

Palynology of Philippine *Amorphophallus* Blume ex Decne. (Araceae) and its taxonomic implications

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Abstract. *Fontarum-Bulawin NF, Medecillo-Guiang MMP, Calaramo MA, Alejandro GJD. 2023. Palynology of Philippine Amorphophallus Blume ex Decne. (Araceae) and its taxonomic implications. Biodiversitas 24: 4095-4109.* *Amorphophallus* is one of the genera of Araceae with diverse pollen characters. This study aimed to investigate the pollen morphological characters of *Amorphophallus* in the Philippines for inferring taxonomical relationships. Light microscopy (LM) and scanning electron microscopy (SEM) were employed to investigate pollen morphology of 14 accessions of *Amorphophallus*. Both acetolyzed and unacetolyzed pollens were studied to compare the outward aspect of pollens and the pollen characters were subjected to cluster analysis. Results revealed that pollen grains were mostly spheroidal, prolate-spheroidal, and subspheroidal in shape. The pollen sizes vary from 3.0 to 50 µm and exhibit very small to medium sizes. The ornamentations of unacetolyzed pollens were psilate and striate, whereas ornamentations in acetolyzed pollens are varied from psilate, rugulate, rugulate hamulate, reticulate, scabrate, striate to verrucate ornamentations. All pollens were inaperturate and monad. Cluster analysis using Euclidean generated 2 major clusters, namely Cluster I consists of *Amorphophallus adamsensis* Magtoto, Mones, Ballada, Austria, R.M.Dizon, Alanguí, Regina, *Amorphophallus* sp.3 (Samar), *Amorphophallus yaoi* A.Galloway, Hett. & Medecillo, *Amorphophallus flammeus* Calaramo, Batuyong, Bulawin & Alejandro, *Amorphophallus urceolatus* Hett. et al. (Ilocos), *Amorphophallus rayongii* Hett. & Medecillo, *Amorphophallus* sp.2 (Romblon), and *Amorphophallus urceolatus* Hett., A.Galloway & Medecillo (Cavite); and cluster 2 consists of *Amorphophallus paeoniifolius* (Dennst.) Nicolson, *Amorphophallus fontarumii* Bulawin, Medecillo & Alejandro, *Amorphophallus rostratus* Hett., *Amorphophallus longispathaceus* Engl. & Gehrm., and *Amorphophallus* sp.1 Based on this study, pollen shape, size, and ornamentation can be used as supplemental characters for the taxonomic identification of *Amorphophallus* in the Philippines.

Keywords: Acetolyzed, *Amorphophallus*, aroids, ornamentation, Philippines, pollen morphology

INTRODUCTION

The family Araceae or aroids is one of the most ecologically important taxa in the forest ecosystem. It comprises more than 3600 species in 144 genera (Diaz-Jimenez et al. 2021) and is distributed worldwide (Ortiz et al. 2019) with diverse habits. The family is best characterized by its inflorescence comprising spadix bearing the flowers, surrounded by a spathe. Several species in the family Araceae are economically important that include food sources, ornamental, fibers, and medicine. According to Gholave et al. (2018), the family nourishes various insects in flowers, resulting in higher pollination.

Amorphophallus is a genus of aroids occurring in tropical forests with a main center of diversity in continental Southeast Asia. A total of 241 species of *Amorphophallus* have been recorded worldwide (Bulawin et al. 2022). *Amorphophallus paeoniifolius* (Dennst.) Nicolson is mostly used in various chronic diseases and extensive pharmacological, safety/toxicity, and

phytochemical were reported by Dey et al. (2022). Tuber extract has been found to positively diminish the apoptosis and spread of breast cancer cells (Sivilla and Santos 2021). The young flower and petiole are used as vegetables and sources of carbohydrates, protein, minerals, vitamins, flavonoids, and fiber (Anil et al. 2023).

Pollen morphological characters are important in identifying plants (Ragho 2020). Palynology is a tool used in the delimitation of species (Atalay et al. 2016) and is utilized as evidence to distinguish taxa and resolve taxonomic problems (Pospiech et al. 2021). Joujeh et al. (2019) proved that pollen characters have taxonomic value and are useful in delineating the species within the genus *Centaurea* (Asteraceae). Furthermore, pollen morphology in *Lamium* (Lamiaceae) is systematically informative at the sectional level, but not at the species and infraspecific level (Atalay et al. 2016). *Amorphophallus's* pollen always lacks an aperture, has a broad size dimension, and manifests a varied exterior (Raman et al. 2017). Morphological evidence signifies that the type of exterior pollen part

frequently explains the infrageneric classification besides pollen morphology and is significant to suprageneric taxonomy (Ulrich et al. 2016).

According to Halbritter et al. (2018b), *Amorphophallus* has the most diverse pollen morphology in Araceae. Moreover, van der Ham et al. (2005) reported 46 species of *Amorphophallus* pollens are inaperturate, monad, have a wide size range, and show highly diverse exine surfaces. The findings produced four main clades, namely the African largely psilate, Asian largely psilate, Asian striate, and Malesian striate clade. Six Philippine species were included in the study belonging to different clades. Then, Ulrich et al. (2016) refuted the earlier report of van der Ham et al. (2005) that pollen of *Amorphophallus* is not resistant to the acetolysis method.

Studies on the pollen morphology of *Amorphophallus* in China also have been reported by Deng et al. (2017). However, studies on the pollen of *Amorphophallus* in the Philippines are scarce and only a few species are included in the earlier reports. Due to the limited species studied, investigation focusing on the pollen of Philippine *Amorphophallus* is important. Currently, there are additional species under the genus have been described (Bustamante et al. 2020a, 2020b, 2021; Hettterscheid et al. 2020; Tamayo et al. 2021; Calaramo et al. 2022; Bulawin et al. 2022). At least, 20 accepted species in the Philippines have been reported (Pelser et al. 2011). Most species are endemic, except *A. paeoniifolius* is distributed and commonly cultivated in other countries (Bulawin et al.

2022). This study aimed to investigate the pollen morphological characters of *Amorphophallus* in the country utilizing a light microscope (LM) and scanning electron microscope (SEM) to infer taxonomical relationships.

MATERIALS AND METHODS

Plant materials

The inflorescences of *Amorphophallus* were gathered from May 2021 to July 2022 with Gratuitous Permit no. 307 and 312. These were the flowering months of most Philippine *Amorphophallus*. Inflorescences were collected from the provinces of Aurora, Bohol, Cavite, Rizal, Romblon, and Samar, as well as in the regions of Boracay, Ilocos, and Panay (Figure 1). Flowers of *Amorphophallus rayongii* Hett. & Medecilo and *A. paeoniifolius* (Bohol) were collected from the Herbarium of Northwestern Luzon (HNUL) and living collections were collected from the botanic garden. All pollens were placed in microtubes with labels and stored in the refrigerator at 5°C (Sudarmono et al. 2016). Matured anthers were collected for flowers with no pollen during the collections and kept in microtubes at 5°C. The 14 accessions of acetolyzed and unacetolyzed pollens of *Amorphophallus* were observed under a light microscope (LM) and scanning electron microscope (SEM) (Table 1).

Table 1. Plant materials used in the study

Collectors	Collection number	Species	Tribe	Source of plant materials	Status
Calaramo, M.A.	MAC # 20104	<i>Amorphophallus adamsensis</i> Magtoto, Mones, Ballada, Austria, R.M.Dizon, Alanguui, Regina	Thomsoniae	Ilocos Norte	Wild
Calaramo, M.A.	MAC # 20105	<i>Amorphophallus flammeus</i> Calaramo, Batuyong, Bulawin & Alejandro	Thomsoniae	Ilocos Norte	Wild
Bulawin, N. F.	NFB # 030	<i>Amorphophallus fontarumii</i> Bulawin, Medecilo & Alejandro	Thomsoniae	Rizal	Wild
Bulawin, N. F.	NFB # 033	<i>Amorphophallus longispathaceus</i> Engl.& Gehrm.	Thomsoniae	Rizal	Wild
Calaramo, M.A.	MAC # 20107	<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson	Thomsoniae	Bohol	Cultivated
Bulawin, N. F.	NFB # 035	<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson	Thomsoniae	Romblon	Cultivated
Calaramo, M.A.	MAC # 20108	<i>Amorphophallus rayongii</i> Hett. & Medecilo	Thomsoniae	Boracay	Wild
Bulawin, N. F.	NFB # 036	<i>Amorphophallus rostratus</i> Hett.	Thomsoniae	Rizal	Wild
Calaramo, M.A.	MAC # 20106	<i>Amorphophallus urceolatus</i> Hett. et al.	Thomsoniae	Ilocos Norte	Wild
Bulawin, N. F.	NFB # 037	<i>Amorphophallus urceolatus</i> Hett., A.Galloway & Medecilo	Thomsoniae	Cavite	Wild
Bulawin, N. F.	NFB # 038	<i>Amorphophallus yaoi</i> A.Galloway, Hett. & Medecilo	Thomsoniae	Panay	Wild
Bulawin, N. F.	NFB # 039	<i>Amorphophallus</i> sp.1	Thomsoniae	Aurora	Wild
Bulawin, N. F.	NFB # 040	<i>Amorphophallus</i> sp.2	Thomsoniae	Romblon	Wild
Bulawin, N. F.	NFB # 031	<i>Amorphophallus</i> sp.3	Thomsoniae	Samar	Wild

