

Pollen morphology of Lecythidaceae in Southeast Asia

WORANART THAMMARONG^{1,2}, PRANOM CHANTARANOTHA³,
JOHN A.N. PARNELL⁴, TREVOR R. HODKINSON⁴ & PIMWADEE PORNPONGRUNGRUENG^{1,*}

ABSTRACT

The pollen morphology of four genera and 33 taxa of Lecythidaceae in Southeast Asia was investigated, including 26 taxa of *Barringtonia*, one taxon each of *Careya* and *Chydenanthus*, and five taxa of *Planchonia* to determine which, if any, taxonomically important characters were present and the implications they have for the systematics of the family. Acetolysed and unacetolysed pollen samples were investigated using light and scanning electron microscopy (SEM). The pollen grains were found to be monads, radially symmetrical, isopolar, small to medium-sized, syntri-colpate or syntri-colporate, prolate spheroidal, oblate spheroidal, subprolate, suboblate or spherical in shape with marginal ridges. Marginal grooves and polar cushions are commonly present in most species. The polar ectoaperture may be open or sealed. The mesocolpial sculpturing is perforate-reticulate. The colpal surface is smooth, with sparsely or densely scattered verrucae-gemmae and with clavate to pilate elements scattered or aligned in longitudinal rows. The results indicated that pollen morphological characters can be used for identification and classification of some closely related species in the genus *Barringtonia*.

KEYWORDS: *Barringtonia*, Brazil nut family, *Careya*, *Chydenanthus*, *Planchonia*, pollen, taxonomy

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INTRODUCTION

The family Lecythidaceae is placed in the order Ericales (APG, 1998, 2016; Schönberger *et al.*, 2005; Reveal & Chase, 2011). It is a family of small to large trees, shrubs or rarely herbs with 22 genera and 325 species (Prance, 2012), distributed mainly in the moist lowland Neotropics, tropical west and east Africa and tropical Asia to north Australia (Prance & Mori, 2004). Ecologically, many species are found in swampy forest areas, montane evergreen and mixed deciduous forests, while a few species occur in open areas. They can be found from sea level to 3,500 m in altitude (Thammarong, 2017). The family is characterised by alternate simple leaves, racemose or panicle inflorescences or solitary flowers that are hermaphroditic, actinomorphic or zygomorphic with numerous stamens connate at

base into a short or long staminal ring and an inferior or half-inferior ovary.

The classification of the family remains uncertain: There is a conflict in the classifications of the APG (2003, 2016), that included the Napoleonaceae and Scytopetalaceae in the Lecythidaceae, and Prance and Mori (2004) who recognised the three as distinct families and divided the Lecythidaceae into three subfamilies; Barringtonioideae (Planchonioideae), Foetidoideae and Lecythidoideae. The latter system was supported by molecular work (Mori *et al.*, 2007). Only subfamily Barringtonioideae occurs in Southeast Asia, where it contains about 70 species in five genera (Thammarong, 2017). Taxonomic information on Southeast Asian Lecythidaceae is, unfortunately, limited to considerations of species diversity and

¹ Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

² Botanical Garden Organization, Queen Sirikit Botanic Gardens, P.O. Box 7, Mae Rim, Chiang Mai 50180, Thailand.

³ Department of Biology and Center of Excellence on Biodiversity (BDC), Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

⁴ Herbarium, Botany Department, School of Natural Sciences, Trinity College Dublin, the University of Dublin and Trinity Centre for Biodiversity Research, Trinity College Dublin, the University of Dublin, Dublin 2, Ireland.

* Corresponding author: ppimwa@kku.ac.th

morphology. This is problematic, as the circumscription of some complex groups of Lecythidaceae taxa based on morphology may be exceptionally difficult (Prance & Mori, 1979; Mori & Prance, 1990; Prance & Kartawinata, 2013). In other words, gross morphology by itself is insufficient to resolve the taxonomic problems in this family. However, pollen morphological characteristics have proven to be diagnostic at species level in some genera in Lecythidaceae such as *Allantoma* Miers, *Barringtonia* J.R.Forst. & G.Forst., *Bertholletia* Bonpl., *Careya* Roxb., *Cariniana* Casar., *Chydenanthus* Miers, *Couratari* Aubl., *Corythophora* R.Knuth, *Couroupita* Aubl., *Crateranthus* Baker f., *Eschweilera* Mart. ex DC., *Foetidia* Comm. ex Lam., *Grias* L., *Gustavia* L., *Lecythis* Loefl., *Petersianthus* Merr. and *Planchonia* Blume (Erdtman, 1952; Muller, 1972, 1973, 1979; Tsou, 1994; John & Kuriakose, 2012). Pollen morphological characters are also useful for separating and explaining subfamilial relationships in Lecythidaceae (Muller, 1972). Moreover, most members of Barringtonioideae have specific pollen characters that differentiate them from all other Angiosperms, i.e. marginal ridges, marginal grooves and a syntricolpate grain (Tsou, 1994). Therefore, this study aimed to investigate pollen morphological characters of the Southeast Asian Lecythidaceae in order to determine diagnostic features and evaluate their taxonomic implications.

MATERIAL AND METHODS

Pollen grain features of four genera and 33 taxa of Lecythidaceae in Southeast Asia were examined using compound light microscopy (LM) and scanning electron microscopy (SEM). The list of voucher specimens included in this study is given in Table 1 and includes 26 taxa of *Barringtonia*, one taxon each of *Careya* and *Chydenanthus*, and five taxa of *Planchonia*. The pollen samples were taken from field collections and herbarium specimens kept at BO, HN and K. Voucher specimens from fieldwork were deposited in KKU. Pollen from field collections were acetolysed according to Erdtman (1960), while pollen from herbarium specimens were studied directly. The acetolysed and unacetolysed pollen grains were examined and photographed using LM (Olympus CH3, Optical Co., Ltd) and a Leo 1450 VP (Cambridge, UK) SEM. Measurements were

made on 5–15 grains per taxon. Terminology and pollen size classes follow Walker & Doyle (1975), Tsou (1994) and Hesse *et al.* (2009); herbarium abbreviations follow Index Herbariorum (Thiers, 2018, continuously updated).

RESULTS

The common pollen morphological characters of each of the studied genera are described below and the palynological features of each species are summarised in Table 2. The outline of Southeast Asian Lecythidaceae pollen features is shown in Fig. 1A–B. Additionally, the definition of some special pollen characters, such as the state of marginal grooves, is shown and explained in Fig. 1C–G and the type of polar ectoaperture in Fig. 1H–I.

Barringtonia (Figs. 2–6A–F)

Pollen grains are monads, radially symmetrical, isopolar, of small to medium size (Polar axis (P) = 22.0–49.0 μm , Equatorial axis (E) = 22.0–49.0 μm), suboblate, subprolate, spherical, prolate spheroidal and oblate spheroidal and syntricolpate except in *B. thailandica* Thammar., Pornp. & Chantar. where it is syntricolporate. Marginal ridges and marginal grooves are usually present except in *B. acutangula* subsp. *spicata* (Blume) Payens, *B. racemosa* (L.) Spreng., *B. schmidtii* Warb. ex Craib and *B. thailandica*. Marginal grooves, when present, are usually of medium size, though they are long in *B. laxiflora* Thammar., Pornp. & Chantar. and *B. tomentosa* Thammar., Pornp. & Chantar. where they are long. Polar cushions are present. Polar ectoaperture sealed or open. Colpial surface is usually smooth or has sparsely or densely scattered verrucae-gemmae elements. Mesocolpial sculpturing is perforate-reticulate.

Careya (Fig. 6G–I)

Pollen grains are monads, radially symmetrical, isopolar, of medium size (P = 35.0–38.0 μm , E = 40.0–48.0 μm), suboblate and syntricolporate. Marginal ridges and marginal grooves are present. Marginal grooves are circular. Polar cushions are absent. Polar ectoaperture open. Colpial surface has clavate to pilate elements scattered at the polar area and aligned in longitudinal rows at the equatorial area. Mesocolpial sculpturing is perforate-reticulate.

