

# Pollen morphology and infrageneric classification of selected *Callicarpa* species (Lamiaceae) from the Philippines and Borneo

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**Abstract.** Danila JS, Alejandro GJD. 2020. Pollen morphology and infrageneric classification of selected *Callicarpa* species (Lamiaceae) from the Philippines and Borneo. *Biodiversitas* 21: 5736-5746. We used pollen grains of ten species representing the controversial genera *Geunsia* Blume and *Callicarpa* L. from the key centers of Malesia - Philippines, and Borneo including other countries in Asia. Herbarium specimens and collected samples from the field were examined using Scanning Electron Microscopy (SEM) in search of new characters to establish conclusive evidence that might contribute to the study of the relationship between *Geunsia* and *Callicarpa*. Based on SEM observations, both the investigated pollen grains of *Geunsia* and *Callicarpa* are mostly small to medium, spheroidal or circular, prolate to oblate, isopolar, and tricolpate. Moreover, the exine surface is mostly coarsely reticulated with a thin perforated colpus edge and sunken finely granulated colpus membrane. However, exine ornamentation shows possible separation of *Geunsia* and *Callicarpa* due to various morphological results observed. Several types of exine ornamentation were found in species of the section *Callicarpa*, i.e., *C. erioclona*, *C. arborea*, *C. macrophylla*, and *C. candicans* which attributes in the projected separation of these species while four species in the section *Geunsia* are united as a group having coarsely reticulate exine. Surprisingly, *C. arborea* samples give additional support to the hypothesis that rugulate exine sculpture might be its plesiomorphic character due to evolutionary evidence of other exine types of this species. On the other hand, pollen size and shape class of the sections of *Geunsia* and *Callicarpa* were both observed with similar features having an average pollen size equivalent to medium and a prolate shape supporting the concept of previous studies to nest *Geunsia* within *Callicarpa*. This reveals that pollen morphology is useful in the study of infrageneric classification within these groups. However, further morphological studies involving larger number of specimens are needed especially in the study of exine to prove its consistency as diagnostic character in the study of phylogenetic relationship.

**Keywords:** Exine, *Geunsia*, palynology, phylogeny, SEM

## INTRODUCTION

*Callicarpa* L. is among the largest genera of the family Lamiaceae with approximately 170 shrub species (Li and Olmstead 2017). The genus is characterized by hairs branched/stellate (simple); inflorescences axillary; flowers polysymmetric, 4(-5) merous; anthers porose; stigma peltate or capitate; and fruit a drupe (Linnaeus, 1753). These plants are made up of an incredibly diverse group of species in terms of their ecology, morphology, and geographical distribution. Among those species that illustrate comparable distributional patterns of *Callicarpa* were found in key centers of Malesia - the Philippines and the island Borneo having 27 and 23 species, respectively (Pelser et al. 2011; Bramley 2013).

Since Linnaeus (1753) established *Callicarpa*, the struggle of taxonomic revision continued due to inconsistency in its infrageneric classification. After several group modifications (Murray 1774; Lamark 1783, 1791; Gaertner 1791; Loureiro 1783; Rauschel 1797), *Callicarpa* has been assigned to a more confident family Verbenaceae (Brown, 1810). In support of Verbenaceae, Briquet (1895) further used calyx characters to form two distinct groups either Tubulosae (tubular) or Cyathimorphae (campanulate) calyx. Furthermore, Chang

(1951) used stamen characters to distinguish either as *Eucallicarpa* with small, ovate, longitudinally dehiscent anthers or *Verticirima* with larger oblong anthers. Later, more infrageneric classifications were established such as Fang's (1982) division of species based on hair types classified as two subgenera: Subgen. *Peiantha* is characterized by simple cymes and curved hair while Subgen. *Callicarpa* with various hair types. Despite all these proposed morphological classifications, *Callicarpa* has been formally transferred and settled in the family Lamiaceae based on floral morphological characters and DNA sequences (Thorne 1992; Cantino et al. 1992). However, the circumscription of the genus *Callicarpa* remains uncertain due to overlapping characters, e.g., stamen and hair types (Chang 1951; Fang 1982). Also, Abraham-Oanes (2002) and Ma et al. (2015) attempted to compare these characters vs pollen morphology but failed to include species from wider samples in the Malesian region.

Several taxonomists like Raj (1983), Wei (2003), and Perveen and Qaiser (2007) attempted to classify *Callicarpa* species through morphology. But the data appears inadequate when only a few species were described. Liu (1985) improved the number of collections, but most samples were examined under a light microscope. In the

study conducted by Ma et al. (2015), pollen grains of *Callicarpa* were studied to determine the taxonomic position of *Callicarpa* but the samples were limited only to *Callicarpa* sect. *Eucallicarpa* and *Verticirima* and involves no samples of Philippine *Callicarpa*.

Later, several taxonomists investigated that *Callicarpa* shows affinities with genus *Geunsia*, distinguished in Blume's (1823) *Catalogus* based on its first known sample, *G. farinosa* Blume. Investigations includes Briquet (1897) testing floral parts either teramerous and pentamerous contradict the relationship of *Callicarpa* and *Geunsia* while Lam (1919), Ridley (1923), Moldenke (1981) considered *Geunsia* and *Callicarpa* as related to each other along with Schauer (1847), Lam and Bakhuizen van den Brink (1921), van Steenis (1967), Burt (1969) who treated the two genera as exactly like the other. Currently, the long-established *Geunsia* has been regarded as synonyms to genus *Callicarpa* (Lam and Bakhuizen van den Brink 1921; Govaert et al. 2007). Due to morphological questionings from leaf arrangement and petiole to the ovary (Lam 1919), section *Geunsia* has been tested its relationship to section *Eucallicarpa* and *Verticirima* (Bramley 2009) suggesting that its relationship between groups were not resolved due to paraphyly of species in the section *Callicarpa*. Lam (1919) and Chang (1951) proposed morphological characters used to distinguished sections *Callicarpa* and *Geunsia* which turn into weak evidence because characters e.g., characters of leaf arrangement and anther appear in numerous sections.