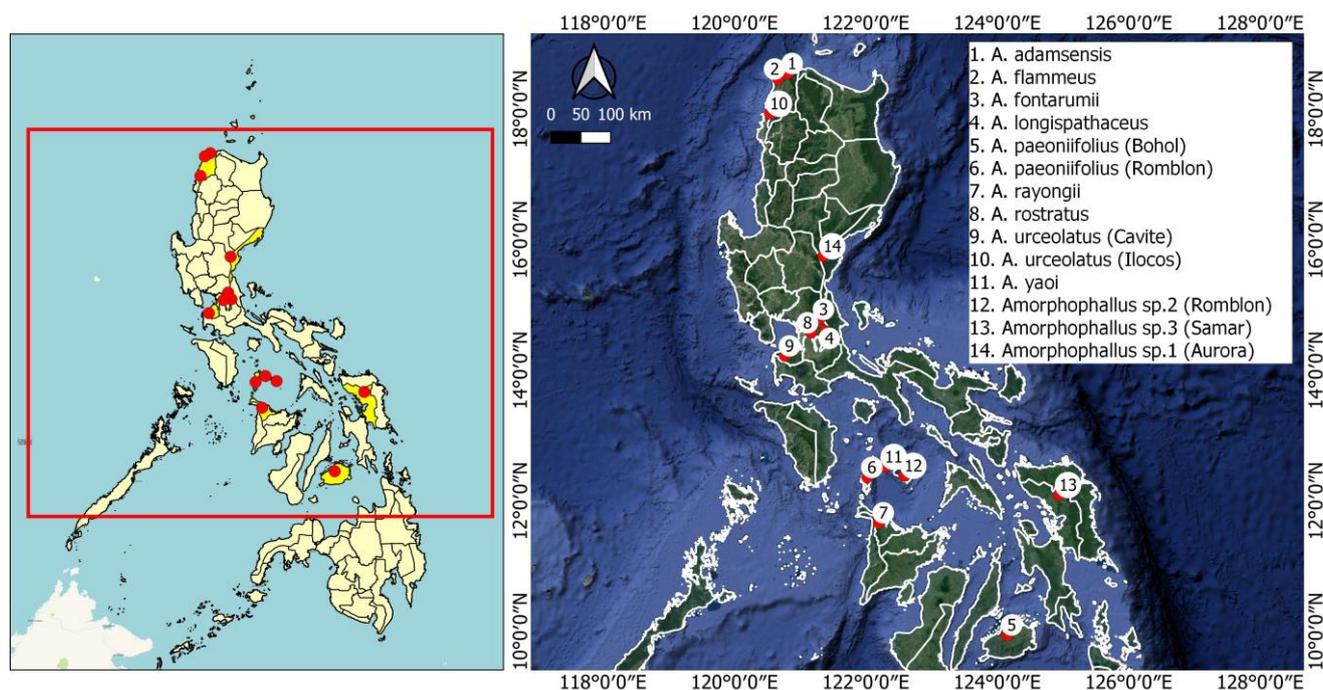


Figure 1. Source of the Philippine *Amorphophallus* used in the study

Procedures

Preparation of pollen specimens for LM

The procedure for preparing the pollen specimens to be observed under LM was followed Halbritter et al. (2018a). Dry pollens were hydrated on a glass slide and observed under LM (HPO) (Olympus CX 23) to find out the initial feature and check the pollen grains' condition. Matured anthers of the flowers were utilized for those flowers without pollen during the collections. Pollens were extracted by pricking the anther with a needle or sharp point, then observed under LM. The method for acetolysis or treated specimens was acetolysis the fast way. The acetolysis mixture was prepared in a small bottle (9 drops of acetic anhydride and one drop of sulfuric acid). A drop of acetolysis mixture was put on the glass slide, then pollens were dispersed on the acetolysis mixture. The slide was heated over the alcohol lamp briefly so the color would not become darker. After heating, the mixture with pollen grains was transferred to another glass slide with a drop of glycerine. Then, the slide was observed under LM. The same procedures were used for matured anther. The anther was placed on the slide with a drop of acetolysis mixture and then pricked by a needle or sharp point to release the pollens. At least 20 pollens were examined under LM for each accession of *Amorphophallus*. Photomicrographs were taken while the specimens were subjected to LM at Roque Laboratory, Graduate School, University of Santo Tomas, Manila, Philippines.

Preparation of pollen specimens for SEM

The procedure described by Halbritter et al. (2018a) was also employed to prepare pollens to be observed under SEM. Acetolysis was done in different methods since

pollen grains must be dried before they can be observed under SEM. The pollens were put in a small test tube (12×75 mm) with a mixture of 9 drops of acetic anhydride and one drop of concentrated sulfuric acid and then heated for 4 minutes at 100°C. Heating of pollens with an acetolyzed mixture was done in a water bath. After heating, the liquid was decanted and washed the residue with acetic acid and three times in water while filtering. It would be much better if the mixture were washed with more water to alleviate the effects of acetolysis mixture into the pollens. Then, the mixture was filtered and air-dried. Air-dried pollens were dispersed at the stub with carbon tape, placed inside the chamber, and observed under SEM (Hitachi TM 3000). Untreated or unacetolyzed pollens were also observed under SEM to compare the features of treated and untreated pollens. At least 20 pollens were examined and measured for each accession of *Amorphophallus*. Intricate pollen images were observed using the higher resolution scanning electron microscope (2000-3000 magnification or more). Photomicrographs were taken while the specimens were subjected to SEM at the Analytical Services Laboratory, Research Center for the Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines.

Pollen parameters observed

This study was focused only on the following parameters, namely: polar axis (P), equatorial axis (E), mean of polar and equatorial axis ($\bar{X}P$, $\bar{X}E$), a ratio of polar and equatorial axis (P/E), and the size of the pollens under higher magnification. The equatorial measurement of the pollen grains was acquired by measuring the grain from one side of the equator to the other. On the one hand, polar measurement was acquired by measuring one pole to the

other. The mean values of the polar and equatorial axes were computed by getting the average of the polar and equatorial axes. The polar and equatorial mean values were computed to find the ratio of the polar and equatorial axes. The ratio was used to identify the shape of the pollen. Pollen characters examined and included in the study were shapes, sizes, pollen sculpture or ornamentations, distribution, and apertures.

Data analysis

All data obtained were analyzed descriptively and pollen terminologies were followed El-Amier (2015), Yao and Zhang (2015), and Halbritter et al. (2018c). Quantitative pollen morphological data were subjected to hierarchical cluster analysis using PAST 4.0 software. Character states were encoded in multistate order and recorded in a binary state. The matrix was analyzed using Euclidean to generate a dendrogram.

RESULTS AND DISCUSSION

The study revealed the polar axis (P), equatorial axis (E), mean of a polar axis ($\bar{X}P$), mean of the equatorial axis ($\bar{X}E$), the ratio of the polar and equatorial axis (P/E), size of the pollens in higher magnification, shapes, sizes, sculpture or ornamentations, distribution, and apertures of pollens from 14 accessions of Philippine *Amorphophallus* (Tables 2, 3, 4, and 5). The general characters of pollen grains of 14 accessions of Philippine *Amorphophallus* are as follows: mostly spheroidal (SPH), prolate-spheroidal (PSP), and subspheroidal (SBS) in shapes. The size of pollen varies from 3.0 to 50 μm , exhibiting very small to medium sizes, for unacetolyzed pollens have a polar axis ranging from 5.82 to 75.0 μm and an equatorial axis ranging from 5.50 to 71.3 μm . On the one hand, the mean value of the polar axis ranged from 10.41 to 66.48, and the mean value of the equatorial axis ranged from 10.50 to

61.69 with a 0.99-1.21 polar and equatorial ratio. However, the ornamentations were psilate and striate. For acetolyzed pollens have a polar axis ranging from 4.39 to 85.0 μm and an equatorial axis ranging from 4.50 to 71.0 μm . The mean value of the polar axis ranged from 10.58 to 57.5, and the mean value of the equatorial axis ranged from 9.20 to 48.35 with a 0.95-1.40 polar and equatorial ratio. The ornamentations are varied, some species possess psilate, rugulate, rugulate hamulate, reticulate, scabrate, striate, and verrucate ornamentation. All pollen grains of 14 accessions of Philippine *Amorphophallus* were inaperturate, and the distributions were all monads. The detailed pollen morphometry and pollen morphology of each *Amorphophallus* accession were presented in Tables 2, 3, 4, and 5. The features of hydrated and acetolyzed pollen grains under LM. Also, the unacetolyzed and acetolyzed pollen grains were carried out under SEM with the size measurement under higher magnification presented in Figures 2, 3, 4, 5, 6 and 7.