Table 1. List of plant materials included in this study.

Species	Voucher
1. <i>Barringtonia acutangula</i> (L.) Gaertn. subsp. <i>acutangula</i>	Thailand, Phangnga, <i>W. Thammarong 536</i> (KKU)
2. <i>B. acutangula</i> subsp. <i>spicata</i> (Blume) Payens	Thailand, Songkhla, <i>W. Thammarong 521</i> (KKU)
3. <i>B. asiatica</i> (L.) Kurz	Thailand, Trang, <i>W. Thammarong 527</i> (KKU)
4. <i>B. augusta</i> Wall. ex Kurz	Thailand, Trat, <i>W. Thammarong 543</i> (KKU)
5. <i>B. calyptrocalyx</i> var. <i>mollis</i> Lauterb.	Indonesia, Moluccas, <i>A.J.G.H. Kostermans 5126</i> (BO)
6. <i>B. confusa</i> Lütjeh. & Ooststr.	Indonesia, Ambon, <i>S.N.</i> (BO-0123478)
7. <i>B. conoidea</i> Griff.	Indonesia, Banka, <i>H.A.B. Bunnemeijer 2088</i> (BO)
8. <i>B. curranii</i> Merr.	Philippines, Palawan, <i>A.D.E. Elmer 13033</i> (BO)
9. <i>B. fusiformis</i> King	Malaysia, Pahang, <i>M.R. Henderson 24848</i> (BO)
10. <i>B. gigantostachya</i> var. <i>megistophylla</i> (Merr.) Payens	Indonesia, Menubar, <i>A.J.G.H. Kostermans 5385</i> (BO)
11. <i>B. lanceolata</i> (Ridl.) Payens	Indonesia, Saggau, <i>A. Elsener 215</i> (BO)
12. <i>B. laxiflora</i> Thammar., Pornp. & Chantar.	Vietnam, Thua Thien Hue, <i>N.T. Hiep et al. 1351</i> (HN)
13. <i>B. longipes</i> Gagnep.	Laos, Bolikhamxai, <i>A.F.G. Kerr 20758</i> (K)
14. <i>B. longisepala</i> Payens	Malaysia, Sarawak, <i>Z.A.A. Hassan 2223</i> (BO)
15. <i>B. macrocarpa</i> Hassk.	Thailand, Phangnga, <i>A.F.G. Kerr 17083</i> (K)
16. <i>B. macrostachya</i> (Jack) Kurz	Thailand, Songkhla, <i>W. Thammarong 534</i> (KKU)
17. <i>B. norshamiae</i> Prance	Malaysia, Pahang, <i>T. Kadim & M. Noor 76</i> (BO)
18. <i>B. novae-hiberniae</i> Lauterb. subsp. <i>novae-hiberniae</i>	Indonesia, Abepura, <i>A.J.G.H. Kostermans & R. Soegeng 427</i> (BO)
19. <i>B. parkinsonii</i> Thammar., Pornp. & Chantar.	Myanmar, Amherst, <i>C.E. Parkinson 5267</i> (K)
20. <i>B. pterita</i> Merr.	Philippines, Tayabas, <i>A.D.E. Elmer 9168</i> (BO)
21. <i>B. racemosa</i> (L.) Spreng.	Thailand, Songkhla, <i>W. Thammarong 528</i> (KKU)
22. <i>B. sarcostachys</i> (Blume) Miq. subsp. <i>sarcostachys</i>	Malaysia, Sabah, <i>W. Meijer 48599</i> (K)
23. <i>B. sarcostachys</i> subsp. <i>dolichosperma</i> (Merr.) Prance	Brunei, Andulau, <i>H.P. Fuchs & J. Muller 21173</i> (K)
24. <i>B. schmidtii</i> Warb. ex Craib	Thailand, Trat, <i>W. Thammarong 545</i> (KKU)
25. <i>B. thailandica</i> Thammar., Pornp. & Chantar.	Thailand, Ubon Ratchathani, <i>W. Thammarong & P. Pornpongrungrueng 557</i> (KKU)
26. <i>B. tomentosa</i> Thammar., Pornp. & Chantar.	Vietnam, Thua Thien Hue, <i>N.T. Hiep et al. 1685</i> (HN)
27. <i>Careya arborea</i> Roxb.	Thailand, Khon Kaen, <i>W. Thammarong 553</i> (KKU)
28. <i>Chydenanthus excelsus</i> (Blume) Miers	Indonesia, Java, <i>A.C. Nolte 4025</i> (BO)
29. <i>Planchonia grandis</i> Ridl.	Malaysia, Potting Yard, <i>M. Nur s.n.</i> (BO-1458742)
30. <i>P. papuana</i> R.Knuth	Indonesia, Kobroor, <i>G. Nooteboom 5910</i> (BO)
31. <i>P. spectabilis</i> Merr.	Philippines, Laguna, <i>A.D.E. Elmer 17492</i> (BO)
32. <i>P. timorensis</i> Blume	Indonesia, Komodo, <i>M. Mughtar 18</i> (BO)
33. <i>P. valida</i> (Blume) Blume	Indonesia, Berau, <i>A.J.G.H. Kostermans 21238</i> (BO)

Chydenanthus (Fig. 6J–K)

Pollen grains are monads, radially symmetrical, isopolar, of medium size (P = 30.0–41.0 µm, E = 30.0–40.0 µm), prolate spheroidal and syntriolate.

Marginal ridges and the marginal grooves are present. Marginal grooves are short. Polar cushions are present. Polar ectoaperture sealed. Colpial surface is smooth. Mesocolpial sculpturing is perforate-reticulate.

Table 2. Comparison of pollen morphological features of the Southeast Asian Lecythidaceae species investigated (P = Polar axis, E = Equatorial axis; Size classes: M = Medium sized-grain, S = Small sized-grain; Shapes: Obl = Oblate spheroidal, Pro = Prolate spheroidal, Sph = Spherical, Subo = Suboblate, Subp = Subprolate; States of marginal groove: Me = Medium, Sh = Short, Lo = Long, Ci = Circular, - = absent; Polar cushion: + = present, - = absent; Colpial sculpturing patterns: 1 = smooth, 2 = abundant rugulae on the two ends and fewer or none of them in the equatorial region, 3 = sparsely or densely scattered verrucae-gemmae, 4 = with clavate to pilate elements scattered or aligned in longitudinal rows, * = additional pollen type found in the present study).

