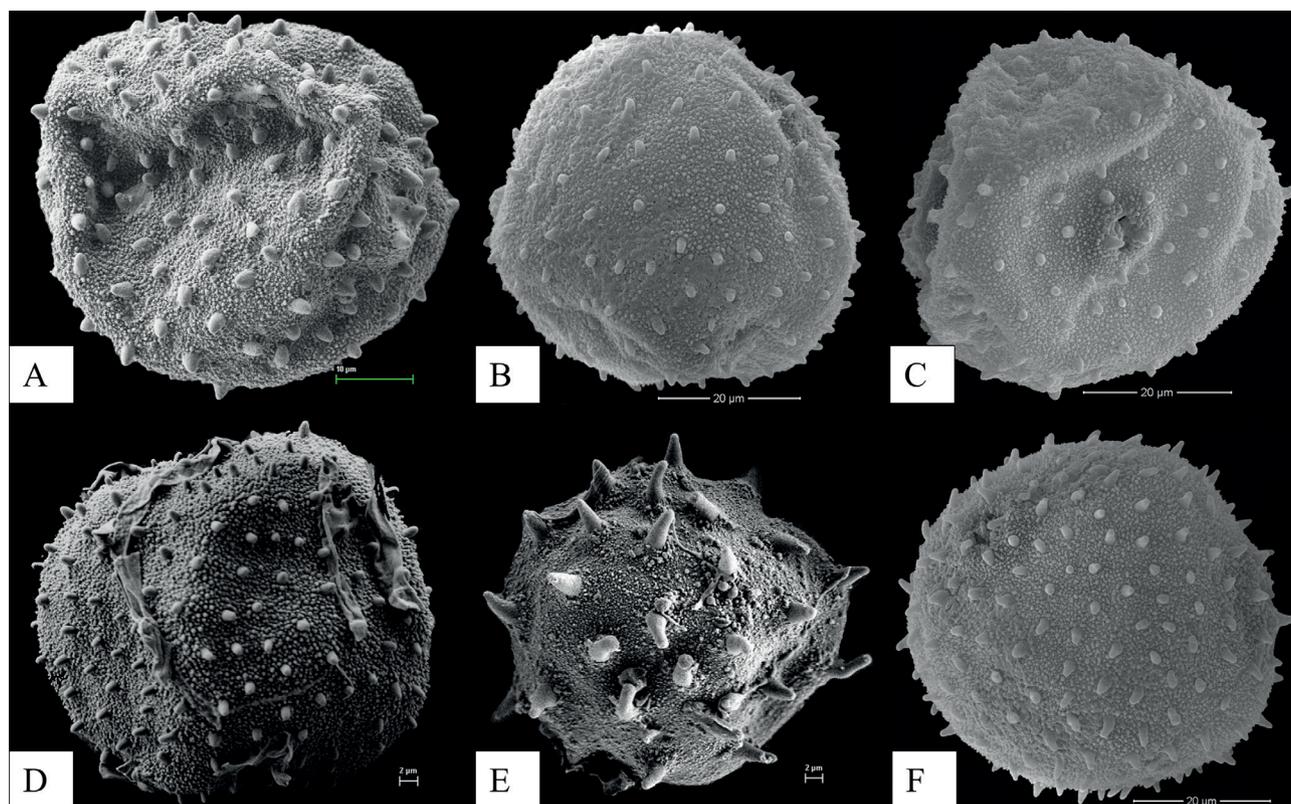


Figure 1. Pollen grains of *Pterospermum* (Malvaceae s.l.) under scanning electron microscope: **A)** *Pterospermum diversifolium*. **B)** *Pterospermum elongatum*. **C)** *Pterospermum jackianum*. **D)** *Pterospermum javanicum*. **E)** *Pterospermum lanceifolium*. **F)** *Pterospermum subpeltatum*.