Palynological characters became a trend in classifying species worldwide. Cantino and Sanders (1986), Abu-Asab

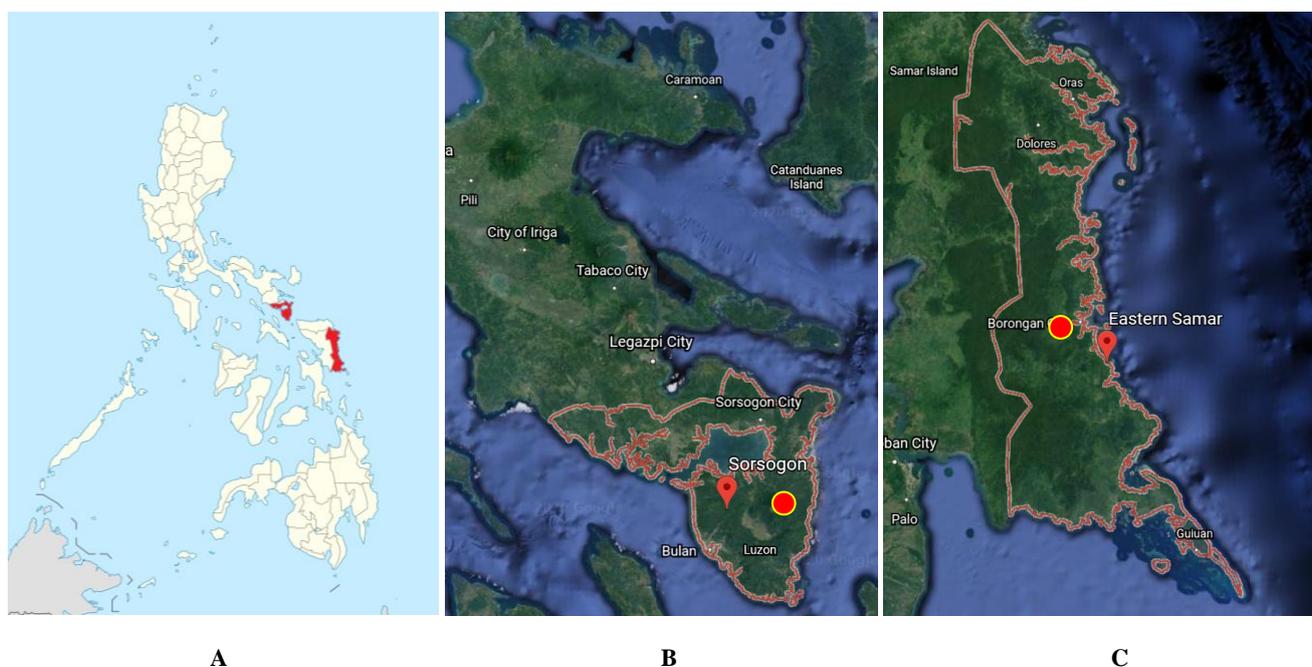
and Cantino (1993), Harley (2004), and Celenk et al. (2008) upholds the study of pollen grain as a useful tool in the classification of the members of the family Lamiaceae. However, previous palynological works of *Callicarpa* by Abraham-Oanes (2002) and Ma et al. (2015) constitute only collections from Malaysia and China, respectively, while neither species from the Philippines nor other parts of Borneo were used in palynology. Thus, immediate palynological reinvestigation to cover species from these two lands is necessary as Ma et al. (2015) pointed out the necessity of the further detailed study of non-Chinese species for a better understanding of *Callicarpa* taxonomic character.

The objective of this paper is to provide a detailed description of the pollen morphology of *Callicarpa* from the Philippines and Borneo using scanning electron microscopy (SEM). Likewise, it will identify the relevant diagnostic character and be able to use this palynological information in the infrageneric classification of *Callicarpa* and its relationship to *Geunsia*.

## MATERIALS AND METHODS

### Study area

Field studies was conducted in Mt. Bulusan, Sorsogon Province and Samar Island Natural Park (SINP), Eastern Samar Province, Philippines to collect pollen grains from fresh inflorescence of *Callicarpa* species (Figure 1).



**Figure 1.** Map of the Philippines (right) showing: A. Mt. Bulusan in Sorsogon Province, B. Samar Island Natural Park (SINP), Samar Province where field collection took place (red dots).

### Plant materials

Pollen grains were mostly taken from the dried specimen from herbarium in Singapore (SIN) while some were collected in a form of fresh inflorescence. Pollen of 10 of 15 taxa was used in scanning electron microscopy. Three to five individuals per species from herbarium were used to represent species of Philippines and Borneo including some samples from India (*C. arborea* Roxb. and *C. macrophylla* Vahl.) and China (*C. candicans* (Burm.f.) Hochr.) for which these samples are included in Bramley (2009) species in Borneo and essential for comparison.

### Procedures

Pollen grains of both herbarium and fresh samples were acetolyzed following combined procedures of Erdtman (1952), Hou (1969), Nilsson (2000), and Hesse et al. (2018). The acetolyzed pollen samples were dehydrated in an ethanol series and mounted in aluminum stubs with double-sided adhesive tape. Pollen grains were described using Hitachi TM-3000 Tabletop Microscope operated at 15 KV at the UST Research Center for the Natural and Applied Sciences (RCNAS). The polar axis and the equatorial axis were measured. The characterization of the pollen of each species like the shape, outline in polar view, and size range was determined by calculating the equatorial diameter (P/E) ratios. Most of the measurements were obtained using the Image J software. Moreover, pollen ornamentation, polarity, amb, aperture type, colpus edge, colpus membrane, exine type, and pollen units were determined. Pollen terminology is based on Punt et al. (2007) descriptions.

## RESULTS AND DISCUSSION

Materials examined for pollen morphological studies of *Callicarpa* and *Geunsia* species are provided in Table 1, while pollen size and shape measurements are presented in Table 2. Representative pollen grains are illustrated in Figures 2–9.

### General description of pollen of *Callicarpa*

Pollen grains of *Callicarpa* is monad, spheroidal while some are circular. Its apertures are typically tricolpate and rarely inaperturate. The sizes of the grain range from small (10–25 µm) to medium size (26–50 µm). The shape classes are mostly subprolate (1.14–1.33 µm) to prolate (1.33–2.00 µm) where areas in some species appear oblate spheroidal (0.88–1.00 µm) and subspheroidal (0.75–1.33 µm) sometimes with irregular infoldings. The number of apertures is mostly 3 but there are recorded species without apertures. The polarity of pollen is mostly isopolar. The outline (amb) demonstrated several types from circular, polygonal to elliptical contour of the pollen either in polar or equatorial view. The colpus has a thin and perforated edge while either sunken, finely or coarsely granulated but rarely raised, coarsely granulated colpus membrane. The exine exhibits reticulated ornamentation either rugosely reticulate, rugulate, micro reticulate, or coarsely reticulate forming muri with lumina.