Species	P (Mean±SD) (µm)	E (Mean±SD) (µm)	Size classes	Shapes	States of marginal groove	Polar cushion	Aperture types	Ecto-aperture types	Colpial sculpturing patterns	Pollen type (according to Tsou (1994))
<i>Barringtonia acutangula</i>	32-37 (34.60±2.07)	28-32(29.60±1.67)	M	Subp	Me	+	syntricolpate	Sealed	3	V
subsp. <i>acutangula</i>										
<i>B. acutangula</i>	32-37 (34.60±2.07)	28-32 (30.00±2.00)	M	Subp	-	+	syntricolpate	Sealed	1	II
subsp. <i>spicata</i>										
<i>B. asiatica</i>	37-48 (43.50±4.65)	33-49 (42.75±7.80)	M	Pro	Me	+	syntricolpate	Sealed	3	V
<i>B. augusta</i>	32-45 (37.83±4.35)	33-36 (34.33±1.03)	M	Pro	Me	+	syntricolpate	Sealed	1	V
<i>B. calyptracalyx</i> var. <i>mollis</i>	24-32 (28.40±3.64)	29-33 (31.00±1.58)	S-M	Obl	Me	+	syntricolpate	Sealed	3	V
<i>B. confusa</i>	24-32 (27.20±2.58)	27-31 (29.00±1.58)	S-M	Obl	Me	+	syntricolpate	Open	1	VI
<i>B. conoidea</i>	22-32 (28.80±3.96)	28-33 (30.20±1.92)	S-M	Obl	Me	+	syntricolpate	Sealed	1	V
<i>B. curranii</i>	29-34 (31.20±1.92)	23-28 (25.20±1.92)	M	Subp	Me	+	Syntricolpate	Sealed	1	V
<i>B. fusiformis</i>	26-33 (29.40±2.88)	22-27 (24.60±2.07)	M	Subp	Me	+	Syntricolpate	Sealed	1	V
<i>B. gigantostachya</i> var. <i>megistophylla</i>	26-31 (28.80±1.92)	28.5-33 (30.50±1.93)	M	Obl	Me	+	Syntricolpate	Sealed	1	V
<i>B. lanceolata</i>	29-33 (31.00±1.58)	23.5-32 (28.10±4.03)	M	Pro	Me	+	Syntricolpate	Sealed	1	V
<i>B. laxiflora</i>	35.5-37.5 (36.5±0.79)	25-37 (33.54±4.99)	M	Pro	Lo	+	Syntricolpate	Sealed	1	IX*
<i>B. longipes</i>	30-34 (31.40±1.67)	30-34 (32.00±1.58)	M	Obl	Me	+	Syntricolpate	Sealed	1	V
<i>B. longisepala</i>	28-33 (30.20±1.92)	26-33 (29.40±2.88)	M	Pro	Me	+	Syntricolpate	Sealed	1	V
<i>B. macrocarpa</i>	30-34 (31.80±1.48)	25-34 (29.80±3.49)	M	Pro	Me	+	Syntricolpate	Open	1	VI
<i>B. macrostachya</i>	41-46 (43.00±2.16)	38.3-49 (44.83±4.65)	M	Obl	Me	+	Syntricolpate	Sealed	1	V
<i>B. norshamiae</i>	29-32.5 (30.80±1.44)	27-32 (29.60±2.07)	M	Pro	Me	+	Syntricolpate	Sealed	1	V

Table 2 (Continued)

Species	P (Mean±SD) (µm)	E (Mean±SD) (µm)	Size classes	Shapes	States of marginal groove	Polar cushion	Aperture types	Ecto-aperture types	Colpial sculpturing patterns	Pollen type (according to Tsou (1994))
<i>B. novae-iberniae</i> subsp. <i>novae-iberniae</i>	33–47 (40.00±6.51)	35–47 (40.20±5.80)	M	Sph	Me	+	Syntricolpate	Open	3	VI
<i>B. parkinsonii</i>	29–36 (31.46±2.75)	30–34 (32.10±1.51)	M	Obl	Me	+	Syntricolpate	Sealed	1	V
<i>B. pterita</i>	32–36 (34.00±1.58)	26–35 (29.80±4.54)	M	Pro	Me	+	Syntricolpate	Sealed	1	V
<i>B. racemosa</i>	30–49 (41.33±7.61)	33–39 (35.50±2.35)	M	Subp	-	+	Syntricolpate	Sealed	1	II
<i>B. sarcostachys</i> subsp. <i>sarcostachys</i>	34–39 (36.40±2.07)	29–35 (32.10±2.65)	M	Pro	Me	+	Syntricolpate	Open	3	VI
<i>B. sarcostachys</i> subsp. <i>dolichosperma</i>	38–46 (41.50±3.39)	31–39 (35.80±3.20)	M	Subp	Me	+	Syntricolpate	Sealed	3	V
<i>B. schmidtii</i>	27–33 (28.85±2.12)	25–33 (28.25±3.59)	M	Pro	-	+	Syntricolpate	Open	1	IV
<i>B. thailandica</i>	28–35 (33.71±2.56)	25–28 (25.57±1.13)	M	Subp	-	+	Syntricolporate	Sealed	1	II
<i>B. tomentosa</i>	26–36.5 (32.24±4.21)	26.5–36.5 (30.96±4.52)	M	Pro	Lo	+	Syntricolpate	Sealed	1	IX*
<i>Careya arborea</i>	35–38 (35.5±1.22)	40–48 (43.83±3.43)	M	Subo	Ci	-	Syntricolporate	Open	4	VIII
<i>Chydenanthus excelsus</i>	30–41 (35.80±4.65)	30–40 (35.6±4.82)	M	Pro	Sh	+	Syntricolpate	Sealed	1	V
<i>Planchonia grandis</i>	30–36 (32.60±2.40)	29–36 (32.00±2.91)	M	Pro	-	+	Syntricolpate	Open	1	IV
<i>P. papuana</i>	33–37 (35.00±1.58)	40–44 (42.00±1.58)	M	Subo	Me	+	Syntricolpate	Open	4	VI
<i>P. spectabilis</i>	30–46 (36.00±6.36)	29–39 (34.80±3.90)	M	Pro	Me	+	Syntricolpate	Sealed	4	V
<i>P. timorensis</i>	31–42 (36.40±4.72)	37–41 (39.00±1.58)	M	Obl	Me	+	Syntricolpate	Open	4	VI
<i>P. valida</i>	42–47 (44.80±2.28)	37.5–49 (45.17±3.85)	M	Obl	Me	+	Syntricolporate	Open	4	VI

Planchonia (Figs. 6L, 7)

Pollen grains are monads, radially symmetrical, isopolar, medium size ($P = 30.0\text{--}47.0\ \mu\text{m}$, $E = 29.0\text{--}49.0\ \mu\text{m}$), suboblate, prolate spheroidal, oblate spheroidal, syntri-colpate except for *P. valida* (Blume) Blume where they are syntri-colporate. Marginal ridges are present and marginal grooves are usually present except for *P. grandis* Ridl. Marginal grooves, when present, are of medium size. Polar cushions are present. Polar ectoaperture open, except for *P. spectabilis* Merr. where it is sealed. Colpial surface typically has clavate to pilate elements

aligned in longitudinal rows except for *P. grandis* where the surface is smooth. Mesocolpial sculpturing is perforate-reticulate.

DISCUSSION AND CONCLUSION

Generally, this work agrees with previous studies on the general features of pollen of Lecythidaceae including Erdtman (1952, 1966), Muller (1972, 1973) and Tsou (1994). The common characters of pollen grains of Southeast Asian Lecythidaceae are that they are monads, isopolar

