Microsc. Microanal. Res. 2019, 32(1) 34-40

Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Journal Home Page: https://www.tci-thaijo.org/index.php/mmres/

Microsc. Microanal. Res.

Microscopic Analysis of the Pollen Morphology of Some *Garcinia Species*

Valerie W. Suwanseree[1,3], Salak Phansiri[2], Yupadee Paopun[2] and Chinawat Yapwattanaphun[3]*

[1] *Graduate School, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand*
[2] *Scientific Equipment and Research Division, KURDI, Kasetsart University, Chatuchak, Bangkok, 10900 Thailand*
[3] *Department of Horticulture, Faculty of Agriculture, Kasetsart University, Chatuchak, Bangkok, 10900 Thailand*

*Corresponding author's e-mail address: agrcwy@ku.ac.th

ARTICLE INFO

Article history
Submitted: 9 April 2019
Revised: 10 August 2019
Accepted: 15 August 2019
Available online: 26 September 2019

Keywords:
Clusiaceae; eurypalynous; *Garcinia cymosa*; *Garcinia dulcis; Garcinia speciosa*

ABSTRACT

Garcinia (Family Clusiaceae) is a large genus comprising almost 400 tropical trees and shrubs, and the pollen of most of them has not been studied. In this research the pollen morphology of 8 *Garcinia* species were studied by light microscope and scanning electron microscope (SEM). The pollen grains were all classified as small; the smallest measuring approximately 12 x 19 µm and the largest 25 x 27 µm. All were spheroid or sub-spheroid. No apertures were visible on 6 of the species, while the other 2 species were 6-colporate. A diversity of exine sculpturing patterns were observed: 3 species were echinate (with spines), 2 were psilate (smooth), one was micro echinate (with very small spines), one was rugulate (with uneven protrusions), and one had an unusual sculpturing pattern that we characterized as "gemmate-micro echinate" because it has gemma, or protuberances of diverse shape and size, and on the surface of these gemma there are tiny spines. The results confirm the finding that Clusiaceae is a eurypalynous family in which the pollen grains show wide diversities of ornamentation patterns and numbers of apertures, even among species in the same genus. The data may be of use in plant systematics and classification.

INTRODUCTION

Garcinia is a genus in the family Clusiasceae (formerly Guttiferae) comprised of evergreen tropical trees and shrubs that originated mainly in Asia, as well as some species from Australia, Africa and Polynesia. There are 397 species in genus *Garcinia* that are accepted by The Plant List. At least 22 *Garcinia* species are found in Thailand [1]. The most well-known species in the genus is *Garcinia mangostana*, or mangosteen, a popular and commercially important fruit crop of Southeast Asia. Mangosteen lacks genetic diversity because the trees do not produce viable pollen and thus cannot be cross-pollinated. This led to our interest in studying the pollen of related species in the same genus, with the hope that some could possibly be used to pollinate mangosteen.

The shape, size, and external sculpturing of pollen grains are distinctive traits that can be useful in classifying plant species. Studying the similarities and differences in pollen ornamentation, pollen shape and pollen size can help botanists understand the evolutionary and phylogenetic relationships of different species of plants [2].

Complete information has not yet been published to describe the pollen of many of the species of genus *Garcinia.* In 1989, Seetharam of Gulbarga University, India, published a report on the pollen characteristics of 300 *Garcinia* species. The author reported that 33% of the species studied had 3-longicolporate pollen, 28% had 4-6-longicolporate pollen, 13% 3-porate pollen, 10% 3-brevicolporate, 6% 4-6 porate (periporate), 5% 4-6 brevicolporate, 2% 4-6 porate (meridional) and 1% 3-colpate. Seetharam theorized that the most common type, 3-longicolporate, should be considered the most primitive and the others derived. Seetharam reported that prolate is the most prevalent pollen shape, so it should be considered the basic type from which spheroidal and oblate pollen shapes evolved. As for the pollen coat, Seetharam's transmission electron microscope (TEM) studies revealed that the exine is divisible into 4 layers: tectum, infratectum, sole and endexine. The author concluded that the more primitive ornamentation character should be reticulate ornamentation, and that lesser-observed foveolate, psilate, clavate, echinate and verrucate ornamentation were derived types [3]. There are some limitations to Seetharam's study, because most observations were made from dried herbarium specimens. Only about 30 *Garcinia* species are found in India. Seetharam's paper includes SEM photos of 5 species (*G. balica, G. dices, G.disepala, G. latissima* and *G. tracancorica*), TEM photos of 2 species (*G. carolinensis* and *G. latissima*) and light microscope (LM) photos of 7 species (*G. acuminata, G. caudata, G. cornea, G. hombroniana, G. kola, G. riedeliana,* and *G. terpnophylla*). The 300 species that were investigated for the research are not listed in Seetharam's article.