**Table 1.** Materials examined for pollen morphological studies of *Callicarpa* and *Geunsia* species

Species	Localities	Acc. no.
<i>Callicarpa</i> species		
<i>C. arborea</i> Roxb.	India	N/A
<i>C. arborea</i> Roxb.	Java, Indonesia	507180
<i>C. arborea</i> Roxb.	Samar, Philippines	JDS001
<i>C. candicans</i> (Burm.f.) Hochr.	Kwangsi, China	451
<i>C. erioclona</i> Schauer	Sorsogon, Philippines	SOR006
<i>C. erioclona</i> Schauer	Bataan, Philippines	4142
<i>C. macrophylla</i> Vahl	India	28
<i>C. pedunculata</i> R. Br.	Aurora, Philippines	JDA001
<i>C. pedunculata</i> R. Br.	Albay, Philippines	18195
<i>Geunsia</i> species		
<i>C. anomala</i> (Ridl.) B.L. Burt	Sarawak, Malaysia	0035867
<i>C. pentandra</i> Roxb.	Selangor, Malaysia	101082
<i>C. scandens</i> (Moldenke) Govaerts	Sabah, Malaysia	144078
<i>G. cumingiana</i> Schauer	Panay, Philippines	31179
<i>G. cumingiana</i> Schauer	Albay, Philippines	18424
<i>G. farinosa</i> Roxb. ex C.B Clarke	Sabah, Malaysia	0152106

### Species-level pollen descriptions for *Callicarpa*/*Geunsia*

#### *Callicarpa* species

*C. arborea* Roxb. (Figure 2 A–J; India)

Pollen unit: monad, size (pollen unit): small to medium-sized (10–25 µm to 26–59 µm), pollen class: tricolpate, polarity: heteropolar, shape: suboblate, oblate spheroidal to oblate and prolate spheroidal, subprolate to prolate, outline in polar view: circular to spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: regulate to microreticulate.

*C. arborea* Roxb. (Figure 2, K; Java, Indonesia)

Pollen unit: monad, size (pollen unit): medium-sized (26–59 µm), pollen class: tricolpate, polarity: heteropolar, shape: subprolate spheroidal, outline in polar view: prolate infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

*C. arborea* Roxb. (Figure 2, L–N; Samar Philippines)

Pollen unit: monad, size (pollen unit): medium-sized (26–59 µm), pollen class: inaperturate, polarity: -, shape: subprolate, outline in polar view: spheroidal, infoldings: not infolded, aperture number: none, aperture type: no aperture, aperture condition: inaperturate, aperture peculiarities: - colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: rugosely reticulate.

*C. candicans* (Burm. F.) Hochr. (Figure 3, A–C; Kwangsi, China)

Pollen unit: monad, size (pollen unit): small to medium-sized (10–25 to 26–59  $\mu\text{m}$ ), pollen class: tricolpate, polarity: isopolar, shape: prolate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: micro reticulate.

*C. erioclona* Schauer (Figure 4, A–B; Sorsogon, Philippines)

Pollen unit: monad, size (pollen unit): small-sized (10–25  $\mu\text{m}$ ), pollen class: inaperturate, polarity: -, shape: oblate, outline in polar view: spheroidal, infoldings: not infolded, aperture number: none, aperture type: no aperture, aperture condition: inaperturate, aperture peculiarities: - colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: rugosely reticulate.

*C. erioclona* Schauer (Figure 4, C–G; Bataan, Philippines)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: tricolpate, polarity: isopolar, shape: oblate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

*C. macrophylla* Vahl. (Figure 5, A–H, Figure 6 A–C; India)

Pollen unit: monad, size (pollen unit): small to medium-sized (10–25 to 26–59  $\mu\text{m}$ ), pollen class: tricolpate, polarity: isopolar, shape: prolate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: rugosely reticulate to rugulate

*C. pedunculata* R. Br. (Figure 7, A–B; Aurora, Philippines)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: colpate, polarity: heteropolar, shape: subprolate to prolate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three armed furrows like aperture, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

*C. pedunculata* R.Br. (Figure 7, C–H; Albay, Philippines)

Pollen unit: monad, size (pollen unit): small-sized (10–25  $\mu\text{m}$ ), pollen class: tricolpate, polarity: isopolar to heteropolar, shape: prolate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane

ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: microreticulate.

### *Geunsia species*

*C. anomala* (Ridl.) B.L. Burt (Figure 8, A–B; Sarawak, Malaysia)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: tricolpate, polarity: heteropolar, shape: prolate, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

*C. pentandra* Roxb. (Figure 8, C–D, Figure 9 A; Selangor, Malaysia)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: colpate, polarity: isopolar, shape: prolate spheroidal to oblate spheroidal, outline in polar view: circular, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, raised granulate ornamentation: coarsely reticulate.

*C. scandens* (Moldenke) Govaerts (Figure 9 B, F; Sabah, Malaysia)

Pollen unit: monad, size (pollen unit): small-sized (10–25  $\mu\text{m}$ ), pollen class: colpate, polarity: heteropolar, shape: prolate, outline in polar view: elliptic, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture colpus edge: thin perforate colpus membrane: sunken, finely granulate membrane ornamented ornamentation: coarsely reticulate.

*G. cumingiana* Schauer (Figure 9, C–E; Panay, Philippines)

Pollen unit: monad, size (pollen unit): small-sized (10–25  $\mu\text{m}$ ), pollen class: tricolpate, polarity: heteropolar, shape: prolate to oblate spheroidal, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: rugulate.

*G. cumingiana* Schauer (Figure 9, F–G; Albay, Philippines)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: tricolpate, polarity: isopolar, shape: prolate spheroidal, outline in polar view: circular, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

*G. farinosa* Roxb. ex C.B Clarke (Figure 9, H–M; Sabah, Malaysia)

Pollen unit: monad, size (pollen unit): medium-sized (26–59  $\mu\text{m}$ ), pollen class: sulcate to tricolpate, polarity: isopolar, shape: oblate spheroidal to prolate spheroidal, outline in polar view: spheroidal, infoldings: aperture(s) sunken, aperture number: three, aperture type: colpus, aperture condition: colpate, aperture peculiarities: aperture membrane ornamented colpus edge: thin perforate colpus membrane: sunken, finely granulate ornamentation: coarsely reticulate.