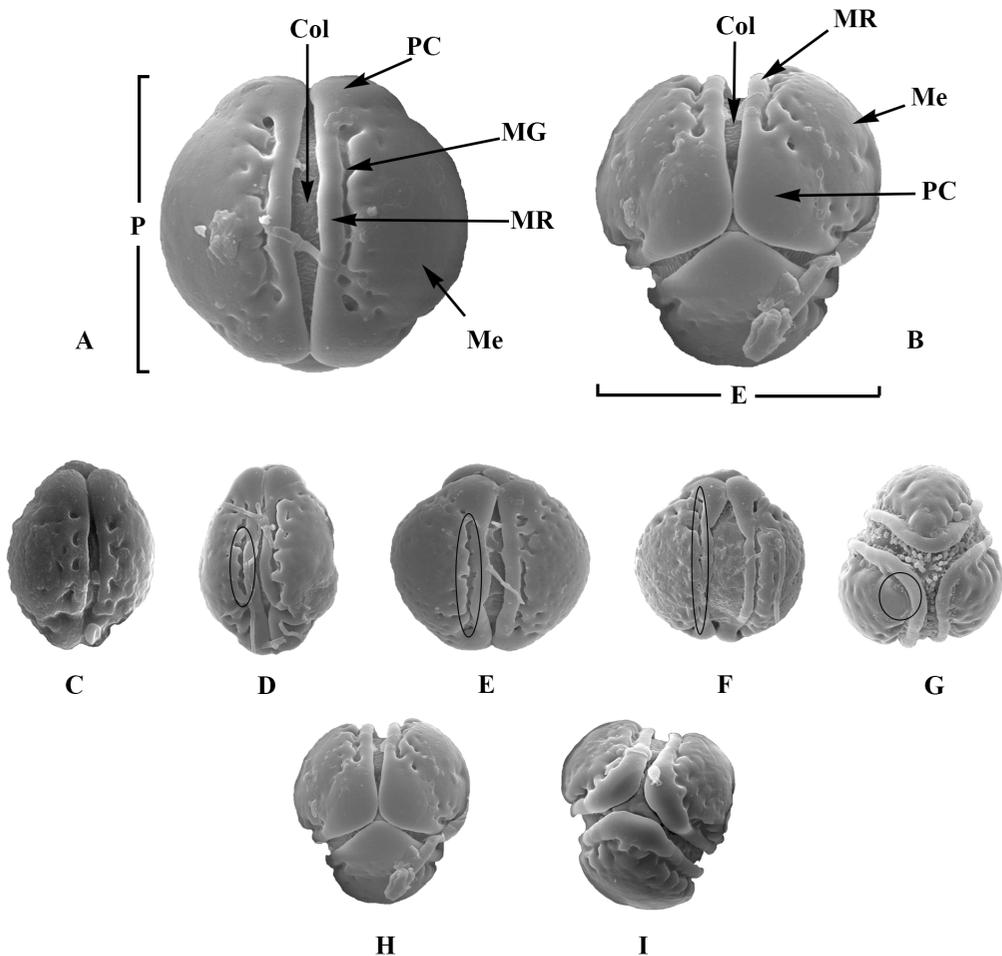


Figure 1. The outline of Southeast Asian Lecythidaceae pollen features (A–B): A. equatorial view, B. polar view (Col = colpium, Me = mesocolpium, MG = marginal groove, MR = marginal ridge, PC = polar cushion, P = polar axis, E = equatorial axis); state of marginal grooves (C–G), marginal groove indicated by a circle: C. marginal grooves absent, D. short marginal groove (marginal grooves are not well developed and restricted to equatorial endoaperturate regions), E. medium marginal grooves (marginal grooves elongated nearly to polar regions), F. long marginal grooves (marginal grooves elongated to polar regions and almost coalesced), G. circular marginal grooves (marginal grooves elongated to polar regions, coalesced and forming a ring enclosing the mesocolpium); polar ectoaperture type (H–I): H. sealed, I. open.

and radially symmetrical. Pollen grain size varies from small to medium ($P = 22.0\text{--}49.0$ and $E = 22.0\text{--}49.0\ \mu\text{m}$). Large-sized grains have been reported by Tsou (1994) but were not present in the species included in this study. Members of *Barringtonia* have small to medium-sized grains while the other genera (*Careya*, *Chydenanthus* and *Planchonia*) have medium-sized grains only. The largest pollen

grain size is found in *B. racemosa* (L.) Spreng. ($P = 30.0\text{--}49.0\ \mu\text{m}$, $E = 33.0\text{--}39.0\ \mu\text{m}$) and the smallest in *B. conoidea* Griff. ($P = 22.0\text{--}32.0\ \mu\text{m}$, $E = 28.0\text{--}33.0\ \mu\text{m}$). Pollen shape is highly variable among species being prolate spheroidal, oblate spheroidal, subprolate, suboblate or spherical. The type of aperture we detected is mostly syntricolpate: that agrees well with Tsou (1994) and Mori *et al.* (2017) who concluded

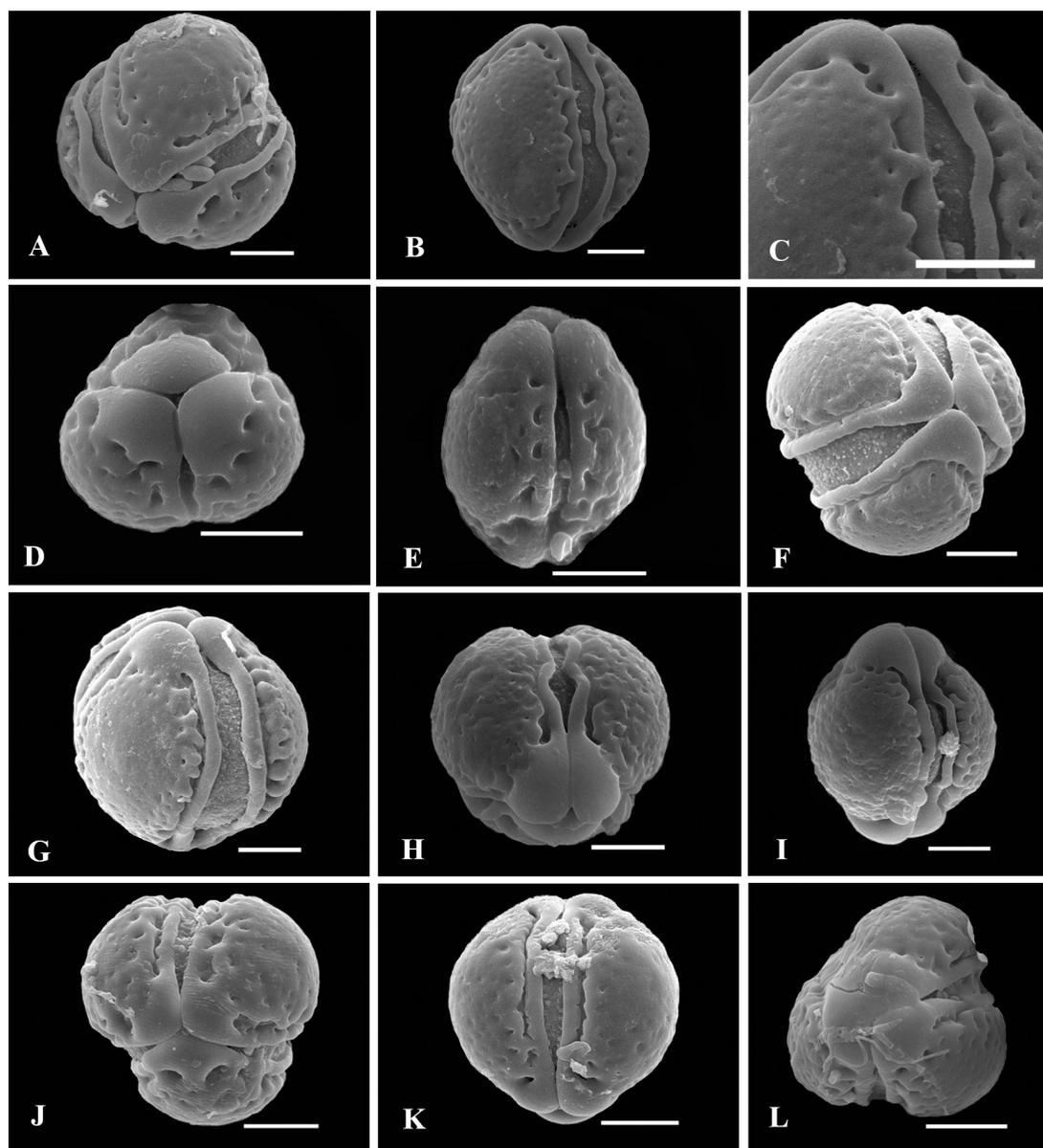


Figure 2. SEM micrographs of pollen grains. A–C: *Barringtonia acutangula* subsp. *acutangula*; D–E: *B. acutangula* subsp. *spicata*; F–G: *B. asiatica*; H–I: *B. augusta*; J–K: *B. calyptrocalyx* var. *mollis*; L: *B. confusa*. A, D, F, H, J, L polar or subpolar view; B, C, E, G, I, K equatorial or subequatorial view. Scale bars = 10 μm .

that the typical aperture type of Barringtonioideae is syntricolpate. However, in the present study, syntricolporate apertures were found in *B. thailandica* (Fig. 6D), *Ca. arborea* Roxb. (Fig. 6I) and *P. valida* (Fig. 7H).

