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POLLEN MORPHOLOGY AND HARMOMEGATHIC CHARACTERS OF *Byttneria* LÖFL. SPECIES (STERCULIACEAE *S. S:* SUBFAM. BYTTNERIOIDEAE)

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ABSTRACT

A palynological study was conducted on seven species of *Byttneria* Löfl. The objectives of this study was to understand the variation in micromorphological and harmomegarthic characteristics of pollen in *Byttneria* species in Malaysia and Singapore. In doing so, more information on the species of Sterculiaceae *s.s* family can be added. Dried pollen samples of seven *Byttneria* species were selected in this study, namely *B. scabrida* Ridl.*, B. pilosa* Roxb.*, B. elliptica* Pohl*, B. curtisii* Oliv.*, B. reinwardtii* Korth.*, B. maingayi* Mast. and *B. jackiana* Wall. Methods involved were acetolysis techniques, single-grain technique, and observation under light microscope and scanning electron microscope. Common characters shared by all studied species were porate pollen class, triporate aperture, and triangular outline. Variation in pollen characteristics includes ratio of exine thickness, pollen size, pore width and height, amb; shape and measurements. These characters are valuable in assisting identification of taxa group that can be used to differentiate between species in the genus. This study proved that pollen morphological characteristics of *Byttneria* have taxonomic values in identification and differentiation of species in subfamily Byttnerioideae and Sterculiaceae *s. s.*

Key words: Palynology, pollen micromorphology, *Byttneria*, Byttnerioideae, Sterculiaceae

INTRODUCTION

Palynology is the study on the structural and applied features of pollens. Pollen grains are the male reproductive structures that are produced by angiosperms (flowering plants) or gymnosperms (naked seeded plants) (Agashe & Coulton 2009). Hyde and Williams (1944) introduced the term palynology as the study of pollen, spore, and any other biological materials using palynological methods or other applications.

According to Payne (1972), pollen grain is a structure unit that is subjected to rapid change in size and shape through the process of loss and uptake of water. Harmomegathy refers to the mechanism that permits the respective change in the shape and size of pollen grains by varying the hydration status. Pollen and spores are characterized by highly variable pollen morphological features, which would assist in precise species identification

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(Agashe & Coulton 2009). Huang (1972) also stated that pollen morphology studies are very useful in many fields such as paleobotany, aeropalynology, forensics science, study on allergies, pharmacopalynology, archaeology, melisopalynology and plant taxonomy.

Previously, studies on pollen morphology of family Sterculiaceae have been conducted by many researchers including Wodehouse (1935), Rao (1950), Erdtman (1952), El-Husseini (2006), Perveen and Qaiser (2009) & Hamdy and Shamso (2010). *Byttneria* is a species in this family with about 130 species can be found mostly in tropical America, South East Asia, Africa, China and Madagascar (Tang *et al*., 2007). Six species can be found in Peninsular Malaysia which are *B. jackiana* Wall., *B. beccarii* Warb., *B. maingayi* Mast., *B. curtisii* Oliv., *B. mastersii* Cristobal and *B. pilosa* Roxb. (Turner 1995).

Characteristics of *Byttneria* leaf venation and trichome has been described by Nurshahidah *et al*. (2013). Study on the pollen of this genus in Malaysia however has not been conducted. Therefore, in this study, pollen from seven species of *Byttneria (B. scabrida*, *B. pilosa*, *B. elliptica*, *B. curtisii*, *B. reinwardtii*, *B. maingayi* and *B. jackiana)* were investigated using observation under light microscope (LM) and scanning electron microscopy (SEM) with emphasis on the morphological and harmomegathic characteristics.

MATERIALS AND METHODS

Dried pollen samples from seven *Byttneria* species of Malaysia namely *B. scabrida*, *B. pilosa*, *B. elliptica*, *B. curtisii*, *B. reinwardtii*, *B. maingayi* and *B. jackiana* were used. Samples were provided by Forest Research Institute Malaysia Herbarium (KEP), Singapore Botanical Garden Herbarium (SING) and Kew Botanical Garden Herbarium (K) based on the availability of flower's bud (Table 1). The pollen samples were acetolysed according to Erdtman (1969), followed by observation under scanning electron microscope (Model Carl Zeiss Supra 55vp) and also photographed under light microscope using digital camera (Olympus BX43F) mounted on the Olympus microscope DP72 (Olympus Soft Imaging Solutions) using Cell B Software under 60× and 100× magnifications.