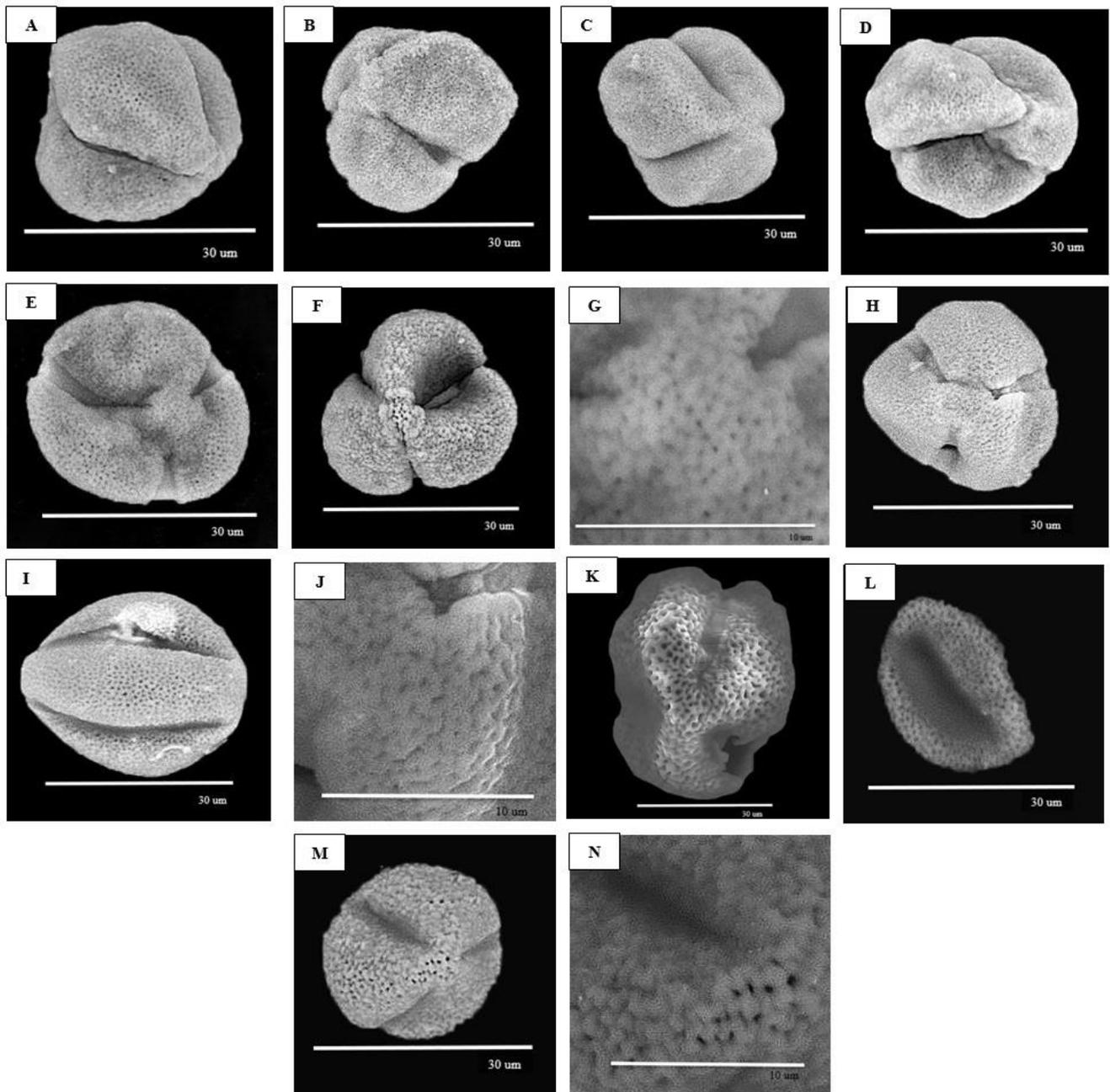
The investigated pollen grains of *Callicarpa* species were studied for the first time except for *C. arborea*, *C. candicans*, *C. macrophylla*, and *C. pedunculata* which were previously described by Ma et al. (2015) in the collection of *Callicarpa* in China. However, the authors of this study used non-Chinese samples of the above-mentioned species except *C. candicans* which was originated in Kwangsi, China. The samples used in this study are mostly collected from Singapore herbarium (SIN) covering a wider range of samples from the Philippines and Borneo including few collections from India and China. Based on SEM observations, most of the investigated pollen of the sections *Geunsia* and *Callicarpa* were medium in size but few small-sized pollens were also observed. Palynological measurements including polar axis (P), equatorial axis (E), and P/E ratio were tabulated to identify the pollen size of each sample based on Erdtman (1971) and Halbritter (2018) pollen size category. Pollen size range (Table 2) in the sections *Callicarpa* (P: 24.00–41.88  $\mu\text{m}$  E: 21.34–33.67  $\mu\text{m}$  P/E: 0.87–1.29  $\mu\text{m}$ ) and *Geunsia* (P: 24.22–37.25  $\mu\text{m}$  E: 18.74–35.25  $\mu\text{m}$  P/E: 0.97–1.36  $\mu\text{m}$ ) showed little or no significant differences in all quantitative characters. The smallest pollen grain of the section *Callicarpa* was recorded in *C. macrophylla* (26.04

$\times$  21.34  $\mu\text{m}$ ) while the largest in *C. candicans* (33.20  $\times$  26.20  $\mu\text{m}$ ). On the other hand, section *Geunsia* showed *C. scandens* (25.42  $\times$  18.74  $\mu\text{m}$ ) and *G. farinosa* (37.25  $\times$  35.25  $\mu\text{m}$ ) produced the smallest and largest pollen grain respectively. Since *Callicarpa* and *Geunsia* appear almost equal in the pollen size category thus, pollen size does not support the supposition that *Callicarpa* species is distinct from *Geunsia* species.