The Barringtonioideae have specialised pollen characters, viz. the presence of marginal ridges,

marginal grooves and polar cushions. These specialised characters and the syncolpatism distinguish the Barringtonioideae from other Angiosperms (Tsou, 1994; Mori *et al.*, 2017). Marginal ridges are present in all studied species and most species have marginal grooves and polar cushions. The marginal grooves are absent in *P. grandis* and four taxa of *Barringtonia*

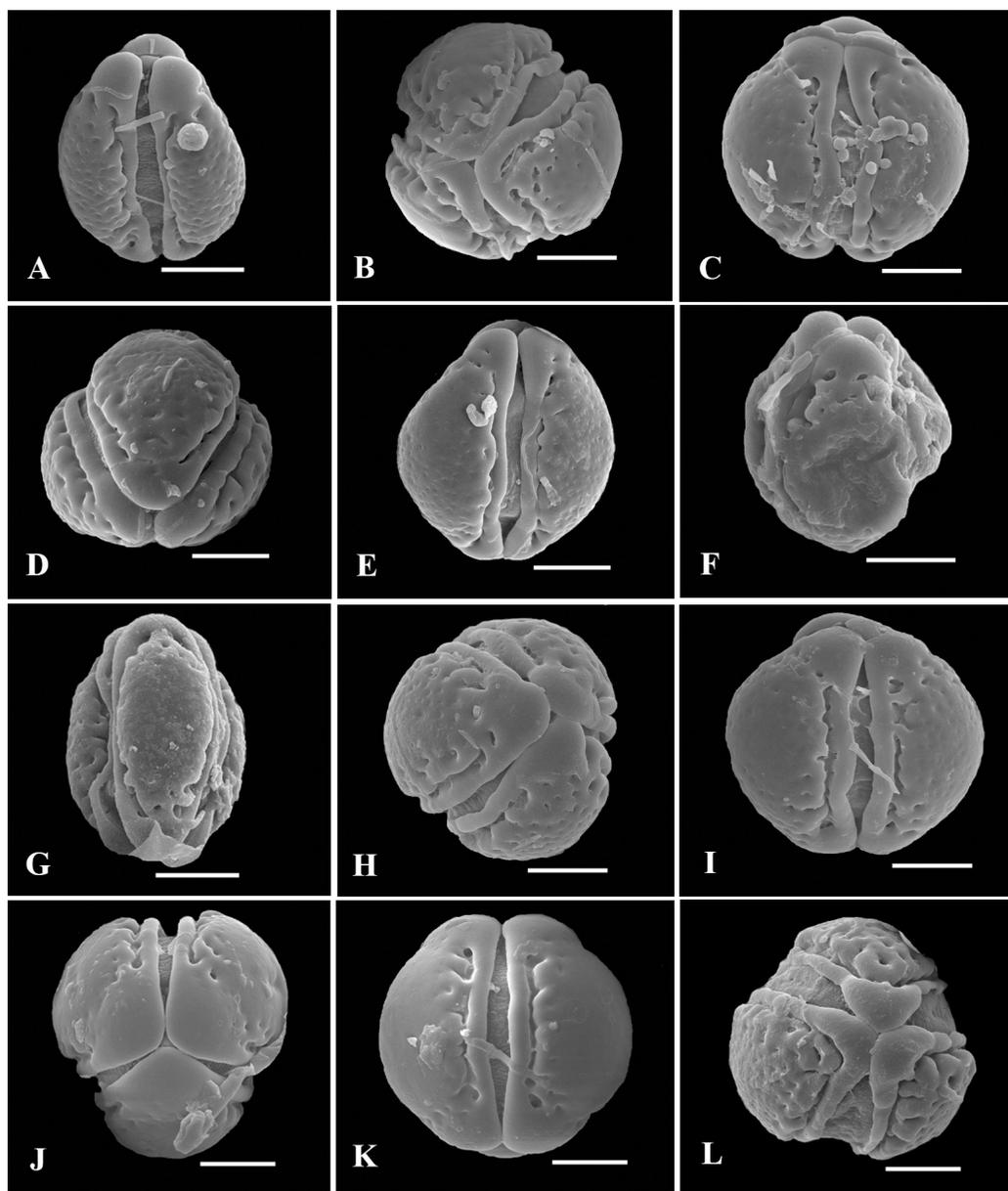


Figure 3. SEM micrographs of pollen grains. A: *Barringtonia confusa*; B–C: *B. conoidea*; D–E: *B. curranii*; F–G: *B. fusiformis*; H–I: *B. gigantostachya* var. *megistophylla*; J–K: *B. lanceolata*. L: *B. laxiflora*. B, D, H, J, L polar or subpolar view; A, C, E–G, I, K equatorial view. Scale bars = 10 μ m.

(*B. acutangula* subsp. *spicata*, *B. racemosa*, *B. schmidtii* and *B. thailandica*). The marginal grooves, when present, are of medium size in most studied species, except in *Ch. excelsus* (Blume) Miers where they are short, in *B. laxiflora* and *B. tomentosa* where they are long and in *Ca. arborea* where they are circular. Polar cushions are present in

all studied species except *Ca. arborea*. Tsou (1994) found that polar cushions were absent in some species of *Barringtonia* but the present study indicated that all Southeast Asian taxa so far studied have polar cushions. The other character that Tsou (1994) used to divide the pollen type of *Barringtonioideae* is the polar ecto-aperture that can be open or sealed.