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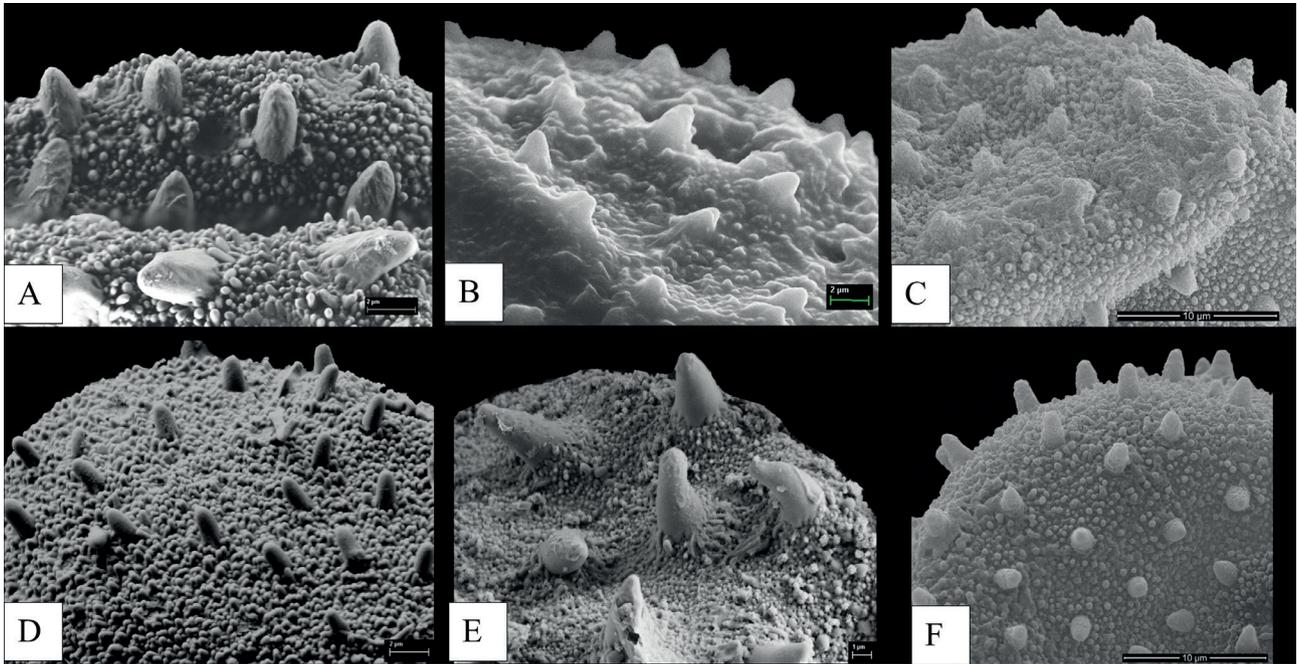


Figure 2. Pollen grains exine ornamentation of *Pterospermum* (Malvaceae s.l.) under scanning electron microscope: **A)** *Pterospermum diversifolium*. **B)** *Pterospermum elongatum*. **C)** *Pterospermum jackianum*. **D)** *Pterospermum javanicum*. **E)** *Pterospermum lanceifolium*. **F)** *Pterospermum subpeltatum*.