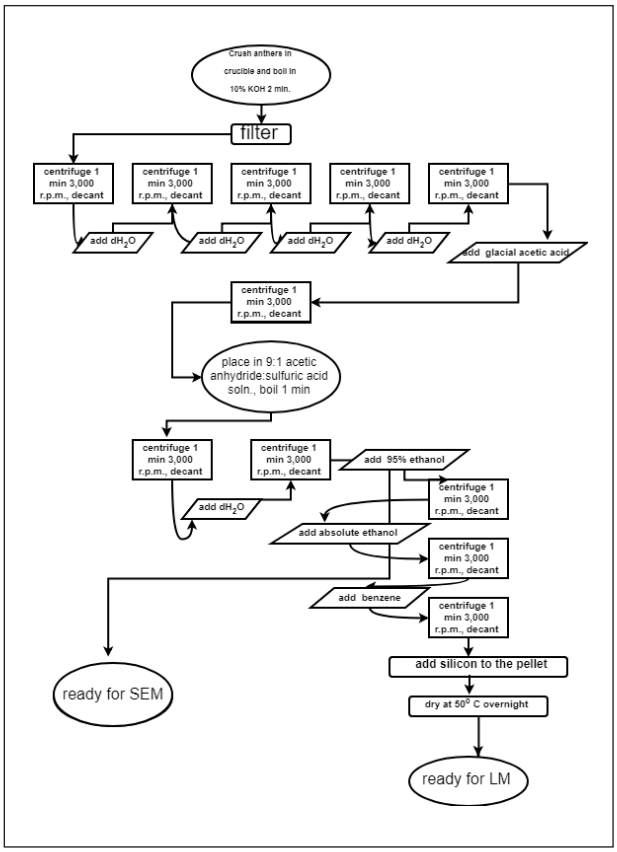
The present study was a preliminary investigation on the morphology of pollen from 8 available *Garcinia* species. Three of the species were identified, while 4 were known only by their accession number from the Bogor Botanic Gardens *Garcinia* collection in Bogor, Indonesia, and the final one was also a sample collected from Bogor Botanical Garden but was not labeled with the accession number on the package after collection, so it is called "unknown *Garcinia* species" in this report.

Acetolysis was used to prepare the LM slides. Acteolysis is a technique for preparing pollen specimens for microscopic analysis. It entails the chemical destruction of cellulose (such as moss, fine rootlets or other organic litter that might be mixed with a pollen sample) as well as the cytoplasmic material inside the pollen grains, for greater visibility, because untreated pollen grains don't reveal all the details needed for description. Acetic anhydride and concentrated sulfuric acid are used for this purpose, and during the acetolysis process the exine layer is also colored for greater visibility under the microscope [4,5].

METHODOLOGY

Garcinia dulcis pollen was obtained from a male tree growing on Kasetsart University's Bangkhen Campus in Bangkok, Thailand, in March, 2016. *Garcinia speciosa* pollen was obtained from Chanthaburi Horticultural Research Center, Chanthaburi, Thailand in February 2016. Pollen from *Garcinia cymosa, Garcinia* sp. accession number 279, *Garcinia* sp. accession number 283, *Garcinia* sp. accession number 287, *Garcinia* sp. accession number 290, and one more unknown *Garcinia* species were collected from the Bogor Botanic Gardens collection run by the Indonesian Institute of Sciences in Bogor, Indonesia, in August 2013.

For LM investigation, the acetolysis procedure of Assistant Professor Dr. Chalermpol Suwanphakdee at the Botany Department, Faculty of Science, Kasetsart University, was used, comprising the following steps.