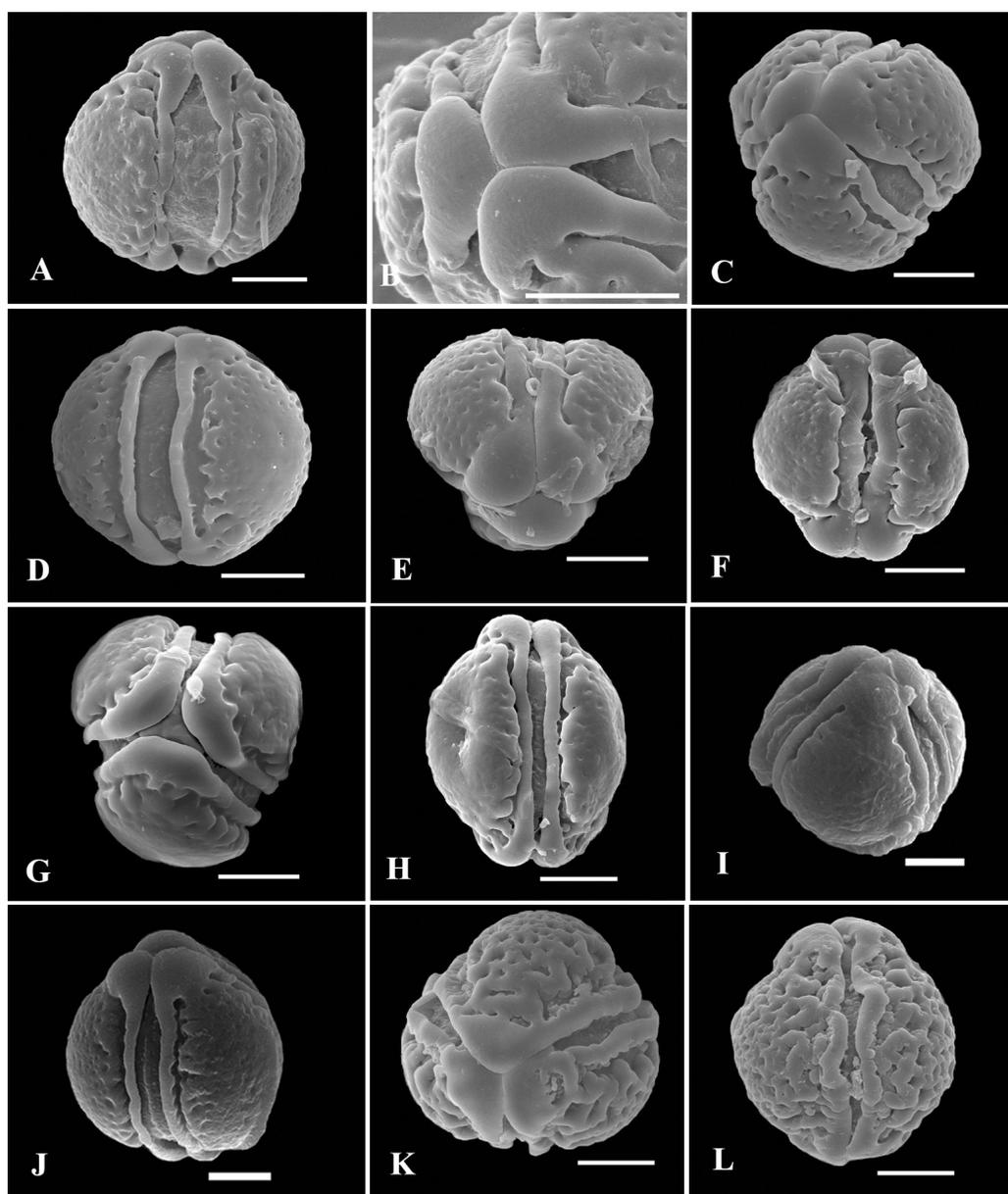


Figure 4. SEM micrographs of pollen grains. A–B: *Barringtonia laxiflora*; C–D: *B. longipes* with sealed polar ectoaperture; E–F: *B. longisepala*; G–H: *B. macrocarpa* with open polar ectoaperture; I–J: *B. macrostachya*; K–L: *B. norshamiae*. B, C, E, G, I, K polar or subpolar view; A, D, F, H, J, L equatorial or subequatorial view. Scale bars = 10 μ m.

Based on the different state of these specialised characters, the pollen morphology of the Barringtonioideae is varied and can be divided into eight types according to Muller (1973) and Tsou (1994) as follows: type I: marginal grooves and polar cushions absent and polar ectoaperture sealed; type II: marginal grooves absent, polar cushions present

and polar ectoaperture sealed; type III: marginal grooves present, polar cushions absent, marginal groove short and polar ectoaperture sealed; type IV: marginal grooves absent, polar cushions present and polar ectoapertures open; type V: marginal grooves and polar cushions present, marginal grooves short to medium and polar ectoaperture sealed; type VI:

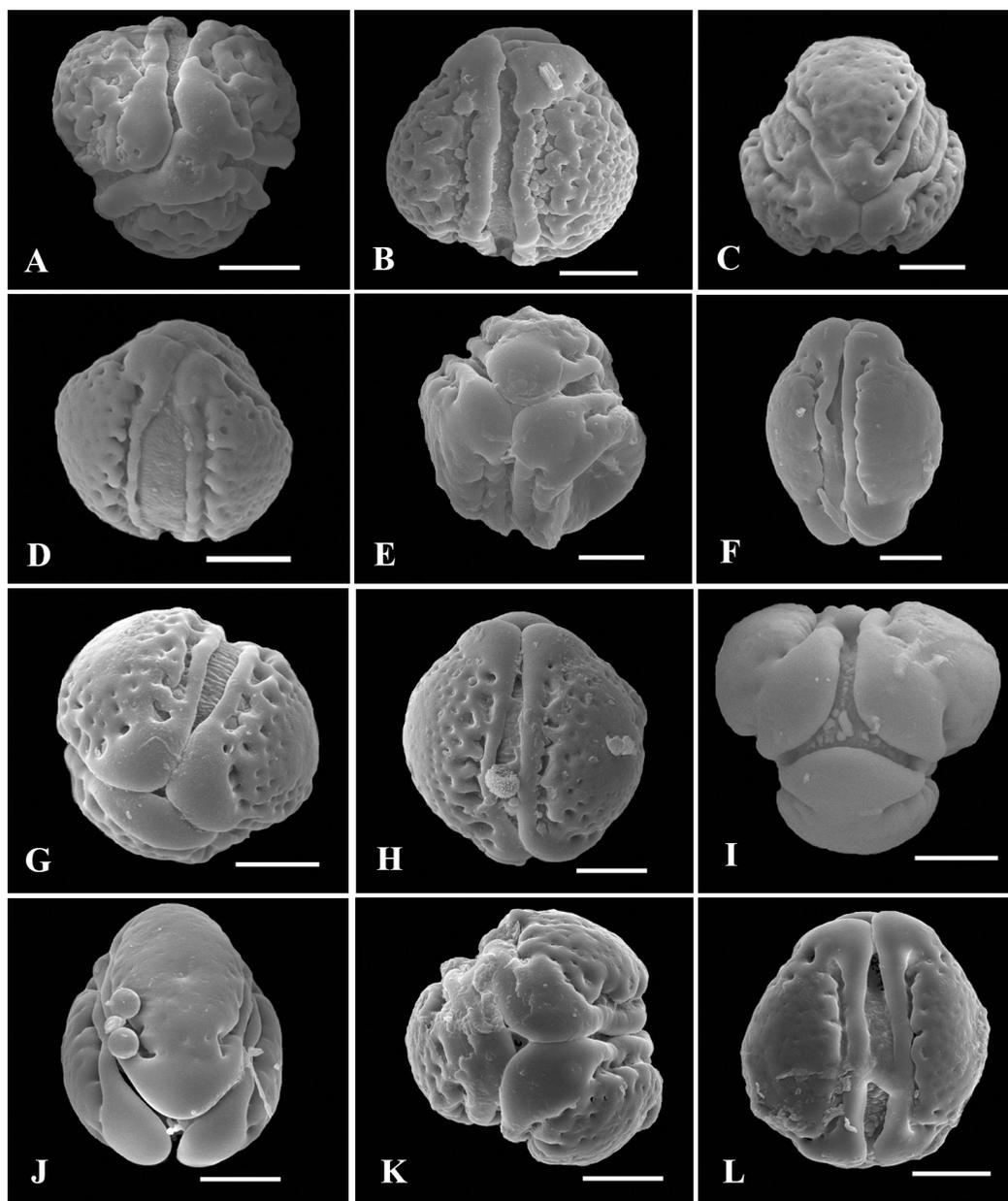


Figure 5. SEM micrographs of pollen grains. A–B: *Barringtonia novae-hiberniae* subsp. *novae-hiberniae*; C–D: *B. parkinsonii*; E–F: *B. pterita*; G–H: *B. racemosa*; I–J: *B. sarcostachys* subsp. *sarcostachys*; K–L: *B. sarcostachys* subsp. *dolichosperma*. A, C, E, G, I, J, K polar or subpolar view; B, D, F, H, L equatorial or subequatorial view. Scale bars = 10 μ m.

marginal grooves and polar cushions present, marginal groove medium and polar ectoaperture open; type VII: marginal grooves present, polar cushions absent, marginal groove long and polar ectoaperture open, and type VIII: marginal grooves present and polar cushions absent, marginal grooves circular and polar ectoaperture open. Of these eight types, five were observed in the Southeast Asian Lecythidaceae

here studied, *i.e.* types II, IV, V, VI and VIII. Additionally, we found a new type in this study herein called type IX in which the marginal grooves and polar cushions are present, the marginal groove is long and the polar ectoaperture is sealed. This additional type is found in *B. laxiflora* and *B. tomentosa*. The most common pollen type in the studied species is type V.