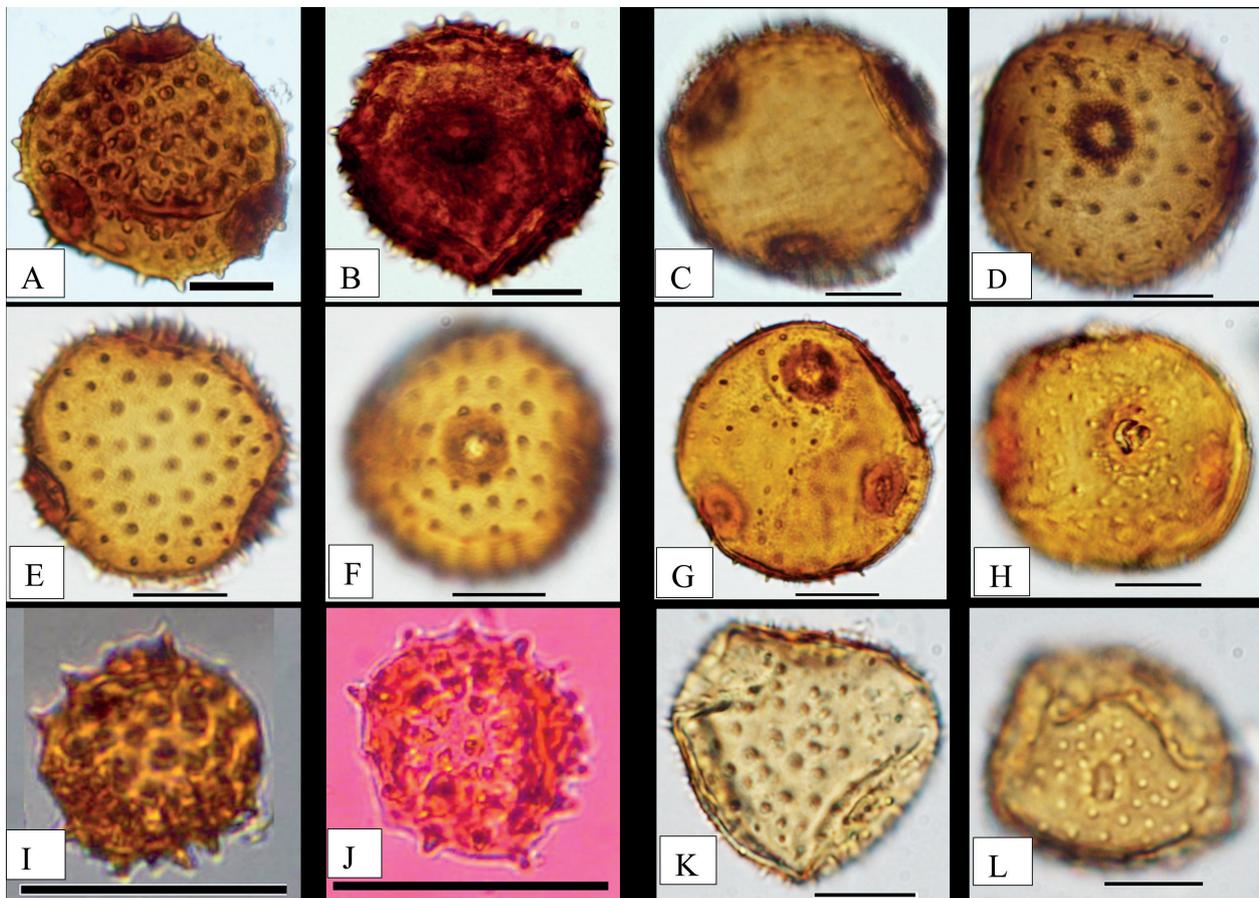


Figure 3. Polar and equatorial view of pollen of *Pterospermum* (Malvaceae s.l.) grains under light microscope: **A-B)** *Pterospermum diversifolium*. **C-D)** *Pterospermum elongatum*, **E-F)** *Pterospermum jackianum*, **G-H)** *Pterospermum javanicum*, **I-J)** *Pterospermum lanceifolium*. **K-L)** *Pterospermum subpeltatum* (Scale bar = **A-B, G-L** = 20 μm; **C-F** = 50 μm).



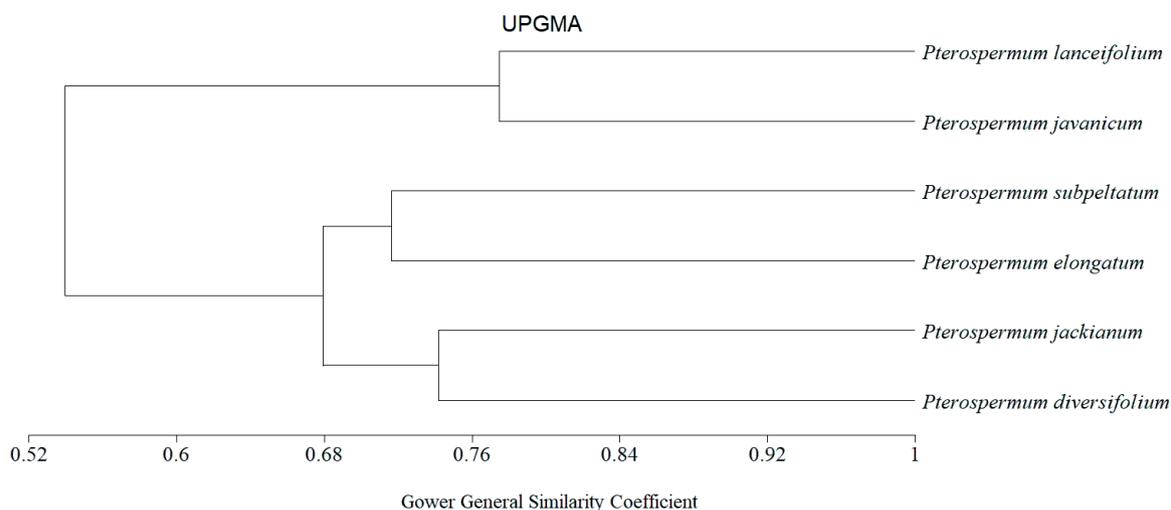


Figure 4. UPGMA cluster analysis on six species of *Pterospermum* using the pollen morphological characteristics.

Table 1. Summary of variations in pollen morphological characteristics in *Pterospermum* species studied.