Amorphophallus adamsensis collection # MAC 20104 (Figure 2A-D, 7A)

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), spheroidal (SPH), subspheroidal (SBS) (acetolyzed); 30 μm , medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to reticulate ornamentation (acetolyzed), consists of a net-like sculpture with lumina of more than 1 μm surrounded by thin ridges in acetolyzed pollens; ridges 1.67-2.04 μm ; lumen 2.12-3.10; polar axis 42.0-58.7 μm , equatorial axis 42.8-56.2 μm ; polar mean value 48.85, equatorial mean value 47.74; polar and equatorial ratio 1.02 (unacetolyzed), polar axis 40.0-54.4 μm , equatorial axis 43.2-52.8 μm ; polar mean value 46.96, equatorial mean value 48.35; polar and equatorial ratio 0.97 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

Table 2. Pollen morphometry of unacetolyzed Philippine *Amorphophallus* accessions using SEM based on Yao and Zhang (2015)

Taxon	P (μm) (min-max)	E (μm) (min-max)	$\bar{X}P$	$\bar{X}E$	P/E	Size (μm)	Shape
<i>A. adamsensis</i>	42.0-58.7	42.8-56.2	48.85	47.74	1.02	30	SPH, PSP
<i>A. flammeus</i>	46.7-59.6	42.0-56.4	50.91	47.48	1.07	30	SPH, PSP
<i>A. fontarumii</i>	5.82-23.9	5.50-17.13	10.41	10.50	0.99	3.0-5.0	SPH, PSP
<i>A. longispathaceus</i>	46.7-57.2	40.2-49.9	51.5	44.57	1.16	30	SPH,PSP, SBP
<i>A. paeoniifolius</i> (Bohol)	66.6-75.0	60.0-71.3	66.48	61.69	1.08	30-50	SPH,PSP, SBS
<i>A. paeoniifolius</i> (Romblon)	66.0-69.0	59.3-65.6	59.76	54.94	1.09	30-50	SPH, PSP
<i>A. rayongii</i>	35.2-52.1	34.1-47.4	41.89	39.25	1.07	30	SPH, PSP
<i>A. rostratus</i>	10.51-18.8	9.00-19.4	13.81	12.84	1.08	20-50	SBP, SBS
<i>A. urceolatus</i> (Ilocos)	46.8-62.0	43.2-51.2	51.51	47.54	1.08	30	SPH,PSP, SBS
<i>A. urceolatus</i> (Cavite)	39.0-48.6	37.7-49.9	44	42.4	1.04	30	SPH, PSP
<i>A. yaoi</i>	43.5-51.2	41.9-49.2	47.61	45.37	1.05	30	SPH,PSP, SBS
<i>Amorphophallus</i> sp.1 (Aurora)	45.6-55.1	37.2-45.8	48.73	40.24	1.21	30	SBS, SBP
<i>Amorphophallus</i> sp.2 (Romblon)	34.7-52.9	33.0-41.1	40.42	36.55	1.11	30	SBS, SBP
<i>Amorphophallus</i> sp.3 (Samar)	42.9-59.1	42.6-53.0	50.66	45.8	1.11	30	SPH,PSP, SBS

Table 3. Pollen morphometry of acetolyzed Philippine *Amorphophallus* accessions using SEM based on Yao and Zhang (2015)

Taxon	P (µm) (min-max)	E (µm) (min-max)	$\bar{X}P$	$\bar{X}E$	P/E	Size (µm)	Shape
<i>A. adamsensis</i>	40.0-54.4	43.2-52.8	46.96	48.35	0.97	30	SPH, SBS
<i>A. flammeus</i>	40.2-51.9	36.0-52.5	44.51	41.45	1.07	30	SPH, SBS
<i>A. fontarumii</i>	4.39-16.2	4.50-12.9	10.58	9.20	1.15	3.0-5.0	SPH, SBS
<i>A. longispathaceus</i>	43.4-55.5	34.1-54.8	48.11	43.45	1.11	30	SPH,SBP, SBS
<i>A. paeoniifolius</i> (Bohol)	69.6-85.0	56.8-71.0	53.75	47.18	1.14	30-50	SPH,PSP, SBS
<i>A. paeoniifolius</i> (Romblon)	58.2-70.5	57.2-63.7	50.08	48.26	1.04	30-50	PSP, SBS
<i>A. rayongii</i>	38.6-68.9	35.4-64.8	53.38	44.96	1.19	30	SPH, SBS
<i>A. rostratus</i>	9.51-52.5	10.9-53.5	24.43	20.94	1.17	20-50	SBP, SBS
<i>A. urceolatus</i> (Ilocos)	37.5-59.0	43.2-48.8	51.56	47.45	1.09	30	SPH, SBS
<i>A. urceolatus</i> (Cavite)	40.8-50.3	48.9-60.3	44.83	43.77	1.02	30	SPH,PSP, SBS
<i>A. yaoi</i>	45.5-65.4	49.1-65.8	57.5	41.04	1.40	30	SPH, SBS
<i>Amorphophallus</i> sp.1 (Aurora)	43.1-55.1	38.6-44.5	48.11	40.76	1.18	30	SBS, SBP
<i>Amorphophallus</i> sp.2 (Romblon)	38.5-46.5	34.9-48.2	34.02	35.93	0.95	30	SBS, SBP
<i>Amorphophallus</i> sp.3 (Samar)	38.2-48.1	37.2-59.3	47.86	47.61	1.06	30	SPH, PSP

Notes: P: polar axis, E: equatorial axis, $\bar{X}P$: mean of the polar axis, $\bar{X}E$: mean of the equatorial axis, P/E: mean of P/mean of E, SPH: spheroidal, PSP: prolate-spheroidal, SBS: subspheroidal, SBP: subprolate

Table 4. Pollen morphology of unacetolyzed Philippine *Amorphophallus* accessions using SEM and LM

Taxon	Physical size	Ornamentation	Distribution	Apertures
<i>A. adamsensis</i>	Medium	Psilate	Monad	Inaperturate
<i>A. flammeus</i>	Medium	Psilate	Monad	Inaperturate
<i>A. fontarumii</i>	Very small	Psilate	Monad	Inaperturate
<i>A. longispathaceus</i>	Medium	Psilate	Monad	Inaperturate
<i>A. paeoniifolius</i> (Bohol)	Medium	Psilate	Monad	Inaperturate
<i>A. paeoniifolius</i> (Romblon)	Medium	Psilate	Monad	Inaperturate
<i>A. rayongii</i>	Medium	Striate	Monad	Inaperturate
<i>A. rostratus</i>	Small to medium	Psilate	Monad	Inaperturate
<i>A. urceolatus</i> (Ilocos)	Medium	Psilate	Monad	Inaperturate
<i>A. urceolatus</i> (Cavite)	Medium	Psilate	Monad	Inaperturate
<i>A. yaoi</i>	Medium	Psilate	Monad	Inaperturate
<i>Amorphophallus</i> sp.1 (Aurora)	Medium	Psilate	Monad	Inaperturate
<i>Amorphophallus</i> sp.2 (Romblon)	Medium	Striate	Monad	Inaperturate
<i>Amorphophallus</i> sp.3 (Samar)	Medium	Psilate	Monad	Inaperturate

Table 5. Pollen morphology of acetolyzed Philippine *Amorphophallus* accessions using SEM and LM