Pollen preparation

Anthers were dissected under dissecting microscope and separated into individual microcentrifuge tubes. Acetolysis mixture of nine parts acetic anhydride and one part of concentrated sulphuric acid were added to the samples and boiled for 5-10 minutes until the pollen turned dark and the mixture turned into brown solution. The anthers were then crushed using glass rod. After the mixture was cooled down, the samples were centrifuged. The residues were washed in acetic acid and water before glycerine was added into the sample to form a suspension.

Single-Grain technique

Single-grain technique following Ferguson *et al*. (2007) was used to get the best pollen position under LM. The same pollen was then transferred onto the SEM stub with a drop of absolute ethanol to remove all traces of glycerine for gold coating and viewing under SEM. The description of pollen morphologies followed Erdtman (1969), Faegri and Iversen (1992), Punt *et al.* (2007) and Hesse *et al.* (2009).

Table 1. List of studied species and respective source

Species	Sources
B. scabrida	Richards, P.W., 1292, 15.08.1932, Ulu Tinjar, Sarawak.
B. pilosa	Ridley, 14697, 24.01.1910, Hulu Terengganu, Terengganu. Evans, I.H.N., s.n. June 1917, Kuala Tekam, Pahang.
B. elliptica	Chew Wee Lek, CWL 56, 28.03.1957, Jalan Kelantan, Terengganu.
B. curtisii	Kochummen, K.M., FRI 16675, 26.03.1973. Bentong, Pahang. Chew, M.Y., FRI 53663, 4.3.2007, Gombak, Selangor. Symington, C.F., FMS 24065, 24.3.1930, Hulu Langat, Selangor.
B. reinwardtii	Fidilis, K., SAN 130009, 25.05.1992, Nabawan, Sabah. Madani, L., SAN 133923, 15.04.1992, Lahad Datu, Sabah.
B. maingayi	Gardette, E., E.G 1510, 6.02.1996, Jelebu, Negeri Sembilan. Leong, T.M., SING 2009-167, 26.02.2009, Bukit Kalang, Singapore. Leong, P., SING 2009-155, 17.02.2009, Mac Ritchie Reservoir, Singapore.
B. jackiana	Everett, B., FRI 13692, 26.11.1969, Pendang, Kedah. Wilkie, No 16874, 10.11.1924, Raub, Pahang.