Pollen morphology	<i>P. diversifolium</i>	<i>P. elongatum</i>	<i>P. jackianum</i>	<i>P. javanicum</i>	<i>P. lanceifolium</i>	<i>P. subpeltatum</i>
Class	Porate	Porate	Porate	Porate	Porate	Porate
Aperture	Triporate	Triporate	Triporate	Triporate	Triporate	Triporate
Shape	Oblate-spheroidal	prolate-spheroidal	Oblate-spheroidal	Subprolate	Subprolate	Oblate-spheroidal
Amb	Circular	Circular	Circular	Convex triangular - Circular	Circular - spheroidal	Circular
Size	52-65 μm (large)	*58-70 μm (large)	40 – 60 μm (medium to large)	30 – 48 μm (medium)	35 – 48 μm (medium)	50 – 70 μm (Large)
P/E value	0.94	1.01	0.97	1.19	1.15	0.98
Average spines length (μm)	2.79 \pm 0.27 μm	2.00 \pm 0.24	2.33 \pm 0.17	1.82 \pm 0.26	*4.50 \pm 0.33	2.19 \pm 0.33
Average Distance between spines (μm)	3.96 \pm 0.47	7.22 \pm 0.84	4.72 \pm 0.42	2.61 \pm 0.38	5.28 \pm 0.45	4.41 \pm 0.59
Exine ornamentation	Echinata-granulate, microgranulate, microspine and baculum present	Echinata-granulate, microgranulate and microspines present	Echinata-granulate-perforate, microgranulate	Echinata-granulate, microgranulate	Echinata-granulate, microgranulate	Echinata-granulate, microgranulate
Annulus	Present clearly around the pore	Present clearly around the pore	Present clearly around the pore	Present clearly around the pore	Present clearly around the pore	Absent
Exine thickness ratio (Exine/E)	0.04 (small)	0.03 (small)	0.04 (small)	0.02 (small)	0.02 (small)	0.04 (small)
Average equatorial diameter (μm)	59.63 (62.11 \pm 0.28) 65.46 μm	61.65 (64.74 \pm 0.29) 67.86	49.74 (55.88 \pm 0.41) 62.18	29.57 (36.28 \pm 0.58) 42.79	34.94 (38.22 \pm 0.22) 40.01	56.29 (62.67 \pm 0.48) 70.68
Average polar axis (μm)	52.69 (58.14 \pm 0.36) 62.88 μm	58.24 (65.24 \pm 0.57) 70.13	44.85 (54.15 \pm 0.44) 60.45	37.02 (43.03 \pm 0.61) 47.42	38.76 (43.86 \pm 0.39) 47.50	53.30 (61.52 \pm 0.58) 69.55

*Highest value

Discussion

All species were collected as dried samples and also in harmomegathy condition. Hence, most pollens appear to be flattened and dehydrated. Our findings demonstrated that the dispersal unit of the pollen grains is monad while the pollen polarity is isopolar. This is parallel with the study performed by Hamdy & Shams (2010), where the pollen grains of Sterculiaceae are monads, radially symmetrical,

isopolar as well as showing reliable variations in size, shape, exine sculpture and type of aperture. This is also supported by previous study on the genus *Byttneria* by Amirul-Aiman et al. (2019) where all species are monad.

The *Pterospermum* species can be separated on the basis of several characteristics such as exine ornamentation, pollen size and pollen shape. The absence of annulus on pollen of *P. subpeltatum* separates the species from other species. This character is considered as diagnostic characteristic that can be used for identification. In addition, the spine

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distribution can be used for delimitation of species. The presence of microspines on pollen exine of *P. elongatum* and the presence of baculum on pollen exine of *P. diversifolium* act as diagnostic characteristics to separate the species from others. Notably, the average spine length of *P. lanceifolium* is the longest among *Pterospermum* in this study. This character is useful to delimitate this species from other species.

One class of pollen is classified for all *Pterospermum* species studied which is porate. The aperture for these porate pollens is trizonoporate or triporate. This is in line with the study done by Hamdy & Shamsou (2010), where the subfamily Dombeyoideae consists of trizonoporate or triporate pollen aperture. Besides, the pollen shape ranges from oblate-spheroidal to subprolate. Based on the pollen shape classified by Erdtman (1952), the pollen shape is determined using ratio between polar axis and equatorial diameter of the pollen. Previous study on other genus in Sterculiaceae s.l. revealed that the pollen structure of *Byttneria* is triporate with small to medium size of pollen, as the shape of pollen ranges from oblate to spheroidal (Amirul-Aiman *et al.* 2019).

Hamdy & Shamsou (2010) mentioned that the pollen size of Dombeyoideae is large. In this study, the pollen of *P. elongatum* has the largest pollen size among these six species while *P. javanicum* has the smallest pollen size. However, the pollen size in this study ranges from 30 – 70 μm , where it falls under the range of medium, medium to large and large in size. According to Erdtman (1946), pollen size is considered as small when its size is below than 25 μm .