Taxon	Physical size	Ornamentation	Distribution	Apertures
<i>A. adamsensis</i>	Medium	Reticulate	Monad	Inaperturate
<i>A. flammeus</i>	Medium	Rugulate hamulate	Monad	Inaperturate
<i>A. fontarumii</i>	Very small	Psilate	Monad	Inaperturate
<i>A. longispathaceus</i>	Medium	Regulate	Monad	Inaperturate
<i>A. paeoniifolius</i> (Bohol)	Medium	Scabrate	Monad	Inaperturate
<i>A. paeoniifolius</i> (Romblon)	Medium	Scabrate	Monad	Inaperturate
<i>A. rayongii</i>	Medium	Striate	Monad	Inaperturate
<i>A. rostratus</i>	Small to medium	Striate	Monad	Inaperturate
<i>A. urceolatus</i> (Ilocos)	Medium	Psilate	Monad	Inaperturate
<i>A. urceolatus</i> (Cavite)	Medium	Psilate	Monad	Inaperturate
<i>A. yaoi</i>	Medium	Verrucate	Monad	Inaperturate
<i>Amorphophallus</i> sp.1 (Aurora)	Medium	Psilate	Monad	Inaperturate
<i>Amorphophallus</i> sp.2 (Romblon)	Medium	Striate	Monad	Inaperturate
<i>Amorphophallus</i> sp.3 (Samar)	Medium	Reticulate	Monad	Inaperturate

***Amorphophallus flammeus* collection # MAC 20105
(Figure 2E-H, 7B)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), spheroidal (SPH), subspheroidal (SBS) (acetolyzed); 30 μm , medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to rugulate hamulate ornamentation (acetolyzed), consists of winding or rounded ridges with different thicknesses that form a maze-like pattern in an irregular

arrangement in acetolyzed pollens; ridges 1.62-1.96; polar axis 46.7-59.6 μm , equatorial axis 42.0-56.4; μm ; polar mean value 50.91, equatorial mean value 47.48; polar and equatorial ratio 1.07 (unacetolyzed), polar axis 40.2-51.9 μm , equatorial axis 36.0-52.5; μm ; polar mean value 44.51, equatorial mean value 41.45; polar and equatorial ratio 1.07 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

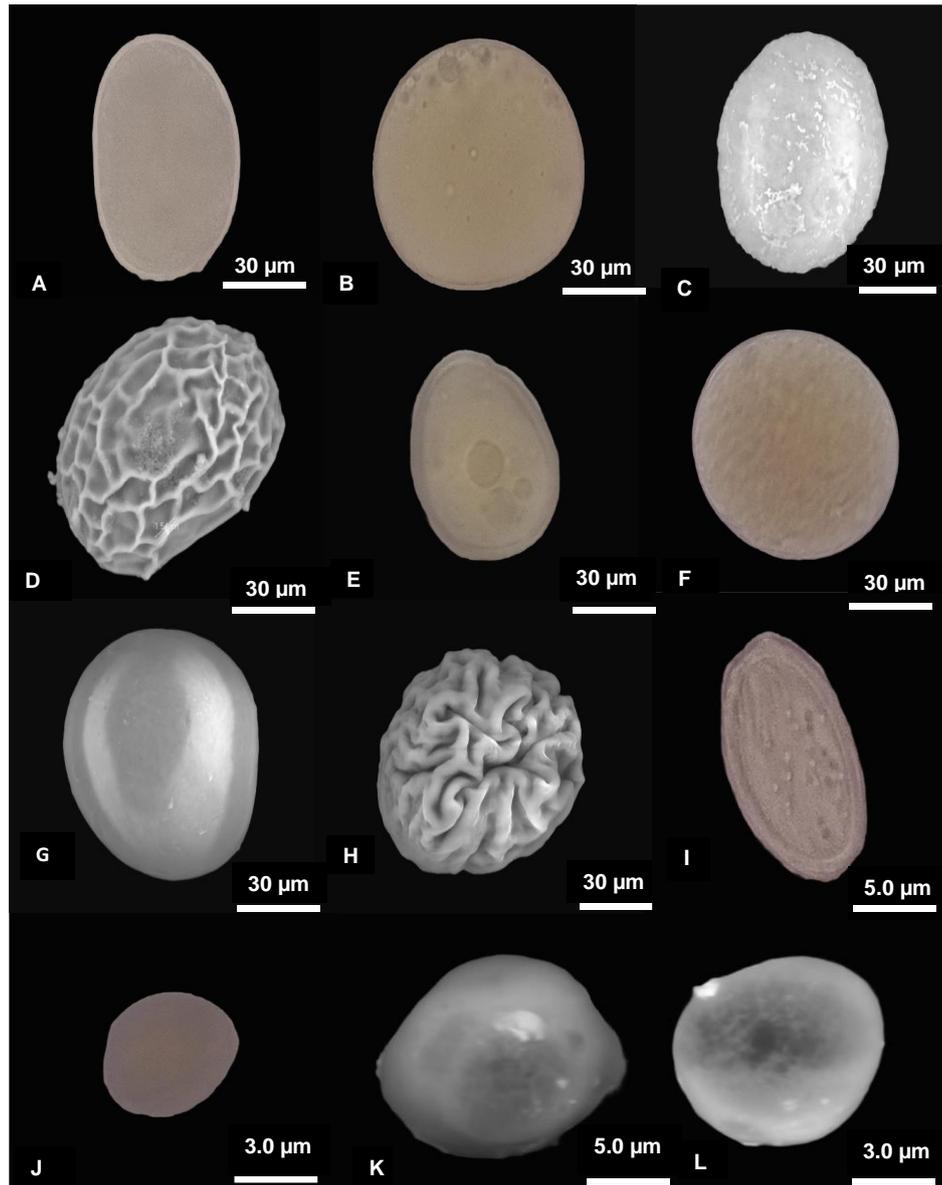


Figure 2. LM and SEM pollen micrographs of Philippine *Amorphophallus* accessions (A-D) *A. adamsensis*: A. LM hydrated equatorial view; B. LM acetolyzed equatorial view; C. SEM unacetolyzed equatorial view; D. SEM acetolyzed equatorial view; (E-H) *A. flammeus*: E. LM hydrated equatorial view; F. LM acetolyzed equatorial view; G. SEM unacetolyzed equatorial view; H. SEM acetolyzed equatorial view; (I-L) *A. fontarumii*: I. LM hydrated equatorial view; J. LM acetolyzed equatorial view; K. SEM unacetolyzed equatorial view; L. SEM acetolyzed equatorial view. Photomicrographs were taken by N. F. Fontarum-Bulawin

***Amorphophallus fontarumii* collection # NFB 030
(Figure 2I-L, 7E)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), spheroidal (SPH), subspheroidal (SBS) (acetolyzed); 3.0-5.0 μm , very small size; ornamentations psilate, with smooth surface both acetolyzed and unacetolyzed pollens; polar axis 5.82-23.9 μm , equatorial axis 5.50-17.13 μm ; polar mean value 10.41, equatorial mean value 10.50; polar and equatorial ratio 0.99 (unacetolyzed), polar axis 4.39-16.2 μm , equatorial axis 4.50-12.9 μm ; polar mean value 10.58, equatorial mean value 9.20; polar and equatorial ratio 1.15 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus longispathaceus* collection # NFB 033
(Figure 3A-D)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP), subprolate (SBP) (unacetolyzed), spheroidal (SPH), subprolate (SBP), subspheroidal (SBS) (acetolyzed); 30 μm , medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to rugulate ornamentation (acetolyzed), consists of more than 1 μm elongated sculpture with an irregular arrangement pattern that is nearly striate and reticulate in acetolyzed pollens; ridges 1.50-1.94; polar axis 46.7-57.2 μm , equatorial axis 40.2-49.9 μm ; polar mean value 51.5, equatorial mean value 44.57; polar and equatorial ratio 1.16 (unacetolyzed), polar axis 43.4-55.5 μm , equatorial axis 34.1-54.8 μm ; polar mean value 48.11, equatorial mean value 43.45; polar and equatorial ratio 1.11 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