SEM analysis was performed at the Botany Department, Faculty of Science, Kasetsart University for *Garcinia dulcis* (acetolysed sample), Bogor accession no. 279, and the unlabeled sample from Bogor Botanical Gardens, and at the Microscopy Lab, Scientific Equipment and Research Division, Kasetsart University Research and Development Institute, for *Garcinia dulcis* (fresh sample), *Garcinia speciosa,* Bogor accession no. 283 and Bogor accession no. 287.

At both locations, the pollen samples were placed on a stub and sputtered with gold using an ion coater, then observed in the SEM and images were recorded at 3,300x to 20,000x.

RESULTS

The size of the pollen of the *Garcinia* species studied was classified as "small." Most had a spheroid to sub-spheroid shape (Table 1).

The exines of the pollen of *Garcinia* species observed had a range of different ornamentation patterns. Three species were echinate, 1 micro echinate, 1 gemmate-microechinate, 2 psilate, and 1 rugulate. Apertures were not visible on most of the species observed (Table 2).

Garcinia cymosa pollen grains (Figures 1, 2 and 3) are oblong to spheroid and hexacolporate. The exine sculpturing was not clearly visible under light microscope investigation, but SEM investigation revealed an interesting surface texture that was rugulate with protuberances of different shapes and sizes, including vertical and horizontal protuberances, often curved, some blunt ended, some more pointed, some with grooves and some without grooves.

A report by Seetharam (1989) [3] stated that of 300 *Garcinia* species studied (the report did not list which species were studied) 76% were colporate and 43% had 6 pores (hexaporate).

Table 1. Size of *Garcinia* pollen in micrometers and polar/ equatorial ratio.

Species	Length – Polar Range (µm) (S.D.)	Length – Equatorial Range (µm) (S.D.)	P/E ratio (range)
G. *dulcis*	21-18 (1.79)	21-26 (1.35)	1.00 -1.22
G. *cymosa*	19-23 (1.49)	12-20 (2.32)	1.05-1.83
G. *speciosa*	21-27 (2.49)	15-25 (3.07)	1.00-1.40
G. sp. 287	15-23 (2.05)	15-23 (2.05)	1.00 -1.05
G. sp. 279	19-20 (0.5)	19-20 (0.5)	1.00

Table 2. Apertures and sculpturing patterns of pollen of 8 *Garcinia* species.

Species	Apertures	Surface ornamentation
G. dulcis	6-colporate	Psilate
G. cymosa	6-colporate	Rugulate
G. speciosa	Non aperturate	Micro ehinate
G. sp. 287	Non aperturate	Psilate
G. sp. 279	Non aperturate	Gemmate-microechinate
G. sp. 290	Non aperturate	Echinate
G. sp. 283	Non aperturate	Echinate
G. sp. unknown	Non aperturate	Echinate

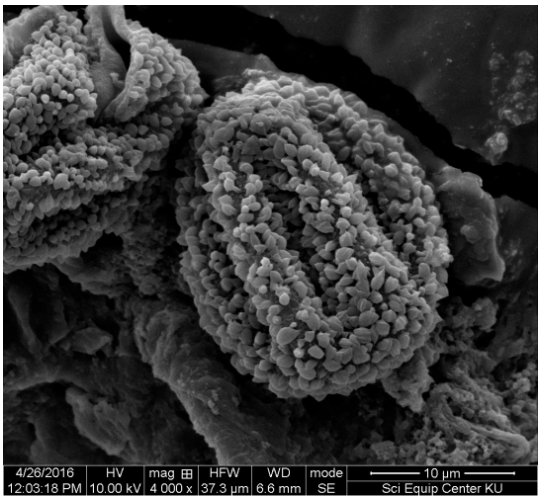


Figure 1. *Garcinia cymosa* SEM image 4,000 x magnification, equatorial view.

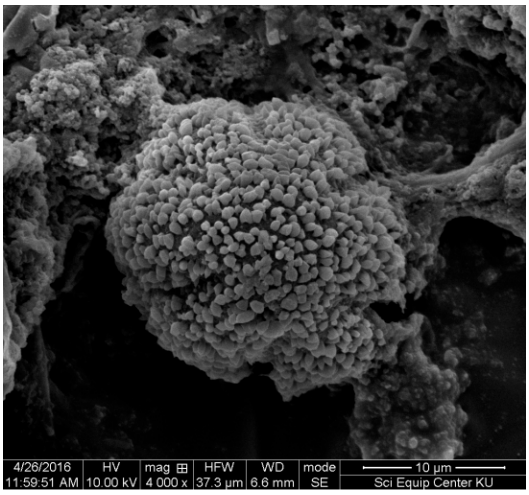


Figure 2. *Garcinia cymosa* SEM image 4,000 x magnification, polar view.