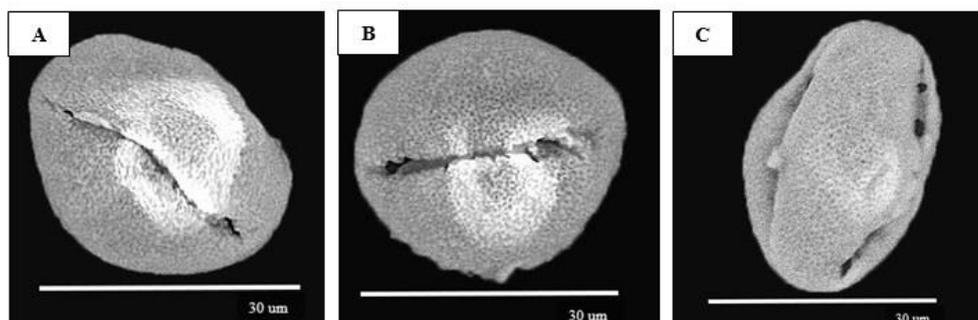
The shape class of the pollen grain was obtained by computing the ratio of the polar (P) and equatorial (E) axis (Erdtman 1952). The study shows that most of the pollen grains are prolate with polar and equatorial axis ratios (P/E) equals to 1.33–2.00  $\mu\text{m}$  shown in species of *C. pedunculata* (Figure 7.A–H), *C. scandens* (Figure 9.B), *G. farinosa* (Figure 9.H–M), *C. anomala* (Figure 8.A–B), *C. macrophylla* (Figure 5.A–H, Figure 6.A–C), and *C. candicans* (Figure 3.A–C). Other shape class includes oblate found in *C. erioclona* (Figure 4.A–G) with P/E: 0.88–1.00  $\mu\text{m}$  while remaining species like *C. pentandra* (Figure 8.C–D, Figure 9.A), *C. arborea* (Figure 2.A–N) and *G. cumingiana* (Figure 9.C–G) have shape variations from oblate (P/E: 0.50–0.75  $\mu\text{m}$ ), suboblate (P/E: 0.75–0.88  $\mu\text{m}$ ), oblate spheroidal (P/E: 0.88–1.00  $\mu\text{m}$ ), prolate spheroidal (P/E: 1.00–1.14  $\mu\text{m}$ ), subprolate (P/E: 1.14–1.33  $\mu\text{m}$ ), and prolate (P/E: 1.33–2.00  $\mu\text{m}$ ). According to Sebsebe and Harley (1992) and Ma et al. (2015), differences in the variations of size are due to acetolysis treatment when the colpal membranes are easily destroyed from hydration and fixation. To reduce destruction in the colpal membranes of highly inconsistent species, *C. pentandra*, *C. arborea*, and *G. cumingiana*, trial and error procedure of Hou (1969) was undertaken in this study to control the acetolysis procedure to reduce the chance of getting too dark images that affect features for observation.

**Table 2.** Pollen grain size and shape measurements of *Callicarpa* and *Geunsia* species

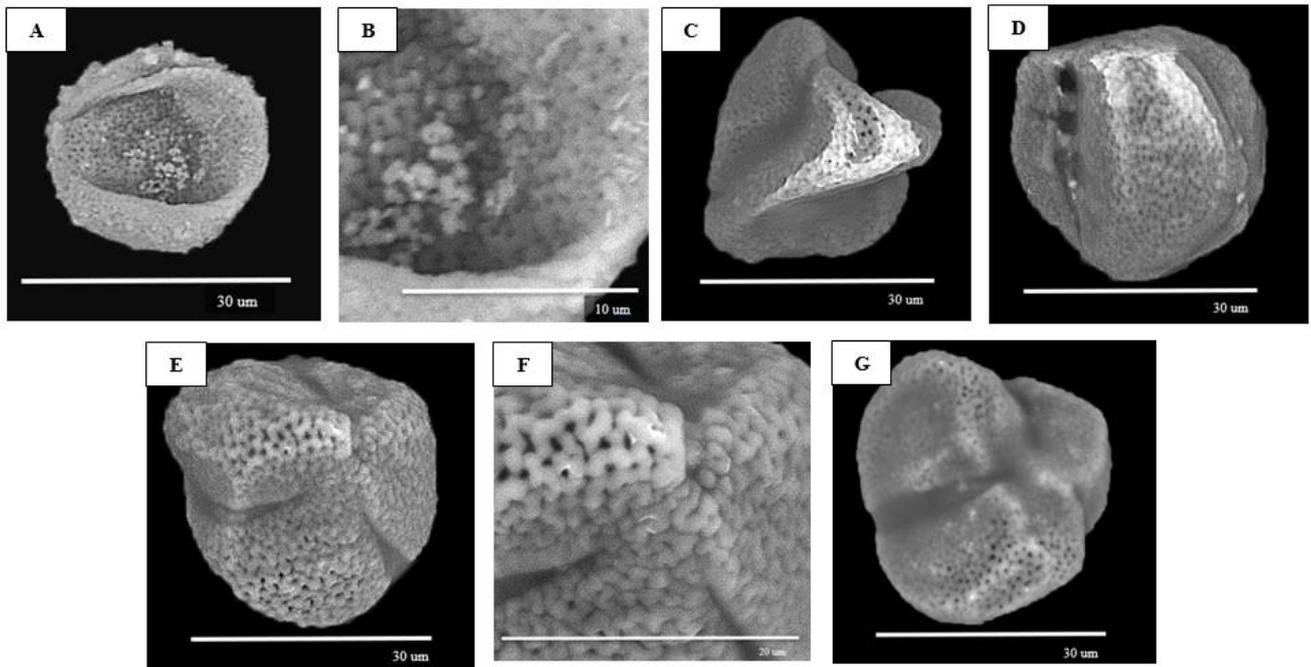
Species	P-value ( $\mu\text{m}$ )	E value ( $\mu\text{m}$ )	P/E ( $\mu\text{m}$ )	Size
<i>Callicarpa</i> species				
<i>C. arborea</i> Roxb.	26.66 (15.98–38.24)	27.10 (23.91–30.31)	0.98 (0.59–1.46)	Medium
<i>C. arborea</i> Roxb.	41.88 (41.88–41.88)	32.55 (32.55–32.55)	1.29 (1.29–1.29)	Medium
<i>C. arborea</i> Roxb.	25.24 (23.15–27.32)	21.74 (19.13–24.34)	1.19 (0.95–1.43)	Medium
<i>C. candicans</i> (Burm.f.) Hochr	33.20 (31.71–36.08)	26.20 (24.05–29.85)	1.28 (1.07–1.50)	Medium
<i>C. erioclona</i> Schauer	24.00 (24.00–24.00)	23.78 (23.78–23.78)	1.04 (1.04–1.04)	Small
<i>C. erioclona</i> Schauer	29.29 (21.54–32.63)	33.67 (32.44–35.82)	0.87 (0.60–0.99)	Medium
<i>C. macrophylla</i> Vahl	26.04 (16.93–30.08)	21.34 (15.98–28.83)	1.28 (0.91–1.88)	Small
<i>C. pedunculata</i> R. Br.	36.55 (31.87–41.23)	25.67 (24.37–26.97)	1.42 (1.31–1.53)	Medium
<i>C. pedunculata</i> R. Br.	23.57 (22.11–25.26)	15.54 (12.90–17.65)	1.53 (1.39–1.64)	Small
Mean values	29.60	25.29	1.21	
<i>Geunsia</i> species				
<i>C. anomala</i> (Ridl.) B. L. Burt	43.55 (37.55–41.88)	35.16 (28.82–41.51)	1.25 (1.19–1.30)	Medium
<i>C. pentandra</i> Roxb.	32.93 (31.21–35.13)	32.77 (30.61–34.03)	1.01 (0.92–1.06)	Medium
<i>C. scandens</i> (Moldenke) Govaerts*	25.42 (25.42–25.42)	18.74 (18.74–18.74)	1.36 (1.36–1.36)	Small
<i>G. cumingiana</i> Schauer	24.22 (23.34–25.10)	19.22 (17.55–20.89)	1.27 (1.20–1.33)	Small
<i>G. cumingiana</i> Schauer	29.69 (26.37–33.01)	30.53 (29.50–31.56)	0.97 (0.89–1.05)	Medium
<i>G. farinosa</i> Roxb. ex C.B Clark	37.25 (27.12–44.10)	35.25 (29.94–45.34)	1.08 (0.72–1.30)	Medium
Mean values	32.18	28.61	1.16	