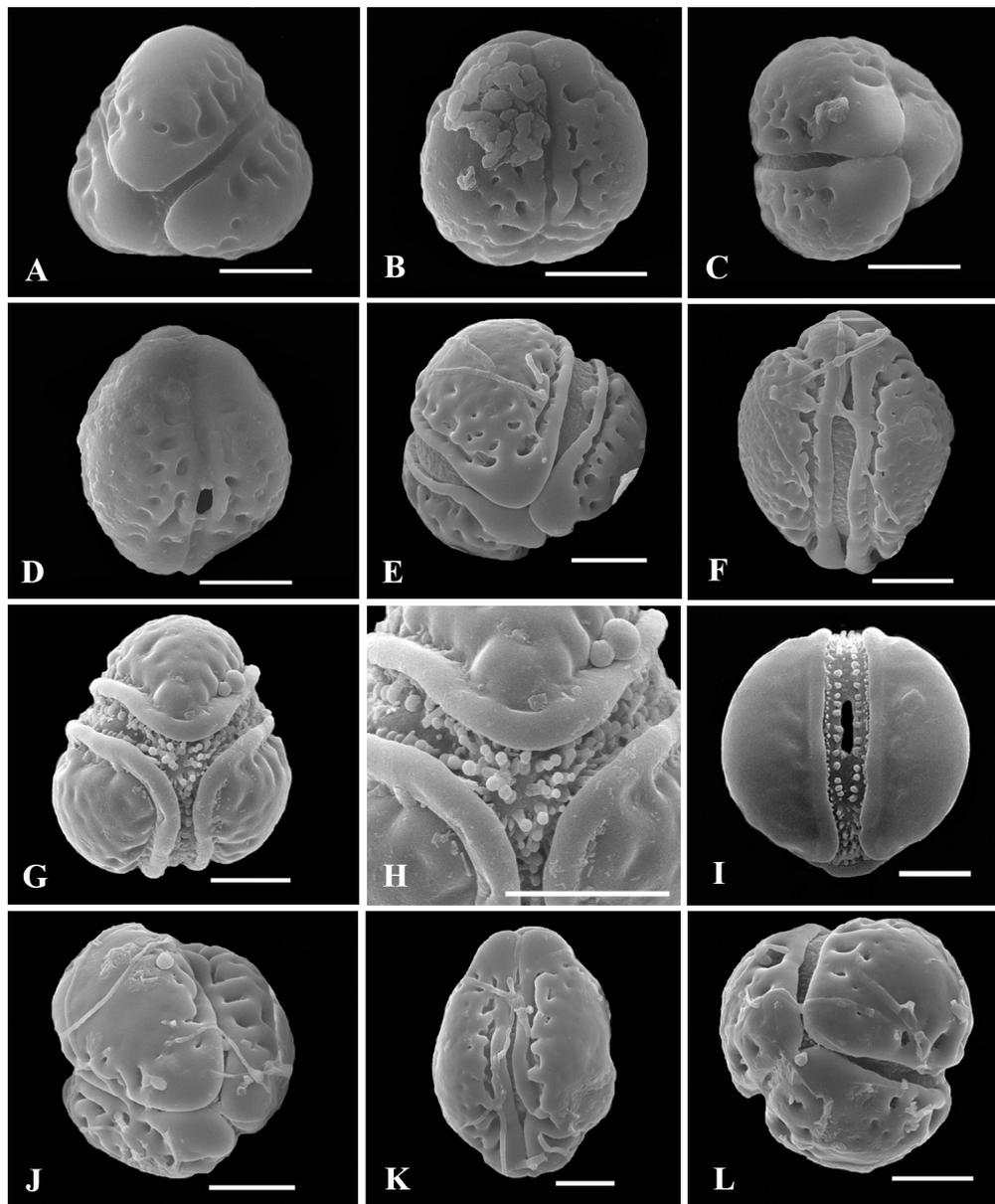


Figure 6. SEM micrographs of pollen grains. A–B: *Barringtonia schmidtii*; C–D: *B. thailandica*; E–F: *B. tomentosa*; G–I: *Careya arborea*; J–K: *Chydenanthus excelsus*; L: *Planchonia grandis*; A, C, E, G, H, J, L polar or subpolar view; B, D, F, I, K, equatorial or subequatorial view. Scale bars = 10 μ m.

Some differences in pollen characters were observed between some species studied by Tsou (1994) and some included in the present study, viz the pollen of *B. asiatica* (L.) Kurz and *B. racemosa* was assigned to the types VI and V, respectively by Tsou (1994) but in the present study they were assigned to the types V and II, respectively and *Ch. excelsus* was assigned to the type II by Tsou (1994) but type V herein. These differences probably arise because the pollen of some species can be variable, even though, in general, the taxa of Barringtonioideae have a single pollen type. For instance, Tsou (1994) also found that *Petersianthus quadrialatus* (Merr.) Merr. has two pollen types. Other possible explanations for the differences between our study and that of Tsou (1994) are that the state of marginal groove when it is short or absent is hard to clearly define and the sealed ectoaperturate type of some species is obscure.

All the studied species have more or less the same mesocolpial sculpturing, as they are perforate-reticulate. Perforate sculpturing is mainly found in the central area of the mesocolpium and reticulate sculpturing is found in the area next to the marginal ridges or marginal grooves. In some figures the surface sculpturing is obscured and seems to be smooth as seen in Figs. 6I and 7I and this is because the images were taken from unacetolysed grains. The colpial surface can be divided into four patterns according to Tsou (1994): (1) the surface is smooth, (2) the surface has abundant rugulae on the two ends and fewer or none in the equatorial region, (3) the surface has sparsely or densely scattered verrucae-gemmae elements and (4) the surface has clavate to pilate elements scattered or aligned in longitudinal rows. Only three of these patterns were observed in the present investigation. But this is because pattern 2 above is found in *Petersianthus* which was not included in the present investigation.

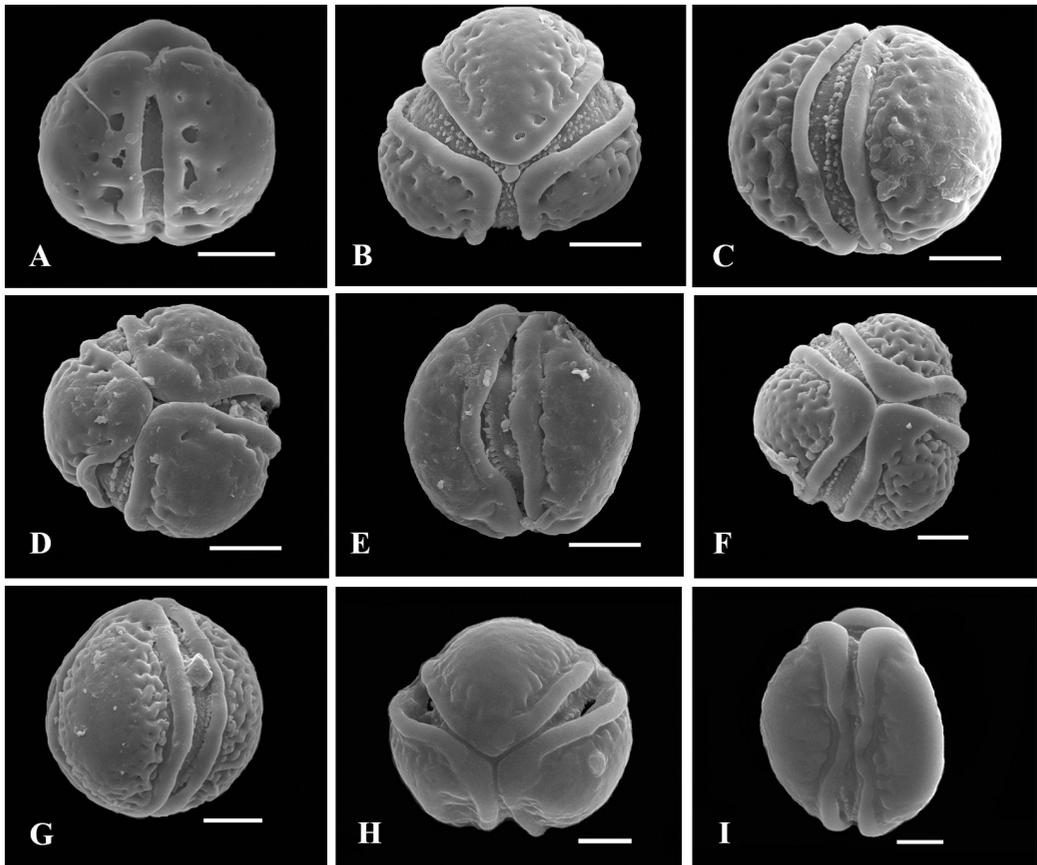


Figure 7. SEM micrographs of pollen grains. A: *Planchonia grandis*; B–C: *P. papuana*; D–E: *P. spectabilis*; F–G: *P. timorensis*; H–I: *P. valida*. B, D, F, H polar or subpolar view; A, C, E, G, I equatorial or subequatorial view. Scale bars = 10 μ m.