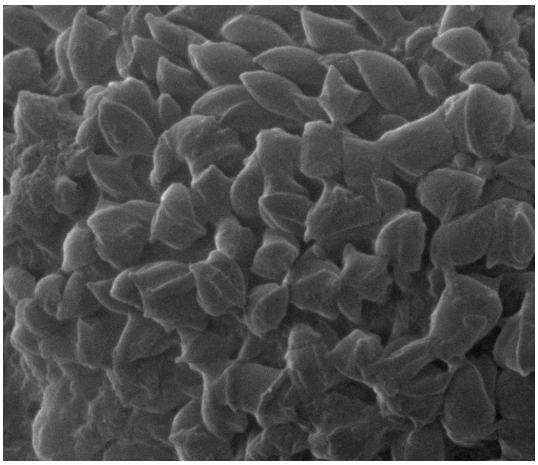


Figure 3. *Garcinia cymosa* SEM image 20,000 x magnification.

Garcinia dulcis pollen (Figures 4, 5 and 6) was almost spheroid with 6 apertures, just like *G. cymosa* but the colpi were not as deeply indented as in *G. cymosa* pollen grains. SEM investigation showed that the surface was psilate, almost entirely smooth but with small indentations.

When viewed under light microscope (Figure 7), the *Garcinia speciosa* pollen grains looked as if they had a prolate shape, but when viewed with SEM (Figures 8 and 9) they were seen to be spheroid. No apertures were apparent. In this investigation we were able to observe 12 *G. speciosa* pollen grains under SEM, and apertures were not visible on any of them. Seetharam (1989) reported that of the 300 *Garcinia* species he studied 76% had 3-longicolporate pollen, 33% had 4-6 longicolporate pollen, 28% had 3-porate pollen, 13% had 3-brevicolporate pollen, 10% had 4-6 porate (periporate) pollen, 6% had 4-6 brevicolporate pollen, and 5% had 4-6 porate pollen, 2% had meridional pollen and 1% had 3-colpate pollen, but Seetharam did not report finding any species with non-apeturate pollen.

Our sample of pollen from *Garcinia* sp. Accession 279 from Bogor Botanic Gardens, Indonesia (Figures 10, 11 and 12) showed rather spheroid to 3-lobed triangular shape, and no apertures were visible on the pollen grains from this specimen either. When viewed under light

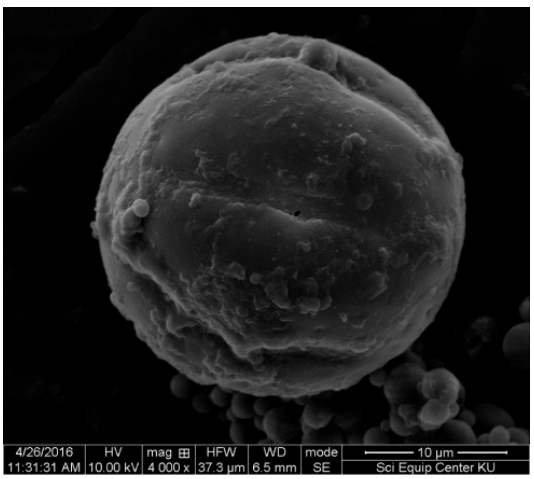


Figure 4. *Garcinia dulcis* SEM image 4,000 x magnification, equatorial view.

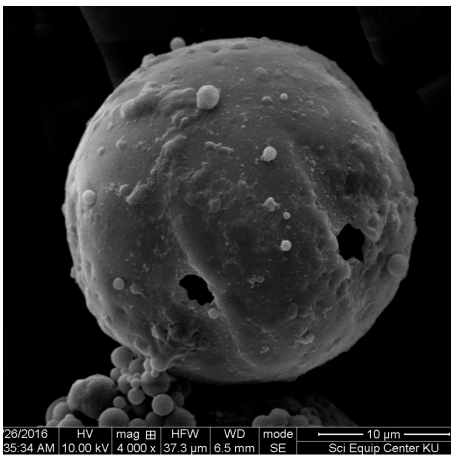


Figure 5. *Garcinia dulcis* SEM image 4,000 x magnification, angled view showing pores.