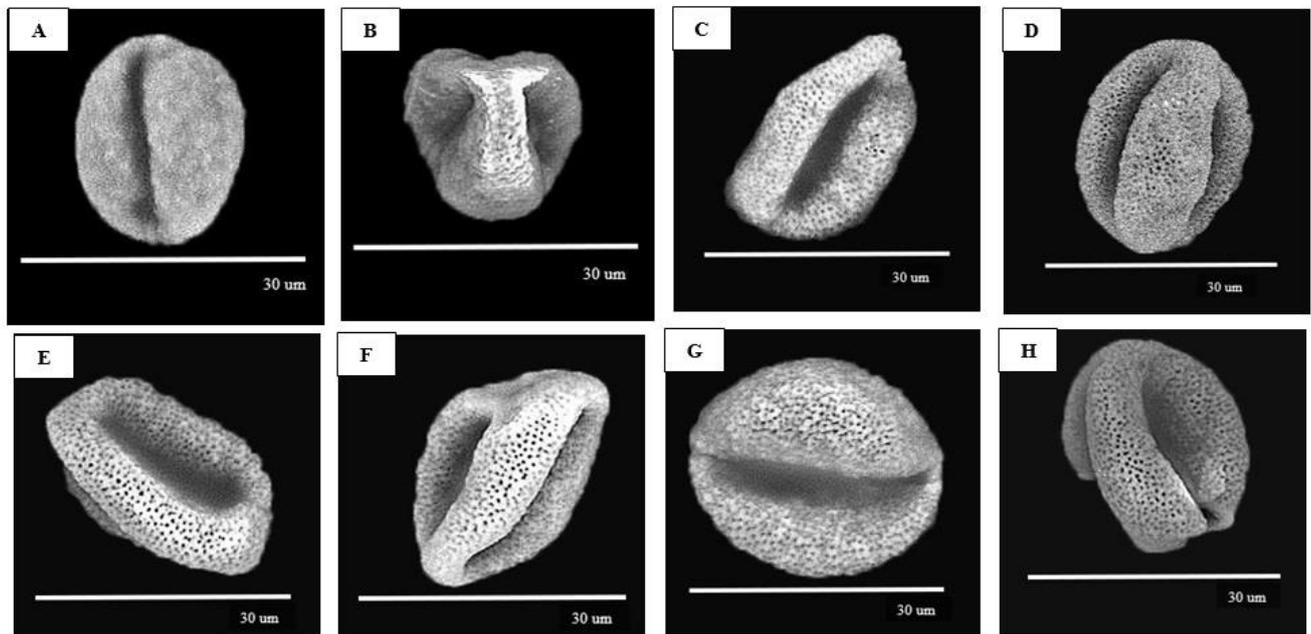
**Figure 2.** SEM micrographs of pollen grains of *Callicarpa*. A–J: *C. arborea* (India): (a– d) equatorial view (e–f) polar view (g) exine surface (h) polar view (i) equatorial view K: *C. arborea* (Java, Indonesia) equatorial view. L–N: *C. arborea* (Samar, Philippines) (l) equatorial view (m) polar view (n) exine surface



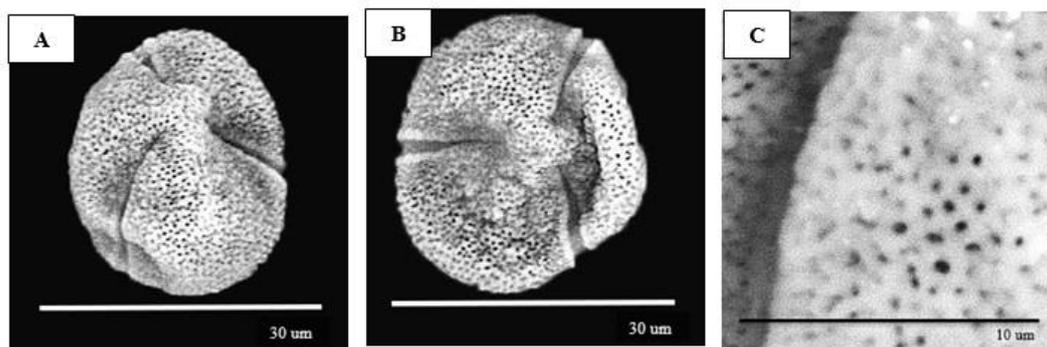
**Figure 3.** SEM micrographs of pollen grains of *Callicarpa*. surface A–C: *C. candicans*, equatorial view