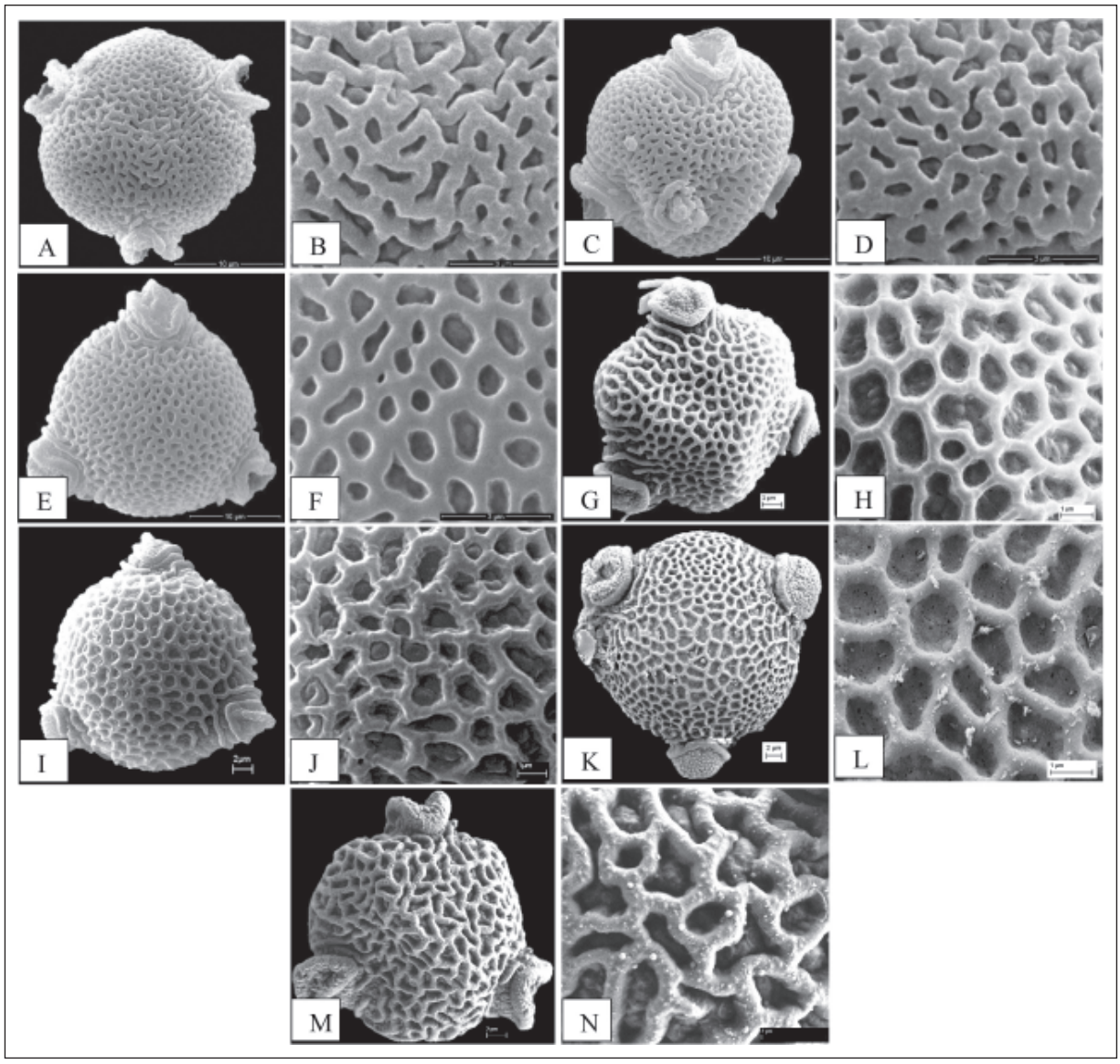

Fig. 1. Pollen grains under SEM and exine ornamentation: A-B) *B. scabrida.* C-D) *B. pilosa*, E-F) *B. elliptica*, G-H) *B. curtisii,* I-J) *B. reinwardtii*, K-L) *B. maingayi*, M-N) *B. jackiana*.

RESULTS AND DISCUSSION

Pollen morphological characteristics under SEM and LM

The pollen morphology as viewed under SEM and LM is described below with illustrations shown in Figures 1 and 2. Similarities and differences in pollen morphological characterictics of the *Byttneria* species are tabulated in Tables 2–5.

***B. scabrida* [Figure 1A & B, Figure 2 i-iv]**

Class: porate. **P/E ratio:** 1.02. **Shape:** prolate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** rounded. **Exine thickness:** 0.77 (1.12 ± 0.28) 1.93 µm. **Exine ornamentation:** reticulate. **Lumen diameter:** 0.24 (0.585 ± 0.34) 1.24 µm. **Murus diameter:** 0.43(0.488 ± 0.06) 0.59 µm. **Pore height:** 2.04 (3.01 ± 0.33) 4.16 µm**. Pore width:** 2.20 (3.11 ± 0.39) 4.31 µm. **Size:** measurements from polar view (P) 21.14 (23.85 ± 0.39) 26.48 µm, measurements from equatorial view (E) 20.25 (23.30 ± 0.37) 25.80 µm.