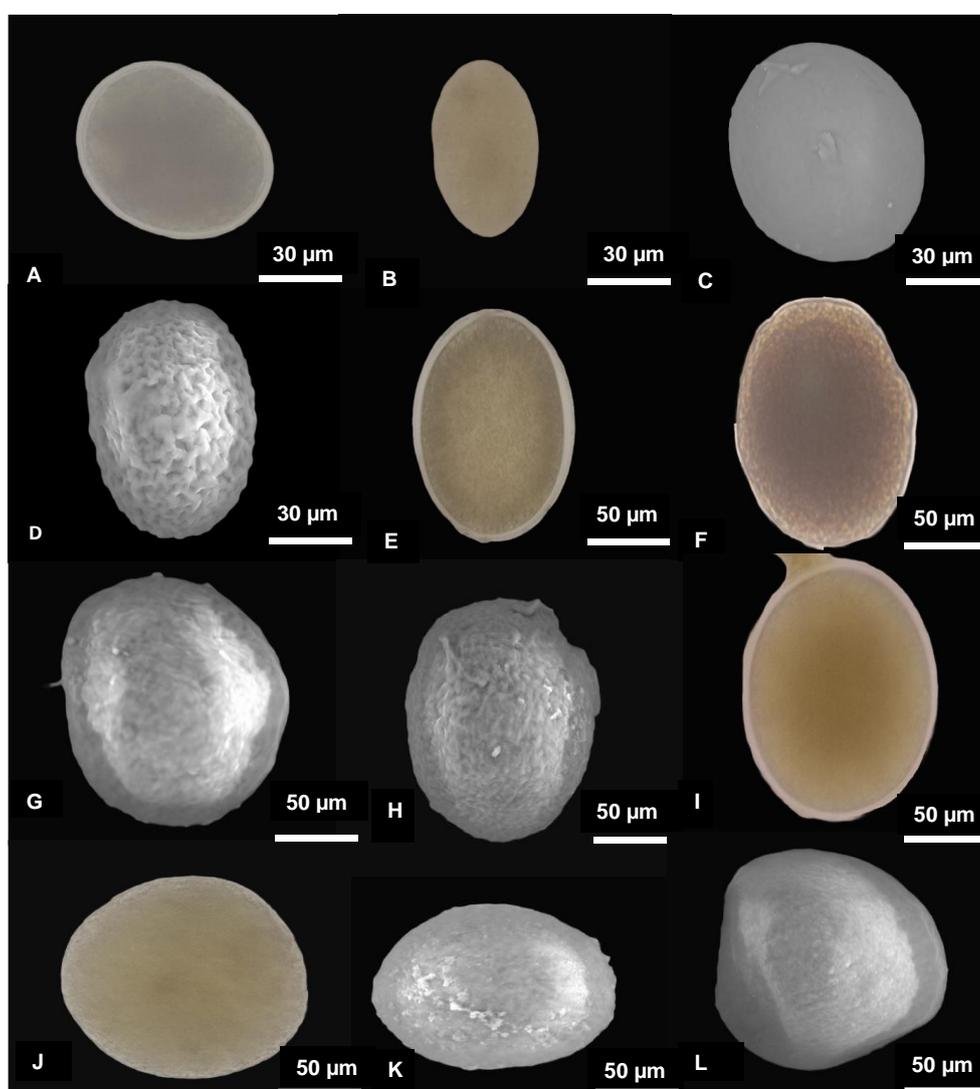


Figure 3. LM and SEM pollen micrographs of Philippine *Amorphophallus* accessions (A-D) *A. longispathaceus*: A. LM hydrated equatorial view; B. LM acetolyzed equatorial view; C. SEM unacetolyzed equatorial view; D. SEM acetolyzed equatorial view; (E-H) *A. paeoniifolius* (Bohol): E. LM hydrated equatorial view; F. LM acetolyzed equatorial view; G. SEM unacetolyzed equatorial view; H. SEM acetolyzed equatorial view; (I-L) *A. paeoniifolius* (Romblon): I. LM hydrated with pollenkitt equatorial view; J. LM acetolyzed equatorial view; K. SEM unacetolyzed equatorial view; L. SEM acetolyzed equatorial view. Photomicrographs were taken by N. F. Fontarum-Bulawin

***Amorphophallus paeoniifolius* (Bohol) collection # MAC 20107 (Figure 3E-H)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP), subspheroidal (SBS) (unacetolyzed), spheroidal (SPH), prolate-spheroidal (PSP), subspheroidal (SBS) (acetolyzed); 30-50 μm , medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to scabrate ornamentation (acetolyzed) with ornamentation elements smaller than 1 μm in all direction in acetolyzed pollens; polar axis 66.6-75.0 μm , equatorial axis 60.0-71.3 μm ; polar mean value 66.48, equatorial mean value 61.69; polar and equatorial ratio 1.08 (unacetolyzed), polar axis 69.6-85.0 μm , equatorial axis 56.8-71.0 μm ; polar mean value 53.75, equatorial mean value 47.18; polar and equatorial ratio 1.14 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus paeoniifolius* (Romblon) collection # NFB 035 (Figure 3I-L, 7F)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), prolate-spheroidal (PSP), subspheroidal (SBS) (acetolyzed); 30-50 μm , medium size; ornamentation psilate, with a smooth surface in some unacetolyzed pollens to scabrate ornamentation (acetolyzed) with elements smaller than 1 μm in all directions in acetolyzed pollens; polar axis 66.0-69.0 μm , equatorial axis 59.3-65.6 μm ; polar mean value 59.76, equatorial mean value 54.94; polar and equatorial ratio 1.09 (unacetolyzed), polar axis 58.2-70.5 μm , equatorial axis 57.2-63.7 μm ; polar mean value 50.08, equatorial mean value 48.26; polar and equatorial ratio 1.04 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to the absence of apertures.

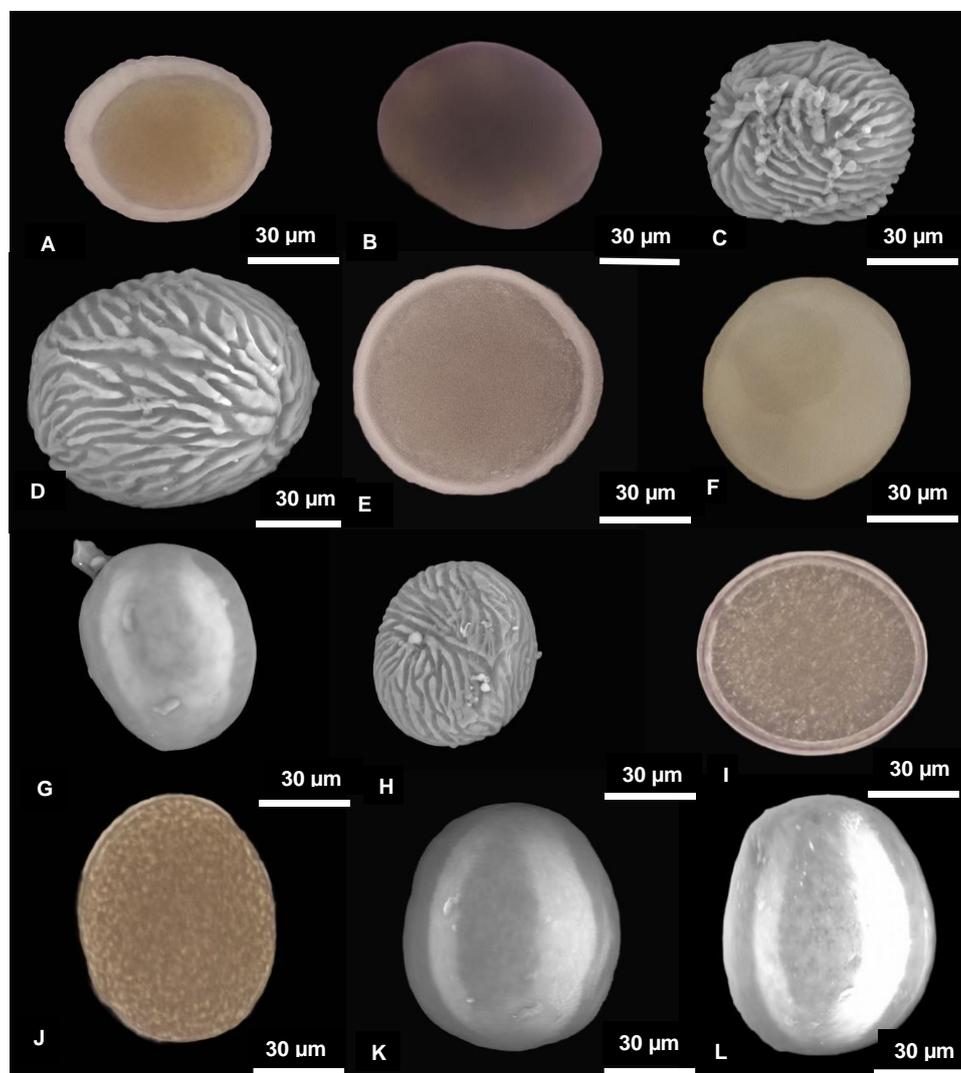


Figure 4. LM and SEM pollen micrographs of Philippine *Amorphophallus* accessions (A-D) *A. rayongii*: A. LM hydrated equatorial view; B. LM acetolyzed equatorial view; C. SEM unacetolyzed polar view; D. SEM acetolyzed equatorial view; (E-H) *A. rostratus*: E. LM hydrated equatorial view; F. LM acetolyzed equatorial view; G. SEM unacetolyzed equatorial view; H. SEM acetolyzed equatorial view; (I-L) *A. urceolatus* (Ilocos): I. LM hydrated equatorial view; J. LM acetolyzed equatorial view; K. SEM unacetolyzed equatorial view; L. SEM acetolyzed equatorial view. Photomicrographs were taken by N. F. Fontarum-Bulawin