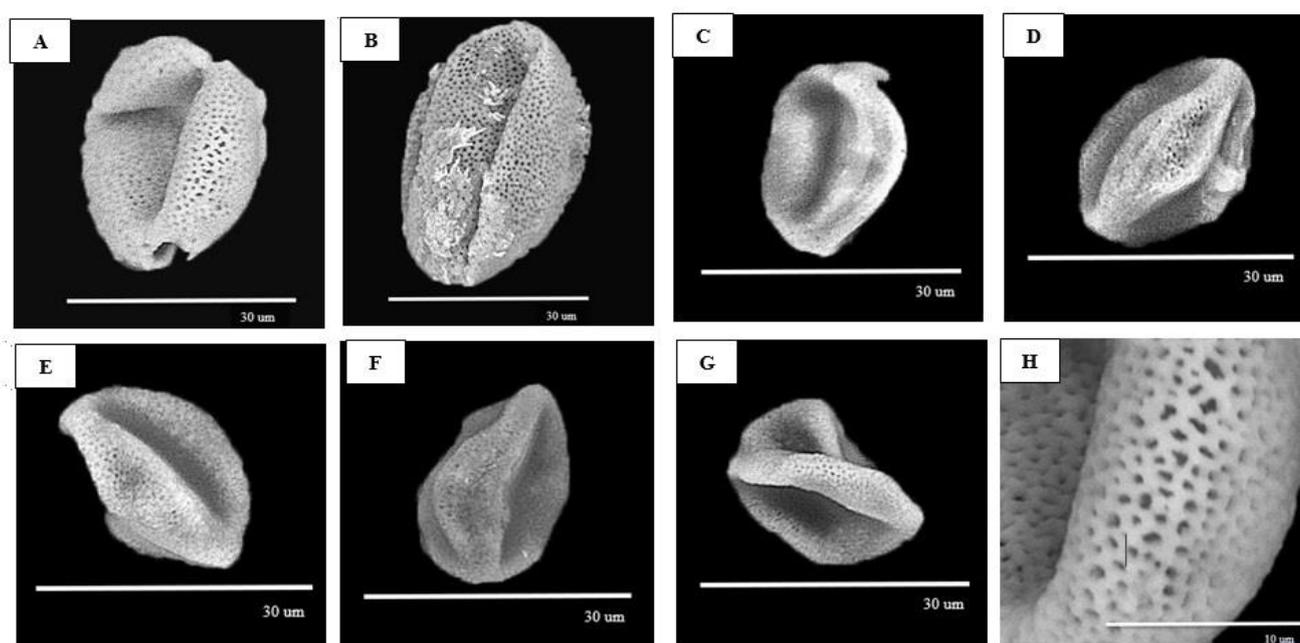
**Figure 4.** SEM micrographs of pollen grains of *Callicarpa*. A–B: *C. erioclona* (Sorsogon, Philippines) (b) exine surface G: *C. erioclona* (Bataan, Philippines) (c,e,g) polar view (d) equatorial view (f) exine surface



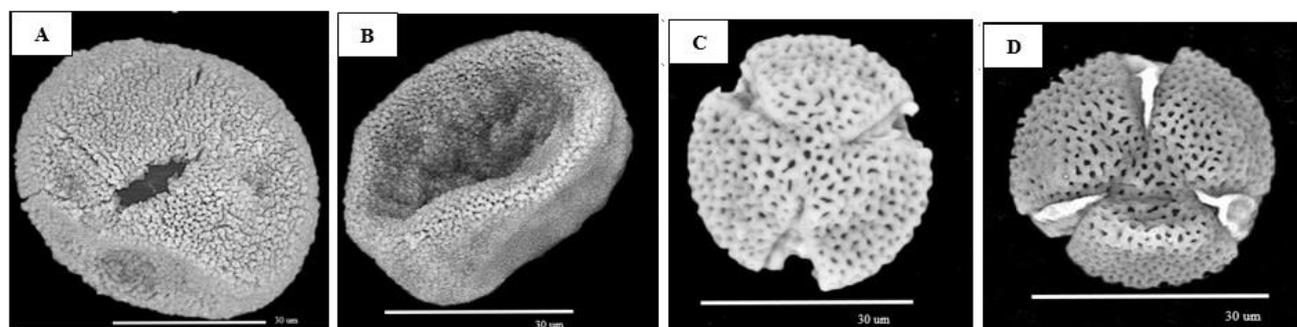
**Figure 5.** SEM micrographs of pollen grains of *Callicarpa*. A–H: *C. macrophylla*. (a–h) equatorial view