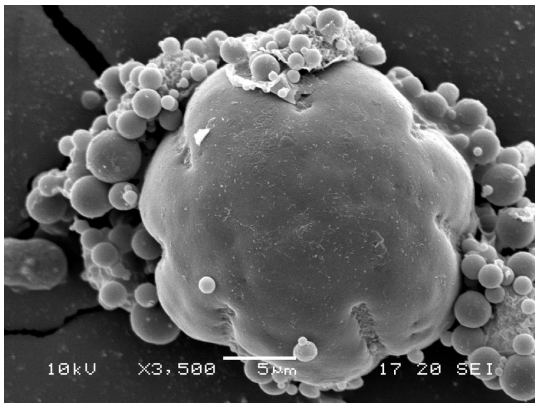


Figure 6. *Garcinia dulcis* SEM image 3,500 x magnification, polar view (globules are wax).

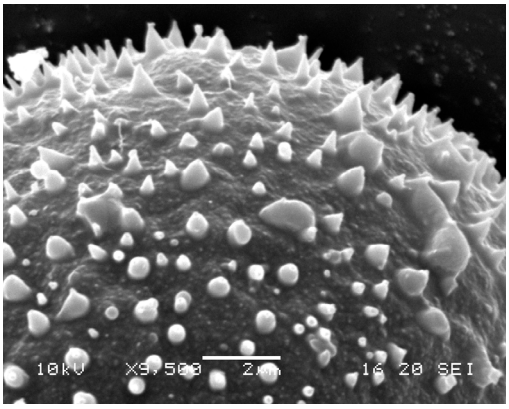


Figure 9. *Garcinia speciosa* SEM image 9,500 x magnification.

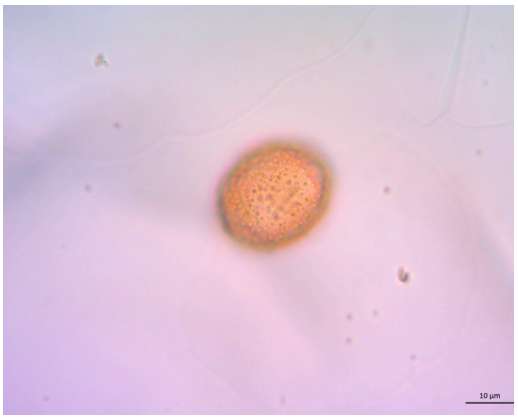


Figure 7. *Garcinia speciosa* LM image 400 x magnification.

Figure 10. Pollen of *Garcinia* sp. Accession 279 from Bogor Botanic Gardens LM image 400 x magnification.

Figure 8. *Garcinia speciosa* SEM image 3,700 x magnification.

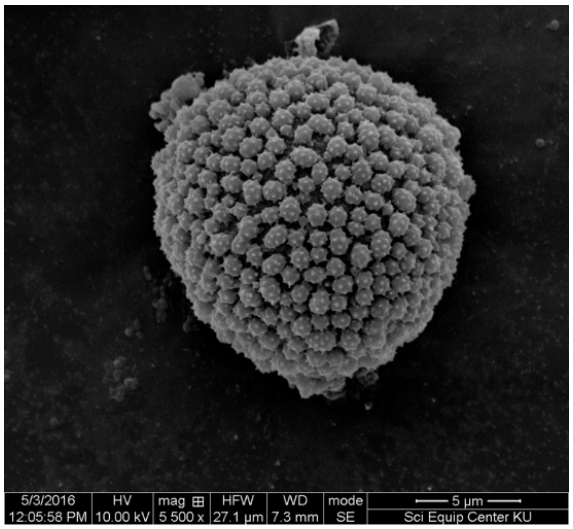


Figure 11. Pollen of *Garcinia* sp. Accession 279 from Bogor Botanic Gardens SEM image 5,500 x magnification.