***Amorphophallus rayongii* collection # MAC 20108 (Figure 4A-D)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), spheroidal (SPH), subspheroidal (SBS) (acetolyzed); 30 µm, medium size; ornamentations striate, with parallel elements separated by grooves in both unacetolyzed and acetolyzed pollens; ridges 1.34-1.92; polar axis 35.2-52.1 µm, equatorial axis 34.1-47.4 µm; polar mean value 41.89, equatorial mean value 39.25; polar and equatorial ratio 1.07 (unacetolyzed), polar axis 38.6-68.9 µm, equatorial axis 35.4-64.8 µm; polar mean value 53.38, equatorial mean value 44.96; polar and equatorial ratio 1.19 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus rostratus* collection # NFB 036 (Figure 4E-H, 7G)**

Pollen grains subprolate (SBP), subspheroidal (SBS) (unacetolyzed), subprolate (SBP), subspheroidal (SBS) (acetolyzed); 20-50 µm, small to medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to striate ornamentation (acetolyzed) with parallel elements separated by grooves in acetolyzed pollens; ridges 1.25-1.46; polar axis 10.51-18.8 µm, equatorial axis 9.00-19.4 µm; polar mean value 13.81, equatorial mean value 12.84; polar and equatorial ratio 1.08 (unacetolyzed), polar axis 9.51-52.5 µm, equatorial axis 10.9-53.5 µm; polar mean value 24.43, equatorial mean value 20.94; polar and equatorial ratio 1.17 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

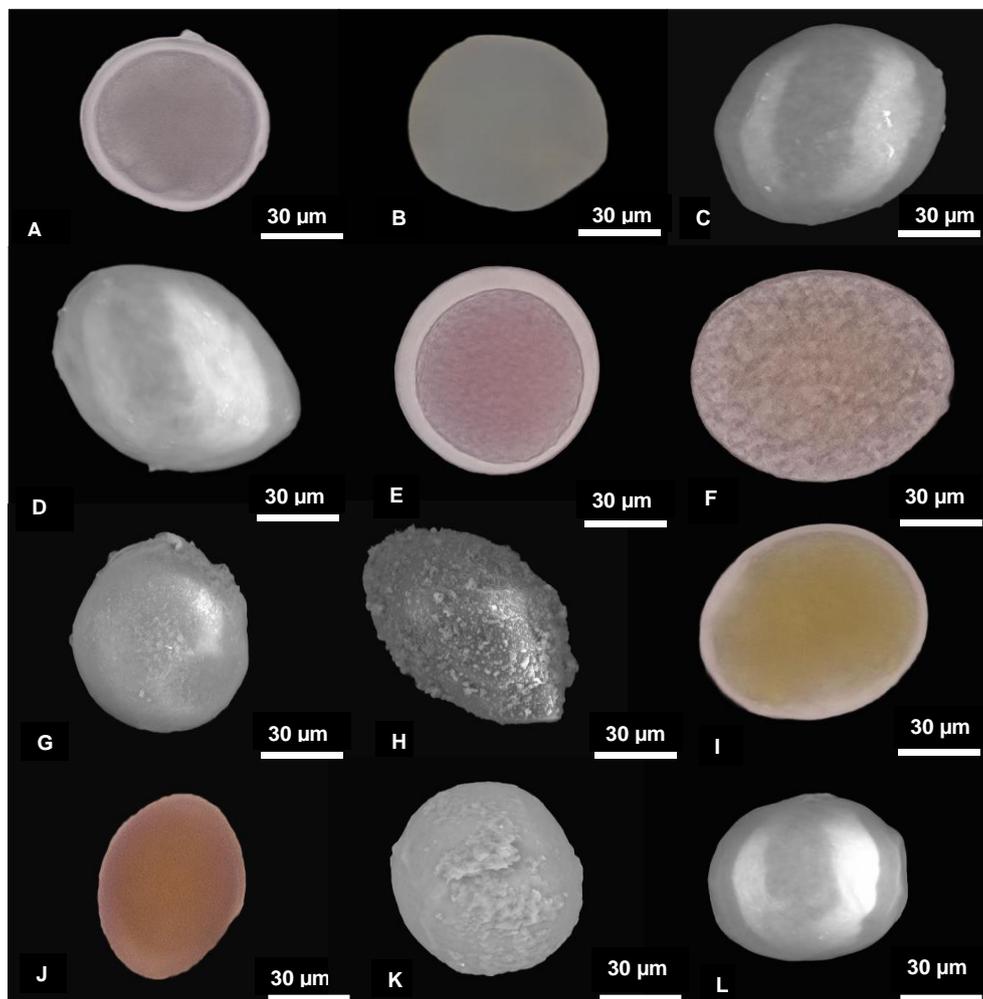


Figure 5. LM and SEM pollen micrographs of Philippine *Amorphophallus* accessions (A-D) *A. urceolatus* (Cavite): A. LM hydrated equatorial view; B. LM acetolyzed equatorial view; C. SEM unacetolyzed equatorial view; D. SEM acetolyzed equatorial view; (E-H) *A. yaoi*: E. LM hydrated equatorial view; F. LM acetolyzed equatorial view; G. SEM unacetolyzed equatorial view; H. SEM acetolyzed polar view; (I-L) *Amorphophallus* sp.1 (Aurora): I. LM hydrated equatorial view; J. LM acetolyzed equatorial view; K. SEM unacetolyzed equatorial view; L. SEM acetolyzed equatorial view. Photomicrographs were taken by N. F. Fontarum-Bulawin

***Amorphophallus urceolatus* (Ilocos) collection # MAC 20106 (Figure 4I-L, 7H)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP), subspheroidal (SBS) (unacetolyzed), spheroidal (SPH), subspheroidal (SBS) (acetolyzed); 30 μ m, medium size; ornamentations psilate, with a smooth surface in both unacetolyzed and acetolyzed pollens; polar axis 46.8-62.0 μ m, equatorial axis 43.2-51.2 μ m; polar mean value 51.51, equatorial mean value 47.54; polar and equatorial ratio 1.08 (unacetolyzed), polar axis 37.5-59.0 μ m, equatorial axis 43.2-48.8 μ m; polar mean value 51.56, equatorial mean value 47.45; polar and equatorial ratio 1.09 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus urceolatus* (Cavite) collection # NFB 037 (Figure 5A-D, 7C)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP) (unacetolyzed), spheroidal (SPH), prolate-spheroidal (PSP), subspheroidal (SBS) (acetolyzed); 30 μ m, medium size; ornamentations psilate, with a smooth surface in both unacetolyzed and acetolyzed pollens; polar axis 39.0-48.6 μ m, equatorial axis 37.7-49.9 μ m; polar mean value 44, equatorial mean value 42.4; polar and equatorial ratio 1.04 (unacetolyzed), polar axis 40.8-50.3 μ m, equatorial axis 48.9-60.3 μ m; polar mean value 44.83, equatorial mean value 43.77; polar and equatorial ratio 1.02 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