***B. pilosa* [Figure 1C & D, Figure 2 v-viii]**

Class: porate. **P/E ratio:** 0.95. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** triangular. **Exine thickness:** 1.07 (1.56 ± 0.23) 2.32 µm. **Exine ornamentation:** reticulate to foveolate. **Lumen diameter:** 0.30 (0.72 ± 0.40) 1.37 µm**. Murus diameter:** 0.44 (0.58 ± 0.10) 0.68 µm. **Pore height:** 2.13 (4.30 ± 1.01) 6.23 µm. **Pore width:** 2.59 (3.90 ± 0.54) 5.41 µm. **Size:** measure-

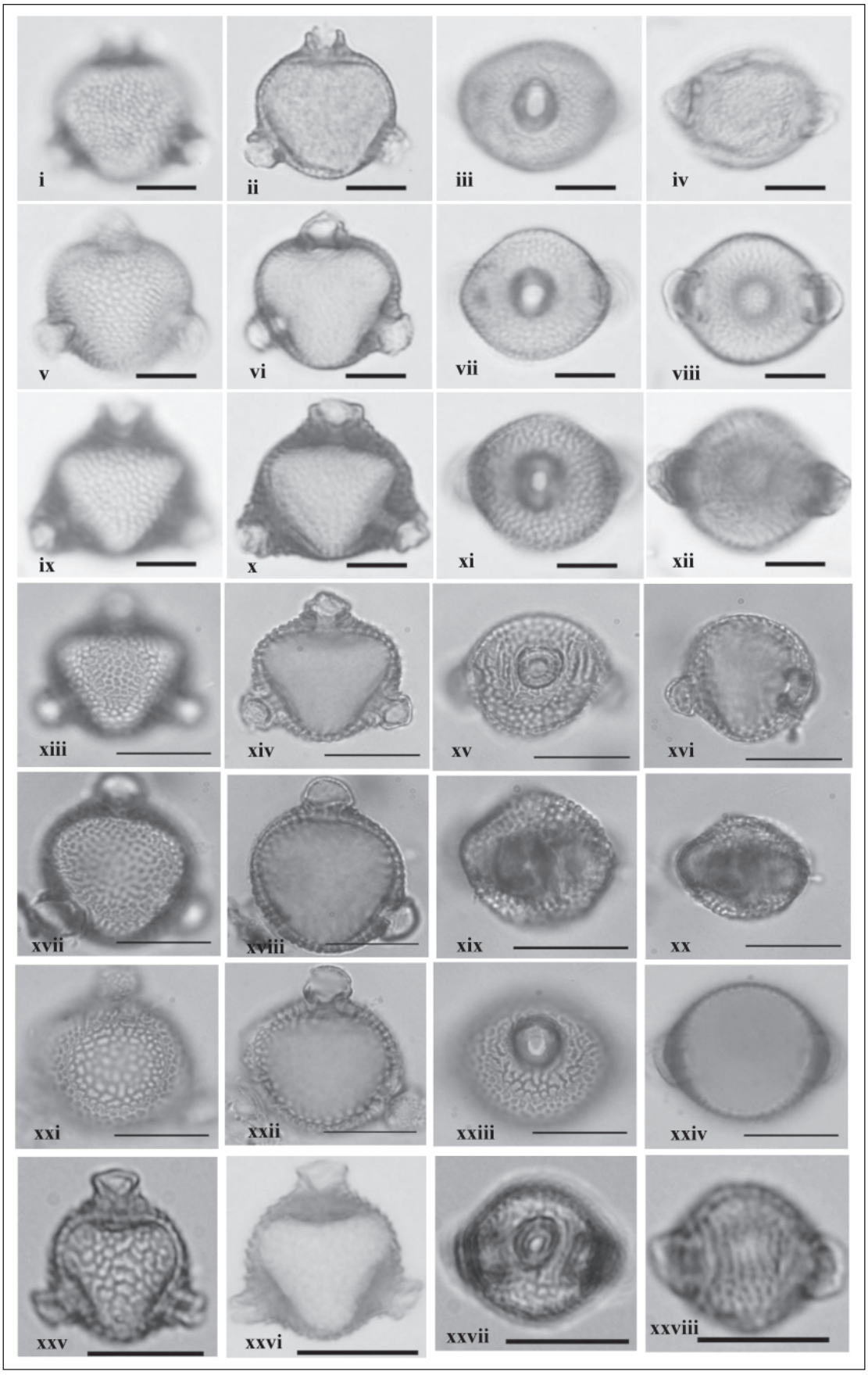

Fig. 2. Pollen grains under LM: i-iv) *B. scabrida* (Scale bar 10 µm), v-viii) *B. pilosa* (Scale bar 10 µm), ix-xii) *B. elliptica* (Scale bar 10 µm), xiii-xvi) *B. curtisii* (Scale bar 20 µm), xvii-xx) *B. reinwardtii* (Scale bar 20 µm), xxi-xxiv) *B. maingayi* (Scale bar 20 µm), xxv-xxviii) *B. jackiana* (Scale bar 20 µm).