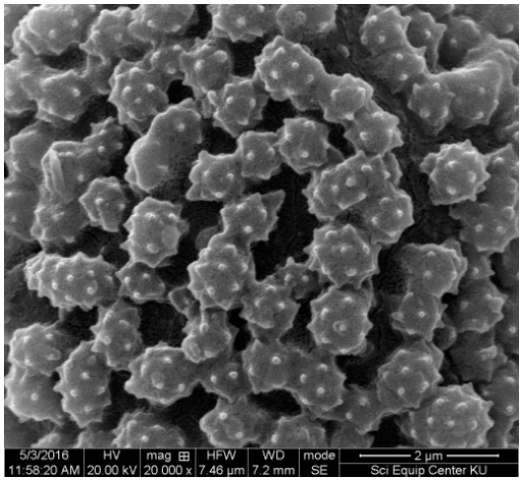


Figure 12. Exine sculpturing pattern of pollen of *Garcinia* sp. Accession 279 from Bogor Botanic Gardens, SEM image 20,000 x magnification.

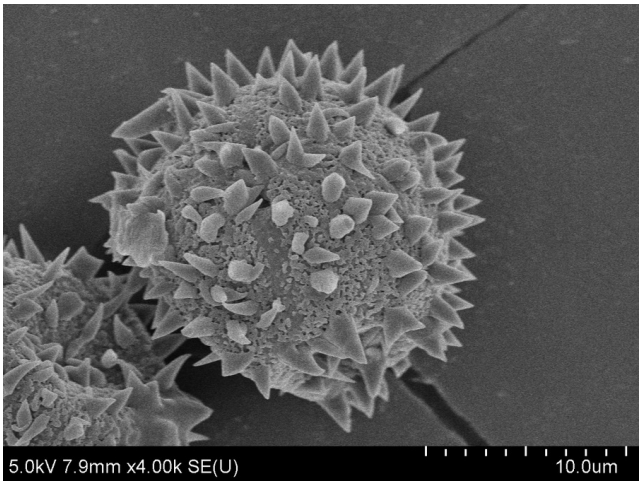


Figure 13. Pollen of *Garcinia* sp. Accession 283 from Bogor Botanic Gardens SEM image 4,000 x magnification.

microscope the exine sculpturing pattern of *Garcinia* sp. Accession 279 pollen grains could not be clearly seen, but using SEM another very interesting ornamentation texture was revealed. We have called this sculpturing pattern "gemmate-microechinate" because it is characterized by gemma, or protuberances of diverse shape and size, and on the surface of these gemma there are tiny spines. This is an unusual exine sculpturing pattern but it shares some similarities with the sculpturing pattern of *Garcinia cymosa* pollen.

The pollen grains of our sample from *Garcinia* sp. Accession 283 from Bogor Botanic Gardens, Indonesia (Figures 13 and 14) were spheroid and no apertures were visible. The exine sculpturing was echinate with large spines with pointed ends and rather broad at the base. The surface between spines was rugulate to gemmate. The sculpturing was similar to the pollen of *G. latissma* as reported by Seetharam (1989).

Like *Garcinia* sp. Accession 283, the pollen from our sample of *Garcinia* sp. Accession 287 from Bogor Botanic Gardens, Indonesia, (Figures 15 and 16) was more or less spheroid (the sample had been stored for a long time and was probably deformed). No apertures were visible. Exine sculpturing was echinate with rather small spines spaced widely apart. The spines were not erect, and most had curved tips, which could have been a result of damage or long term storage of the pollen grains. The exine surface in between spines was verrucate. It looks similar to the pollen grains of *G. tracancorica* in the report of Seetharam (1989).

The pollen grains of our sample from *Garcinia* sp. Accession 290 from Bogor Botanic Gardens, Indonesia, (Figure 17) were spheroid and no apertures were visible. Exine sculpturing was echinate with rather large spines with pointed tips and broad bases. Unfortunately, no SEM photos were obtained from this sample so the surface sculpturing pattern between spines cannot be described. The sculpturing is similar to that of *Garcinia* sp. Accession 283 from Bogor Botanic Gardens, Indonesia, described above.