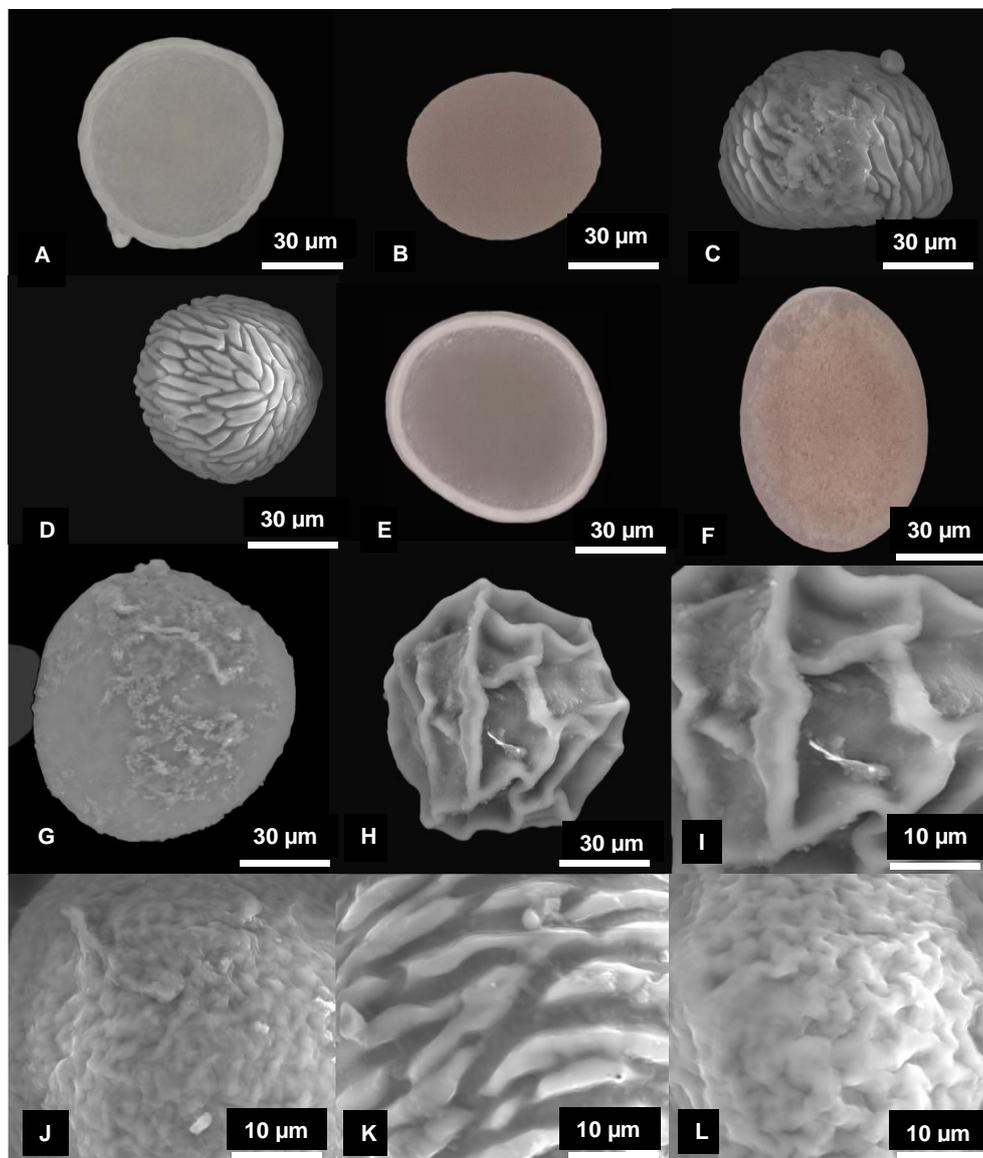


Figure 6. LM and SEM pollen micrographs of Philippine *Amorphophallus*: (A-D) *Amorphophallus* sp.2 (Romblon), A. LM hydrated equatorial view; B. LM acetolyzed equatorial view; C. SEM unacetolyzed equatorial view; D. SEM acetolyzed equatorial view; (E-H) *Amorphophallus* sp.3 (Samar): E. LM hydrated equatorial view; F. LM acetolyzed equatorial view; G. SEM unacetolyzed equatorial view; H. SEM acetolyzed equatorial view; (I-L) SEM detailed ornamentations: I. *Amorphophallus* sp.3 (Samar); J. *A. paeoniifolius* (Bohol); K. *A. rayongii*; L. *A. longispataceus*. Photomicrographs were taken by N. F. Fontarum-Bulawin

***Amorphophallus yaoi* collection # NFB 038 (Figure 5. E-H, Figure 7. D)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP), sub spheroidal (SBS)(unacetolyzed), spheroidal (SPH), sub spheroidal (SBS) (acetolyzed); 30 µm, medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to verrucate ornamentation (acetolyzed), consists of a wart-like, not constricted sculpture wider than its height in acetolyzed pollens; polar axis 43.5-51.2 µm, equatorial axis 41.9-49.2 µm; polar mean value 47.61, equatorial mean value 45.37; polar and equatorial ratio 1.05 (unacetolyzed), polar axis 45.5-65.4 µm, equatorial axis 49.1-65.8 µm; polar mean value 57.5, equatorial mean value 41.04; polar and equatorial ratio 1.40 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus* sp.1 (Aurora) collection # NFB 039 (Figure 5I-L, 7J)**

Pollen grains subspheroidal (SBS), subprolate (SBP) (unacetolyzed), subspheroidal (SBS), subprolate (SBP) (acetolyzed); 30 µm, medium size; ornamentations psilate, with a smooth surface in both unacetolyzed and acetolyzed pollens; ridges 1.52-1.85; polar axis 45.6-55.1 µm, equatorial axis 37.2-45.8 µm; polar mean value 48.73, equatorial mean value 40.24; polar and equatorial ratio 1.21 (unacetolyzed), polar axis 43.1-55.1 µm, equatorial axis 38.6-44.5 µm; polar mean value 48.11, equatorial mean value 40.76; polar and equatorial ratio 1.18 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

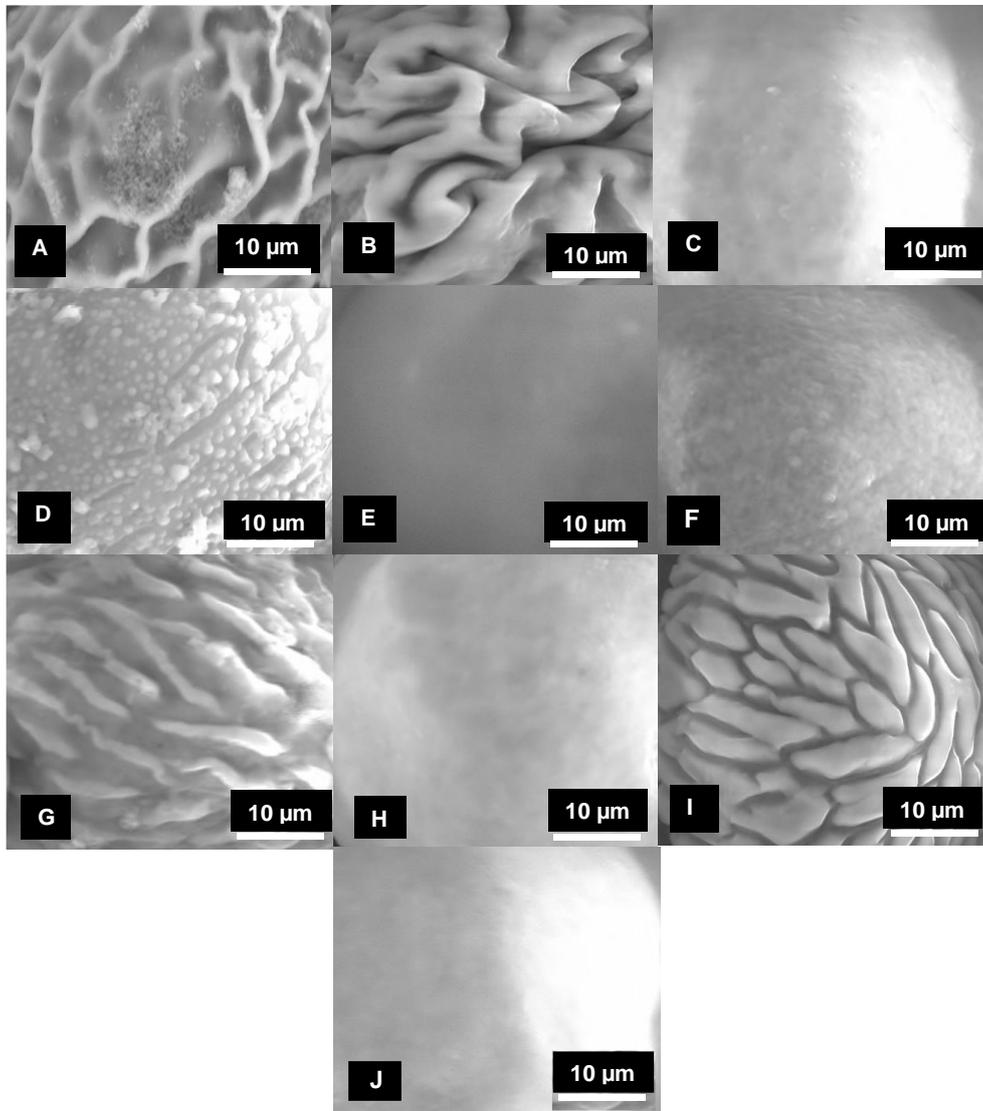


Figure 7. SEM detailed pollen ornamentations of Philippine *Amorphophallus* accessions: A. *A. adamsensis*; B. *A. flammeus*; C. *A. urceolatus*; D. *A. yaoi*; E. *A. fontarumii*, F. *A. paeoniifolius* (Romblon); G. *A. rostratus*, H. *A. urceolatus* (Ilocos); I. *Amorphophallus* sp.2 (Romblon); J. *Amorphophallus* sp.1 (Aurora). Photomicrographs were taken by N. F. Fontarum-Bulawin