Table 2. Pollen class, size, and amb of *Byttneria* species studied

Species	Pollen class	Pollen size	Amb
B. scabrida	Triporate	20 – 26 µm (small – medium)	Rounded
B. pilosa	Triporate	22 – 27 µm (small – medium)	Triangular
B. elliptica	Triporate	25 – 30 µm (small – medium)	Triangular
B. curtisii	Triporate	20 – 32 µm (small – medium)	Triangular
B. reinwardtii	Triporate	21 – 36 µm (small – medium)	Rounded
B. maingayi	Triporate	27 – 36 µm (medium)	Rounded
B. jackiana	Triporate	23 – 39 µm (small – medium)	Rounded

Table 3. P/E value of *Byttneria* species studied

Species	P mean value (µm)	E mean value (µm)	P/E	Pollen shape
B. scabrida	21.14 (23.85) 26.48	20.25 (23.30) 25.80	1.02	Prolate-spheroidal
B. pilosa	23.05 (24.42) 25.21	22.33 (25.63) 26.73	0.95	Oblate-spheroidal
B. elliptica	22.54 (24.58) 27.06	25.18 (27.20) 30.64	0.90	Oblate-spheroidal
B. curtisii	20.63 (27.95) 29.76	20.71 (26.44) 31.22	1.06	Prolate-spheroidal
B. reinwardtii	24.00 (24.89) 26.65	21.72 (27.78) 35.02	0.90	Oblate-spheroidal
B. maingayi	30.14 (32.45) 34.29	27.71 (31.81) 35.83	1.02	Prolate-spheroidal
B. jackiana	22.18 (23.88) 26.96	23.85 (25.42) 28.82	0.94	Oblate-spheroidal

P = Length at polar view, E = Length at equatorial view.

Table 4. Exine thickness of *Byttneria* species studied

Species	Mean value (µm)	Minimum thickness (µm)	Maximum thickness (µm)	Value of exine thickness (Exine/P)	Value ratio (Exine/P)
B. scabrida	1.12	0.77	1.93	0.047	< 0.050 (small)
B. pilosa	1.56	1.07	2.32	0.064	0.05–0.1 (medium)
B. elliptica	1.85	1.27	2.73	0.075	0.05–0.1 (medium)
B. curtisii	2.32	1.59	3.26	0.083	0.05–0.1 (medium)
B. reinwardtii	2.32	1.55	3.16	0.071	0.05–0.1 (medium)
B. maingayi	2.23	1.6	3.14	0.069	0.05–0.1 (medium)
B. jackiana	2.68	1.54	3.96	0.112	0.1–0.25 (big)

ments from polar view (P) 23.05 (24.42 ± 0.12) 25.21 µm, measurements from equatorial view (E) 22.33 (25.63 ± 0.21) 26.73 µm.

***B. elliptica* [Figure 1E & F, Figure 2 ix-xii]**

Class: porate. **P/E ratio:** 0.90. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** triangular. **Exine thickness:** 1.27 (1.85 ± 0.39) 2.73 µm. **Exine ornamentation:** reticulate to foveolate. **Lumen diameter:** 0.41 (0.97 ± 0.26) 1.42 µm. **Murus diameter:** 0.43 (0.63 ± 0.15) 0.82 µm. **Pore height:** 2.27 (4.47 ± 0.44) 5.62 µm. **Pore width:** 1.27 (1.85 ± 0.39) 2.73 µm. **Size:** measurements from polar view (P) 22.54 (24.58 ± 0.30) 27.06 µm, measurements from equatorial view (E) 25.18 (27.20 ± 0.40) 30.64 µm.