Lastly, we have included an SEM image from another sample of an unidentified *Garcinia* species from Bogor Botanic Gardens, Indonesia (Figure 18). It was unidentified because V.S. made a data recording mistake and no accession number was written on the package, but the only samples that were collected were *Garcinia* so it should still be representative of the genus. The pollen from this sample was also

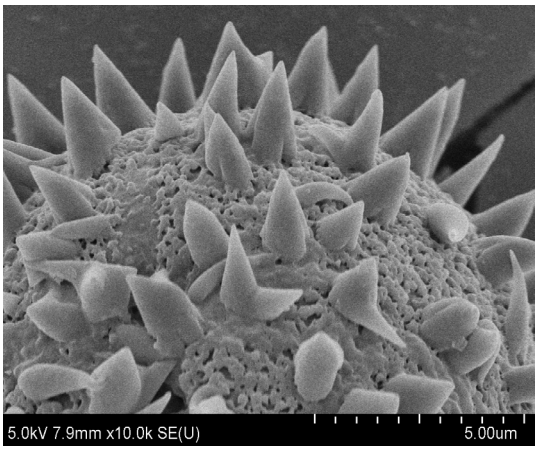


Figure 14. Pollen of *Garcinia* sp. Accession 283 from Bogor Botanic Gardens SEM image 10,000 x magnification.

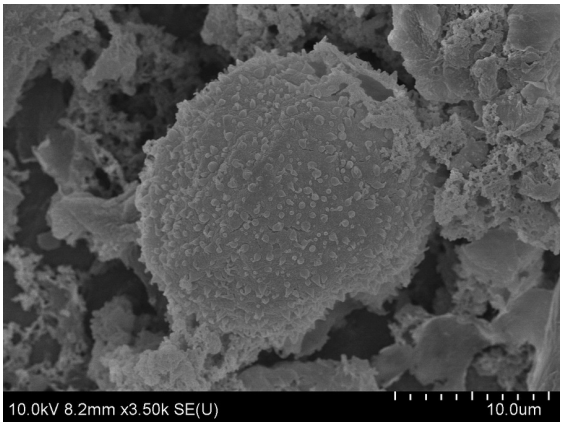


Figure 15. Pollen of *Garcinia* sp. Accession 287 from Bogor Botanic Gardens SEM image 3,500 x magnification.

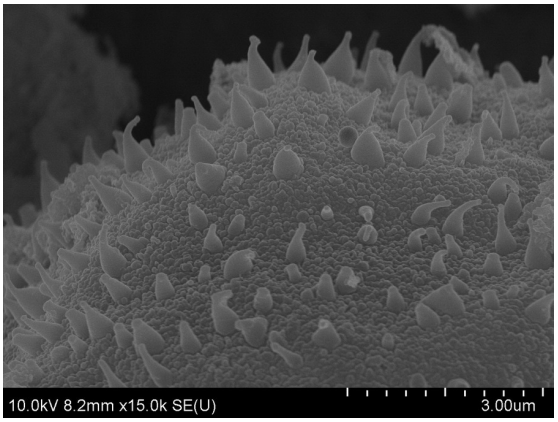


Figure 16. Exine sculpturing of the pollen of *Garcinia* sp. Accession 287 from Bogor Botanic Gardens SEM image 15,000 x magnification.

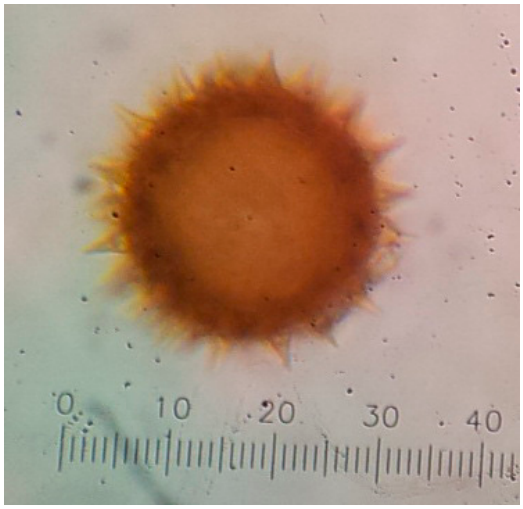


Figure 17. Pollen of *Garcinia* sp. Accession 290 from Bogor Botanic Gardens light microscope image 400 x magnification.