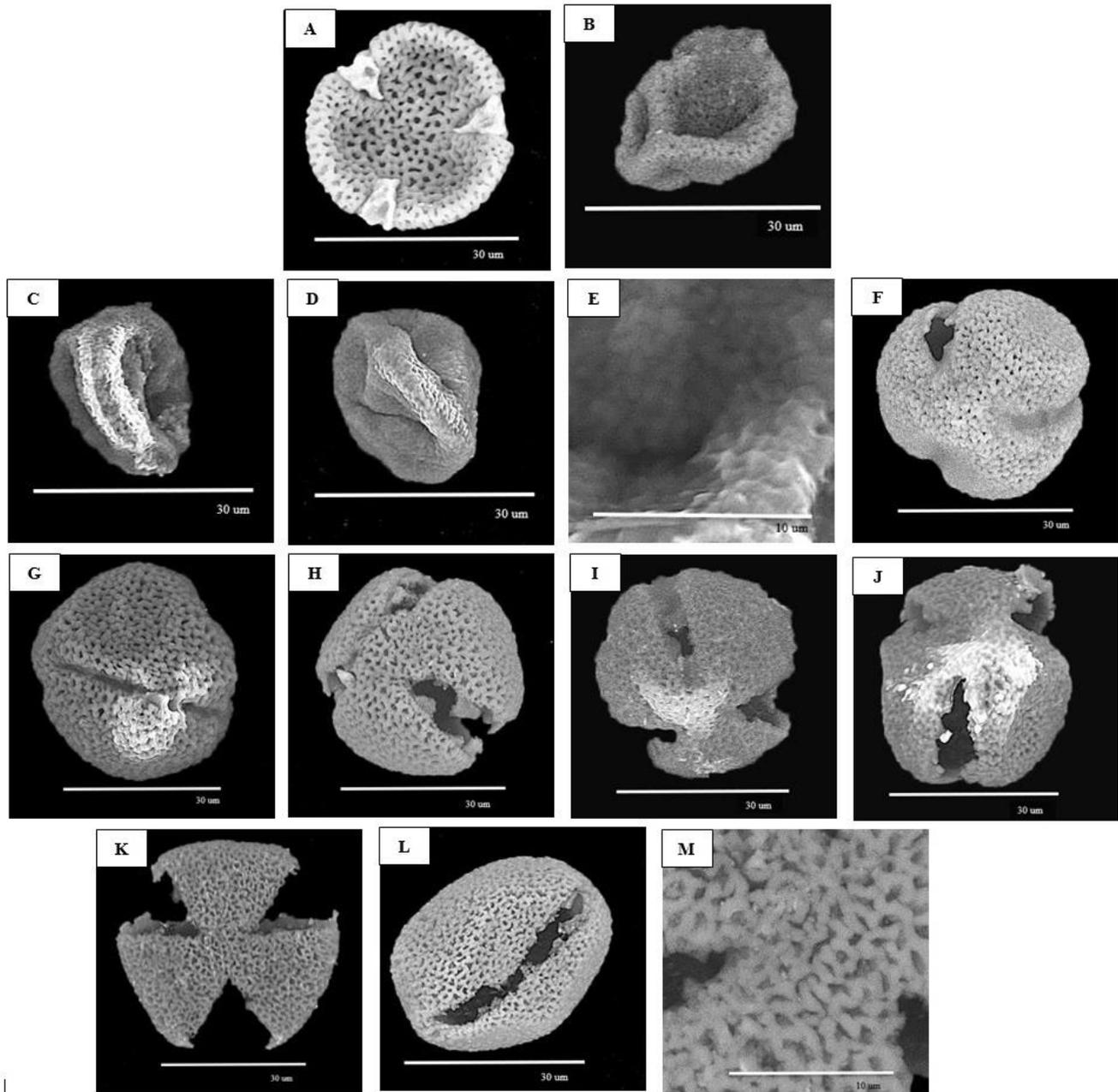
**Figure 6.** SEM micrographs of pollen grains of *Callicarpa*. A–C: *C. macrophylla* (a–b) polar view (c) exine surface.



**Figure 7.** SEM micrographs of pollen grains of *Callicarpa*. A–B: *C. pedunculata* (Aurora, Philippines) (a–b) equatorial view C– H: *C. pedunculata* (Albay, Philippines) (c–g) equatorial view (h) exine surface



**Figure 8.** SEM micrographs of pollen grains of *Geunsia*. A–B: *C. anomala*: (a) exine surface (b) monad showing inaperturate face. C–D: *C. pentandra*, polar view



**Figure 9.** SEM micrographs of pollen grains of *Geunsia*. A: *C. pentandra*, polar view B: *C. scandens*, equatorial view C–E: *G. cumingiana* (Panay, Philippines) (c-d) equatorial view (e) exine surface F–G: *G. cumingiana* (Albay, Philippines) (f) polar view (g) equatorial view H–M: *G. farinosa*. (h–k) polar view (l) equatorial view (m) exine surface

Species belong to *Geunsia* group include *C. scandens*, *C. pentandra*, and *C. anomala*, all have prolate in shape except *G. farinosa* from Borneo which has prolate spheroidal to oblate spheroidal and *G. cumingiana* from the Philippines has prolate to oblate spheroidal. Some inconsistent species like *C. scandens* achieved additional support from this study when recent phylogenetic study (Bramley 2009) of *Callicarpa* surprisingly included *C. scandens* in the section *Geunsia* where initially most of its flower and fruit morphology was more of *Callicarpa*. On the other hand, members of *Callicarpa* which include *C. arborea*, *C. erioclona*, *C. macrophylla*, and *C.*

*candicans* also show various shapes from prolate, prolate spheroidal to oblate. Both sections of *Geunsia* and *Callicarpa* were demonstrated with samples having prolate in shape which supports the suggestion of Bramley (2009) to nest *Geunsia* within *Callicarpa*. However, the authors suggest extending wider samples to increase the reliability of shape class as a diagnostic character since other samples show variations in shape.

The exine of *Callicarpa* shows considerable variation in texture which can be of strong diagnostic value. Coarsely reticulate is the most common type of tectum surface ornamentation among the observed species, it occurs in 7

species (*C. pedunculata*, *G. farinosa*, *C. pentandra*, *C. anomala*, *C. scandens*, *G. cumingiana*, and *C. erioclona*). Other types of surface ornamentation include microreticulate (*C. candicans*), and rugulate (*C. arborea* and *C. macrophylla*). The exine sculpture of two members of section *Geunsia*, *C. pentandra* and *C. scandens* examined in the present study share interesting similarities with samples originally described as *G. cumingiana* and *G. farinosa*. This shows evidence that exine ornamentation provides evidence to support that *C. pentandra*, *C. scandens*, *G. farinosa*, and *G. cumingiana* were united to support the synonymy of *Geunsia* and *Callicarpa*. On the other hand, pollen grains of *C. arborea* of the section *Callicarpa* are characterized by their rugulate exine sculptures, although coarsely reticulate, rugosely reticulate, and microreticulate exine sculptures were also observed in a few *C. arborea* species. This may give additional support to the hypothesis that rugulate exine sculptures might be plesiomorphic character state of *C. arborea* and the coarsely reticulate, rugosely reticulate and microreticulate exine sculptures might have evolved independently more than once as it may show evolutionary evidence in this species.

Various types of exine ornamentation were also observed in other members of the section *Callicarpa*, i.e., *C. erioclona*, *C. macrophylla*, and *C. candicans* which attributes to the projected separation of these species. Exine sculptures of the taxa of *Geunsia* are consistently similar with coarsely reticulate exine surface while section *Callicarpa* had a wide range of variations which might support the concept that *Geunsia* and *Callicarpa* as separate genus. The result of exine ornamentation reveals that this character is useful for the infrageneric classification of *Callicarpa*.

In conclusion, pollen morphology of the genus *Callicarpa* has shown interesting diagnostic value, thus, allowing evidence to support its relationship with *Geunsia*. Likewise, pollen morphology does support the supposition of other studies to recognize *Geunsia* as synonyms of *Callicarpa* when pollen size and shape class were observed. However, exine ornamentation does provide evidence to differentiate *Callicarpa* from *Geunsia* which may result in separation of genera. But authors suggest a better sampling of the section *Callicarpa* and *Geunsia* be able to gather more concrete evidence in its phylogenetic relationship.

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