***B. curtisii* [Figure 1G & H, Figure 2 xiii-xvi]**

Class: porate. **P/E ratio:** 1.06. **Shape:** prolate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** triangular **Exine thickness:** 1.59 (2.32 ± 0.32) 3.26 µm. **Exine ornamentation:** reticulate, perforate. **Lumen diameter:** 0.86 (1.13 ± 0.22) 1.44 µm. **Murus diameter:** 0.34 (0.56 ± 0.19) 0.73 µm. **Pore height:** 2.25 (3.37 ± 0.34) 4.41 µm. **Pore width:** 5.23 (5.72 ± 0.13) 6.11 µm. **Size:** measurements from polar view (P) 20.63 (27.95 ± 0.48) 29.76 µm, measurements from equatorial view (E) 20.71 (26.44 ± 0.93) 31.22µm.

***B. reinwardtii* [Figure 1I & J, Figure 2 xvii-xx]**

Class: porate. **P/E ratio:** 0.90. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Outline:** triangular.

Ambiture: rounded. **Exine thickness:** 1.55 (2.32 ± 0.37) 3.16 µm. **Exine ornamentation:** reticulate. **Lumen diameter:** 0.87 (2.05 ± 0.42) 3.15 µm. **Murus diameter:** 0.42 (0.64 ± 0.20) 0.88 µm. **Pore height:** 2.75 (4.17 ± 0.40) 5.58 µm. **Pore width:** 4.72 (6.27 ± 0.45) 8.76 µm. **Size:** measurements from polar view (P) 24.00 (24.89 ± 0.18) 26.65 µm, measurements from equatorial view (E) 21.72 (27.78 ± 0.86) 35.02 µm.

***B. maingayi* [Figure 1K & L, Figure 2 xxi-xxiv]**

Class: porate. **P/E ratio:** 1.02. **Shape:** prolate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** rounded. **Exine thickness:** 1.6 (2.23 ± 0.29) 3.14 µm. **Exine ornamentation:** reticulate, perforate. **Lumen diameter:** 1.6 (2.23 ± 0.29) 3.14 µm**. Murus diameter:** 0.4 (0.66 ± 0.17) 0.85 µm. **Pore height:** 3.91 (5.61 ± 0.54) 8.61 µm. **Pore width:** 5.88 (7.00 ± 0.26) 8.43 µm. **Size:** measurements from polar view (P) 30.14 (32.45 ± 0.24) 34.29µm, measurements from equatorial view (E) 27.71 (31.81 ± 0.52) 35.83µm.

***B. jackiana* [Figure 1K & L, Figure 2 xxv-xxviii]**

Class: porate. **P/E ratio:** 0.94. **Shape:** oblate-spheroidal. **Aperture:** triporate. **Outline:** triangular. **Ambiture:** rounded. **Exine thickness:** 1.54 (2.68 ± 0.56) 3.96 µm. **Exine ornamentation:** reticulate. **Lumen diameter:** 1.22 (1.73 ± 0.44) 3.11 µm. **Murus diameter:** 0.66 (0.82 ± 0.15) 1.10 µm. **Pore height:** 2.80 (4.62 ± 0.42) 5.95 µm. **Pore width:** 2.87 (5.42 ± 0.55) 6.79 µm. **Size:** measurements from polar view (P) 22.18 (23.88 ± 0.34) 26.96 µm, measurements from equatorial view (E) 23.85 (25.42 ± 0.43) 28.82 µm.

The pollen of seven species of *Byttneria* from this study is monad with the dispersal unit of pollen consists of only a single pollen grain. This observation fits the description of pollen of Sterculiaceae which are monads, radially symmetrical, isopolar and showing reliable variations in shape, size, exine sculpture and aperture type previously described by Hamdy and Shamso (2010).

Pollen class for the studied species is porate with the aperture of trizonoporate or triporate. According to Hesse *et al.* (2009), pollen class is an artificial grouping of pollen grains that shares a single distinctive character. Pollen sizes of these species are deduced by following the size of pollen described by Hesse *et al.* (2009). In this study, the pollen for all species ranged from small to medium except for *B. maingayi* with medium sized pollen. The smallest pollen size measured in this study is shown by *B. scabrida* and *B. curtisii* with approximately 20 µm and the largest is recorded in *B. reinwardtii* and *B. maingayi* with some pollen reached more than 35 µm. The sizes in both SEM and LM differs in all studied species. The size under SEM is comparatively smaller than under LM. Mohd-Arrabe' and Noraini (2013) stated that the smaller pollen size viewed under SEM was due to the extra procedure involved when processing same pollen previously viewed by LM.