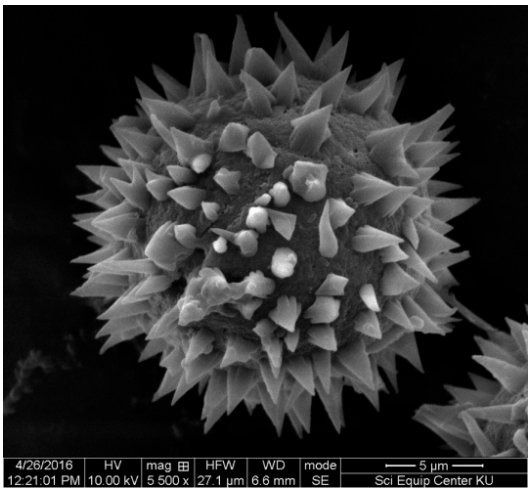


Figure 18. Pollen of unidentified *Garcinia* sp. from Bogor Botanic Gardens SEM image 4,000 x magnification.

spheroid and no apertures were visible. Exine sculpturing was echinate with very large spines with pointed tips and broad bases. Not all the spines were uniform, as some were divided with concave ridges and others were entire. The sculpturing is similar to that of *Garcinia* sp. Accession 283 from Bogor Botanic Gardens, Indonesia, and *Garcinia* sp. Accession 290 from Bogor Botanic Gardens, Indonesia, but the spines were larger.

In conclusion, the pollen grains of the *Garcinia* species studies were all small sized; the smallest measuring about 12 x 19 µm and the largest 25 x 27 µm. All were spheroid or sub-spheroid. From our observed samples, 6 of the species were likely non aperturate because no apertures were visible, while the other 2 species were 6-colporate.

DISCUSSION

There was great diversity of sculpturing patterns observed in this genus. Our very limited sampling showed 3 species with echinate sculpturing, 2 that were psilate, 1 that was micro echinate, 1 with rugulate sculpturing, and 1 that we have characterized as "gemmate-micro echinate." The most unusual sculpturing pattern observed in this study was the latter (gemmate-micro echniate), on *Garcinia* sp. Accession 279 from Bogor Botanic Gardens, Indonesia.

Only a few *Garcinia* species were studied in this research and complete data were not obtained. Many of the species were not positively identified but labelled only with an accession number. However, our observations were for the most part consistent with the findings of Seetharam (1989) [3], who reported that of the 300 species he studied, 63% of the pollen grains were spheroidal in shape. All of the samples in the present research were found to be spheroidal to subspheroidal.

Seetharam reported that 28% of the species he studied had pollen grains that were 4-6 longicolporate. In the present research, 2 species were found to have 6-longicolporate pollen grains, but on the other 6 species no apertures were visible, which is in contrast to the previous research findings, as Seetharam did not report any non aperturate species.

As for research on the pollen of other genera in Family Clusiaceae, Premathilake and Nilsson (2001) [6] reported that the pollen of *Calophyllum walker* was 35-41 x 33-42 µm (larger than the *Garcinia* pollen observed in the present research), 3-colporate, and with scabrate-perforate sculpturing.

In a report on 10 species in genus *Tovomita*, Marinho *et al.* [7] reported that the size range was medium size, Equatorial 15-30 µm x Polar 17-42 µm, which is not much larger than the *Garcinia* pollen observed in the present research. All of the *Tovomita* pollen were observed to be tripcolporate, however, which was different from the *Garcinia* species samples we observed. As for sculpturing pattern, Marinho and colleagues divided the 10 *Tovomita* species into 5 groups, namely 1) tectate, psilate (1 species); 2) tectate, psilate-rugulate (1 species); 3) semitectate, perforate (3 species); 4) semitectate, foveolate (4 species); and 5) semitectate, microreticulate (1 species). Marinho *et al.* [7] wrote: "The pollen grains of Clusiaceae s.l. show wide diversities of ornamentation patterns and numbers of apertures, even among species in the same genus – which characterizes as a eurypalynous family."

The findings from our observations of 8 *Garcinia* species support this conclusion.

CONCLUSION

In terms of shape (spheroid) and size (small), the pollen grains of *Garcinia* species studied were similar to previous reports. Apertures were not visible on several of the samples studied, which was different from previous reports. In terms of surface ornamentation, a wide variety was observed, which is consistent with other studies that found *Garcinia* to be a eurypalynous genus, within Clusiaceae, a euryplaynous family.

ACKNOWLEDGEMENTS

Thanks to Mr. Pichai Sirisangsawang for assistance with SEM operation and to Assistant Professor Dr. Chalermpol Suwanphakdee and Mr. Charernsak Puhin at the Botany Department, Faculty of Science, Kasetsart University, for guidance in light microscopy and acetolysis techniques.

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