Furthermore, this study discovered that the sizes of pollen grains under LM and SEM are different. The size of pollen grains viewed under SEM is smaller than the size viewed under LM. This might be due to the shrinking or reduction in size of pollen grains during the acetolysis procedure and critical point drying procedure prior to SEM observation (Mohd-Arrabe' & Noraini 2013). Wodehouse effect or generally known as harmomegathic effect is also the reason why the pollen sizes differ under LM and SEM. Pollen morphology can change to accommodate the pressure changes within the pollen grains in response to dry and wet conditions (Hesse *et al.* 2009). Phumphumirat *et al.* (2018) studied on the pollen morphology and harmomegathic character of *Gnetum* species. They found that the sizes of dehydrated and hydrated pollen were different. Similarly, the pollen size of *Byttneria* was affected by the harmomegathic effect as reported by Amirul-Aiman *et al.* (2019).

The pollen exine ornamentations found in this study are echinate-granulate, microgranulate and perforate. Hesse *et al.* (2009) stated that this type of echinate exine ornamentation consists of pointed element that is longer or wider than 1 μm . Punt *et al.* (2007) and Erdtman (1952) suggested the term 'spinose' to define spines that are longer than 3 μm and 'spinules' for the smaller characters. Our findings also supported the study by Hamdy & Shamsou (2010) that reported the echinate-granulate exine

ornamentation of *Pterospermum acerifolium*. There is also a variation between the distances of spines for each studied species. The pollen of *P. elongatum* has the highest average spine distance while *P. javanicum* has the shortest one. This character can also be used as species classification and identification tool.

Based on the cluster analysis performed using the pollen characteristics, it was found that two clusters are separated at the similarity coefficient of 0.54. *P. lanceifolium* is closer to *P. javanicum* with similarity coefficient of 0.775 but they are separated from other four species. This might be because these two species share the same pollen size group which is medium size pollen as compared to other *Pterospermum*. They also have the same pollen shape which is subprolate. The other cluster consists of pollen with circular amb and large pollen size except *P. jackianum* (medium to large in pollen size). In this second cluster, two subclads are formed. *P. subpeltatum* and *P. elongatum* are separated from *P. jackianum* and *P. diversifolium* with similarity coefficient of 0.679. We predict that this two subclads are divided due to the tip shape of the spine. Both *P. subpeltatum* and *P. elongatum* have slightly sharp spine tip while *P. jackianum* and *P. diversifolium* have slightly round spine tip.

Perveen & Qaiser (2009) in their findings on pollen study of two species of *Melhanina* (Dombeyoideae) explained that their two species were divided into two pollen types based on the polar length. They also explained that the pollen of Dombeyoideae is more or less stenopalynous where the pollen grains are usually triporate, spheroidal-sub-oblate along with echinate tectum with the variation in polar length. Previously, the family Sterculiaceae has been divided by Erdtman (1952) into eight pollen types which are Eriolaeneae, Fremontodendreae, Dombeyae, Hermanniae, Byttneriae, Lasiopetaleae, Helicterterae and Sterculiae.

On the basis of our data, we conclude that several pollen characters can be considered as the diagnostic characteristics in pollen delimitation. The results obtained also show that pollen morphological characteristics have some taxonomic values that can be used especially in identification and differentiation of species in *Pterospermum*. Further study should be conducted to understand the differences and similarities of more species under the same genus and subfamilies. More species and genus under the Malvaceae need to be added in order to support or discard the treatment of Sterculiaceae, Tiliaceae, Bombacaceae and Malvaceae as a separate family rather than the merging of these families based on molecular data, as observed by Judd & Manchester (1997), Bayer *et al.* (1999) and Bayer & Kubitzki (2003). In conclusion, this study has shown that pollen morphological characteristics of *Pterospermum* have taxonomic values that can be used especially in identification and differentiation of species in subfamily Dombeyoideae as well as in the family of Malvaceae s.l..



Acknowledgements

We thank Herbarium of Forest Research Institute Malaysia, Kepong (KEP) and Herbarium Royal Botanic Garden, Kew (Kew) for samples and Faculty of Science and Technology, Universiti Kebangsaan Malaysia for providing the facilities to conduct this study. We also thank Scanning Electron Microscopy Unit FST UKM and Centre of Research Institute and Management (CRIM). Authors also thank RIGS 16-095-0259 Grant (IIUM), GUP-2017-035 (UKM) and GP-IPM/2021/9697300 for the funding provided. Special thanks for Ministry of Higher Education Malaysia and Universiti Putra Malaysia.

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