Since all the studied species were dried specimens collected from the herbaria and also quite old, the pollen structures and sizes might get affected by the dehydration process. This is known as harmomegathic effects. Wodehouse (1935) described the term harmomegathy as the alterations of pollen form or the changes in the degree of hydrations of pollen grains. According to Payne (1972), harmomegathic effect occurs in all parts of the pollen walls, not only on the apertures. The thin aperture membranes will usually show the more obvious change. Since the pollen of *Byttneria* species do not comprise of any obvious thin apertures or colpus, the harmomegathic effects occur on the pollen walls in some species thus making the pollen measurement quite different from one another even in the same species.

In this study, we discovered three types of exine sculpturing or ornamentation for these species which are reticulate, reticulate to foveolate, and reticulate-perforate. *B. scabrida, B reinwardtii* and *B. jackiana* have reticulate exine, while *B. pilosa* and *B. elliptica* displayed reticulate to foveolate exine, whereas *B. curtisii* and *B. maingayi* comprise of reticulate-perforate exine sculpturing. According to Hesse *et al.* (2009), reticulate exine ornamentation is a network-like pattern formed by exine elements which is the muri, where the lumina is usually wider than 1 µm. Foveolate refers to the pollen wall with foveolae, roundish lumen more than 1 µm in diameter with distance between two adjacent lumina is larger than their diameter. This is a contrast with the exine sculpturing of the *Waltheria*, another genus under the subfam. Byttnerioideae. In the study conducted by Saba and dos Santos (2015), they found the exine sculpturing for this genus to be suprareticulate and microechinate to echinate. Five of the *Byttneria* species in this study showed medium value ratio of exine thickness while in *B. scabrida* and *B. jackiana* showed small ratio value and big ratio value of exine thickness respectively. Exine ornamentation is very useful in taxonomical study. A study by Talip (2008) managed to differentiate *Shorea* species by using the exine ornamentation characteristics.

By following the pollen shapes classification by Erdtman (1952), we found that the shape of these species comprises of oblate-spheroidal and prolate-spheroidal shape. The pollen shape determination was done using the ratio between the polar axis and equatorial diameter of the pollen. As the P/E index of these seven species are 0.90, 0.90, 0.94, 0.95, 1.02,

1.02 and 1.06 for *B. elliptica, B. reinwardtii, B. jackiana, B. pilosa, B. scabrida, B. maingayi* and *B. curtissi* respectively, the indexes fall under the range of 0.88–1.00 and 1.00–1.14 in the classification, thus considered as oblate-spheroidal and prolate-spheroidal shape.

Dichotomous key of *Byttneria* species identification was constructed using pollen morphological characteristics as below:

1a. Porate class, triporate aperture, amb rounded..... 2
1b. Porate class, triporate aperture, amb triangular 5
2a. Exine ornamentation reticulate-perforate..... ..... *B. maingayi*
2b. Exine ornamentation reticulate..... 3
3a. Pollen shape prolate-spheroidal..... *B. scabrida*
3b. Pollen shape oblate-spheroidal..... 4
4a. Ratio value of exine thickness; medium (0.05-0.1)..... *B. reinwardtii*
4b. Ratio value of exine thickness; big (0.1-0.25) ..... *B. jackiana*
5a. P/E value more than 0.1..... *B. curtisii*
5b. P/E value less than 0.1..... 6
6a. Pollen size ranges from 22 – 27 µm..... *B. pilosa*
6b. Pollen size ranges from 25 – 30 µm..... ..... *B. elliptica*

CONCLUSIONS

This study showed that pollen morphological characteristics of *Byttneria* has taxonomic values especially for the identification and differentiation of species in subfamily Byttnerioideae as well as in the family of Sterculiaceae *s.str*. More species and genera under the Sterculiaceae need to be added in order to support or discard the treatment of Sterculiaceae as a separate family rather than its merging to Malvaceae with other families such as Tiliaceae and Bombacaceae as observed by Judd and Manchester (1997), Bayer *et al.* (1999) and Bayer and Kubitzki (2003) based on molecular data.

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