Although pollen morphological features cannot be used for specific identification for all species studied, the data do permit some species to be distinguished or confirm infraspecific classification. For example, *Ca. arborea* has distinct pollen characters compared to the other genera of Barringtonioideae. Moreover, in the case of *Barringtonia*, the pollen characters are helpful in resolving the taxonomic status of some closely related taxa as well as the status of the subspecies. For instance, the complex group of *B. acutangula* subsp. *acutangula*, *B. acutangula* subsp. *spicata*, *B. schmidtii* and *B. thailandica* which have similar gross morphology can be easily distinguished by means of the presence of marginal grooves, polar ectoaperture, apertural system and pollen shape. The two subspecies of *B. acutangula* can be distinguished by the type of pollen: the pollen of *B. acutangula* subsp. *acutangula* is type V, while that of *B. acutangula* subsp. *spicata* is type II. Similarly, the type and shape of pollen can be used to distinguish the two subspecies of *B. sarcostachys* (Blume) Miq. The pollen of *B. sarcostachys* subsp. *sarcostachys* is type VI and the shape is prolate spheroidal, whilst that of *B. sarcostachys* subsp. *dolichosperma* (Merr.) Prance is type V and the shape is subprolate.

One other taxonomic implication of our study relates to *B. schmidtii*, treated as a synonym of *B. acutangula* subsp. *spicata* by a number of authors (Payens, 1967; Prance, 2012; Prance & Kartawinata, 2013) but later reinstated as a distinct species by Thammamong *et al.* (2015) based on gross morphology and fruit characters (4-angled, ribless and puberulent in *B. schmidtii* and globose, 8-ribbed and glabrous in *B. acutangula* subsp. *spicata*). The present study found that pollen provides more characters for distinguishing these two taxa, as the pollen of *B. acutangula* subsp. *spicata* is type II and subprolate in shape, while that of *B. schmidtii* is type IV and prolate spheroidal in shape.

In conclusion, pollen morphological characters provide valuable taxonomic characters for the family Lecythidaceae.

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REFERENCES

- APG [Angiosperm Phylogeny Group] (1998). An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* 85: 531–533.
- _____. (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.
- _____. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.
- Erdtman, G. (1952). Pollen morphology and plant taxonomy: Angiosperms. Almqvist & Wiksell, Stockholm.
- _____. (1960). The acetolysis method: a revised description. *Svensk Botanisk Tidskrift* 54: 561–564.
- _____. (1966). Pollen morphology and plant taxonomy: Angiosperms. Hafner Publishing, New York.
- Hesse, M., Zetter, R., Halbritter, H., Weber, M., Buchner, R., Frosch-Radivo, A. & Ulrich, S. (2009). Pollen terminology: an illustrated handbook. Springer, Wien & New York.
- John, L. & Kuriakose, M.E. (2012). A morphological analysis of south Indian Lecythidaceae. *International Journal of Current Research* 4: 4–7.
- Mori, S.A. & Prance, G.T. (1990). Lecythidaceae—part II: the zygomorphic-flowered New World genera (*Couropita*, *Corythophora*, *Bertholletia*, *Couratari*, *Eschweilera*, & *Lecythis*). *Flora Neotropica Monograph* 21 (II). The New York Botanical Garden, New York, pp. 1–373.
- Mori, S.A., Tsou, C.H., Wu, C.C., Cronholm, B. & Anderberg, A.A. (2007). Evolution of Lecythidaceae with an emphasis on the circumscription of Neotropical genera: information from combined *ndhF* and *trnL-F* sequence data. *American Journal of Botany* 94: 289–301.

- Mori, S.A., Kiernan, E.A., Smith, N.P., Kelley, L.M., Huang, Y.-Y., Prance, G.T. & Thiers, B. (2017). Observations on the phytogeography of the Lecythidaceae clade (Brazil nut family). *Phytoneuron* 30: 1–85.
- Muller, J. (1972). Pollen morphology evidence for subdivision and affinities of Lecythidaceae. *Blumea* 20: 350–355.
- _____. (1973). Pollen morphology of *Barringtonia calyptrocalyx* K.Sch. (Lecythidaceae). *Grana* 13: 29–44.
- _____. (1979). Pollen. In: G.T. Prance & S.A. Mori. Lecythidaceae. Part I, The actinomorphic-flowered New World Lecythidaceae (*Asteranthos*, *Gustavia*, *Grias*, *Allantoma* & *Cariniana*). *Flora Neotropica Monograph* 21. The New York Botanical Garden, New York, pp. 72–76.
- Payson, J.P.D.W. (1967). A monograph of the genus *Barringtonia* (Lecythidaceae). *Blumea* 15: 158–263.
- Prance, G.T. (2012). A revision of *Barringtonia* (Lecythidaceae). *Allertonia* 12: 1–164.
- Prance, G.T. & Kartawinata, E.K. (2013). Lecythidaceae. In: H.P. Nooteboom & P.C. van Welzen (eds.), *Flora Malesiana* 21, pp. 1–118. Naturalis Biodiversity Center, Leiden.
- Prance, G.T. & Mori, S.A. (1979). Lecythidaceae—part I: the actinomorphic-flowered New World Lecythidaceae (*Asteranthos*, *Gustavia*, *Grias*, *Allantoma*, & *Cariniana*). *Flora Neotropica Monograph* 21. The New York Botanical Garden, New York, pp. 1–270.
- Prance, G.T. & Mori, S.A. (2004). Lecythidaceae. In: K. Kubitzki (ed.), *The families and genera of vascular plants* 6, pp. 221–232. Springer-Verlag, Berlin & Heidelberg.
- Reveal, J.L. & Chase, M.W. (2011). APG III: bibliographical information and synonymy of Magnoliidae. *Phytotaxa* 19: 71–134.
- Schönenberger, J., Anderberg, A.A. & Sytsma, K.J. (2005). Molecular phylogenetics and patterns of floral evolution in the Ericales. *International Journal of Plant Sciences* 166: 265–288.
- Thammarong, W. (2017). Systematics of Lecythidaceae in Southeast Asia. Ph.D. Dissertation. Khon Kaen University, Khon Kaen.
- Thammarong, W., Chantaranonthai, P. & Pornpong-rungrueng, P. (2015). A new species of *Barringtonia* (Lecythidaceae) from Thailand and taxonomic notes on *B. schmidtii*. *Phytotaxa* 239: 73–81.
- Thiers, B. (2018, continuously updated). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden. Available from <http://sweetgum.nybg.org/ih/>. [accessed 17 March 2018].
- Tsou, C.H. (1994). The classification and evolution of pollen types of Planchonioideae (Lecythidaceae). *Plant Systematics and Evolution* 189: 15–27.
- Walker, J.W. & Doyle, J.A. (1975). The bases of angiosperm phylogeny: palynology. *Annals of the Missouri Botanical Garden* 62: 664–723.