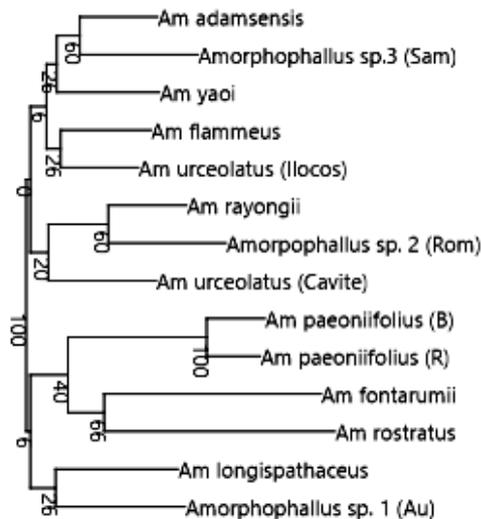


Figure 8. Hierarchical cluster analysis of the Philippine *Amorphophallus* accessions based on quantitative data

***Amorphophallus* sp.2 (Romblon) collection # NFB 040 (Figure 6A-D, 7I)**

Pollen grains subspheroidal (SBS), subprolate (SBP) (unacetolyzed), subspheroidal (SBS), subprolate (SBP) (acetolyzed); 30 μm , medium size; ornamentations striate, with parallel elements separated by grooves in both unacetolyzed and acetolyzed pollens; ridges 1.45-1.65; polar axis 34.7-52.9 μm , equatorial axis 33.0-41.1 μm ; polar mean value 40.42, equatorial mean value 36.55; polar and equatorial ratio 1.11 (unacetolyzed), polar axis 38.5-46.5 μm , equatorial axis 34.9-48.2 μm ; polar mean value 34.02, equatorial mean value 35.93; polar and equatorial ratio 0.95 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

***Amorphophallus* sp.3 (Samar) collection # NFB 031 (Figure 6. E-H)**

Pollen grains spheroidal (SPH), prolate-spheroidal (PSP), subspheroidal (SBS) (unacetolyzed), spheroidal (SPH), prolate-spheroidal (PSP) (acetolyzed); 30 μm , medium size; ornamentations psilate, with a smooth surface in some unacetolyzed pollens to reticulate ornamentation (acetolyzed), consists of a net-like sculpture with lumina of more than 1 μm surrounded by thin ridges in acetolyzed pollens; ridges 1.50-2.01 μm ; lumen 2.11-2.30; polar axis 42.9-59.1 μm , equatorial axis 42.6-53.0 μm ; polar mean value 50.66, equatorial mean value 45.8; polar and equatorial ratio 1.11 (unacetolyzed), polar axis 38.2-48.1 μm , equatorial axis 37.2-59.3 μm ; polar mean value 47.86, equatorial mean value 47.61; polar and equatorial ratio 1.06 (acetolyzed); distribution monad, dispersed by an individual unit; and inaperturate due to absence of apertures.

The resulting dendrogram revealed two (2) major clusters. Cluster 1 consists of *A. adamsensis*, *Amorphophallus* sp.3 (Samar), *A. yaoi*, *A. flammeus*, *A. urceolatus* (Ilocos), *A. rayongii*, *Amorphophallus* sp.2 (Romblon) and *A. urceolatus* (Cavite), and cluster 2

consists of *A. paeoniifolius*, *A. fontarumii*, *A. rostratus*, *A. longispathaceus* and *Amorphophallus* sp.1 (Figure 8).

Discussion

According to Soares et al. (2013) and El-Amier (2015), the shape of pollen grains based on the polar and equatorial ratio was classified as spheroidal (0.88-1.14 μm), prolate (1.33-2.00 μm), oblate (0.50-0.75 μm), oblate-spheroidal (0.88-1.00 μm), prolate-spheroidal (1-1.14 μm), perprolate (>2 μm), suboblate (0.75-0.88 μm), subprolate (1.14-1.33 μm), subspheroidal (0.75-1.33 μm), and peroblate (<0.50 μm) (Soares et al. 2013; El-Amier 2015). Four pollen shapes of *Amorphophallus* are identified in this study under LM and SEM observations. These are the spheroidal (SPH), prolate-spheroidal (PSP), sub-spheroidal (SBS), and sub-prolate (SBP) (Table 2 and Table 3). This finding is similar to the work of van der Ham et al. (2005) on *Amorphophallus* pollens. Circular, elliptic, lobate, triangular and polygonal are the common shapes of pollen (Pospiech et al. 2021). However, Ulrich et al. (2016) mentioned that *Amorphophallus* pollens have an oblate shape with an elliptic outline and apices at the center. The 14 pollens of Philippine *Amorphophallus* were hydrated and acetolyzed. Hydrated pollens under LM were fully dilated due to the emerging in water. The pollen wall and the protoplast were distinct in Figure 2, Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7.

The protoplasmic content of pollen was protected by thick endexine. Some hydrated *Amorphophallus* pollens do not have pollen walls because when pollens are rehydrated, the exterior wall is removed immediately (Ulrich et al. 2016). Pacini and Franchi (2020) stated that oval grains change into spherical shapes when immersed in water because of absorption. Harmomegathy is the cause of changes in the shape and volume of pollen grains due to in and out water movement in the pollen wall. Based on the study of Phuphumirat et al. (2018), during wet and dry environmental conditions, there are changes in osmotic pressure within the pollen grains, which lead to morphological variations of the pollens like deflation and inflation of the exine, and it is hard to visualize the exact shape of hydrated pollens since apertures, pollen wall, layers of pollen wall, size, ornamentation and pollenkitt influence it. Whereas pollen walls in acetolyzed pollens carried out under LM and SEM were removed due to the reaction of the acetolysis mixture. The detailed pollen shapes and sculpture or ornamentation were observed in acetolyzed pollens subjected to SEM compared to acetolyzed pollens, which were observed under LM.

According to Ulrich et al. (2016) and Alzer et al. (2021), the pollen size was classified as very small (<10 μm), small (10-25 μm), medium (26-50 μm), and large (51-100 μm). Hydration and preparation technique will affect the size of grains, which is why sizes vary from very small to very large (Ulrich et al. 2016; Halbritter et al. 2018c; Pospiech et al. 2021). Ulrich et al. (2016) reported that the common size of pollens in the genus *Amorphophallus* ranges from medium to large, and the factors that affect pollen size are the intensity of hydration, the methods in preparing the specimens and natural differences.

Nevertheless, the size of the pollen from 14 Philippine *Amorphophallus* accessions ranges from 3.0-50 μm (very small to medium) (Table 2, Table 3, Table 4 and Table 5). Pollens with a very small size is *A. fontarumii*, small to medium size is *A. rostratus*, and most species are medium size. The sizes of the *Amorphophallus* plant vary from very small to large (Shavkat o'g'li 2022). Besides, pollen size evolves from small to large. In the Philippines, *A. fontarumii* is the smallest size of *Amorphophallus* (Bulawin et al. 2022), which is why it has a very small size of pollens. Variation in the measurement of pollens is noticeable. The study acquired the equatorial measurement of the pollen grains by measuring the grain from one side of the equator to the other. Pollen measurements vary for species collected from different environmental conditions and localities, within or between a group of plants, and in the same flower with different anthers. One of the significant pollen characteristics that can be used is size. However, extra care is needed in observing the size since it could be affected by the environment or medium used to prepare pollen specimens (Tuler et al. 2017). In describing the size of pollen grains, consider the medium in which the grains are treated because the medium may also affect the shape and volume of pollen grains (Pacini and Franchi 2020). The size of fresh pollens might differ from the size of fossil pollens of the same species due to fossilization processes (Demske et al. 2013). According to Ulrich et al. (2016), the smallest *Amorphophallus* size is striate type, while the largest pollens are psilate and verrucate. Psilate pollen is greater in size, more oval and with greater volume than the striate type. In our study, a very small *Amorphophallus* was psilate, and the small to medium size were striate, while those pollens with medium size were striate, psilate, rugulate, reticulate, rugulate hamulate, verrucate, and scabrate. Size and shape were essential to establish the type of pollen.

Pollens of 14 species of Philippine *Amorphophallus* were acetolyzed to view the sculpture or ornamentation of each species. LM and SEM were used to examine the pollen of *Amorphophallus*. Different methods of microscopy have advantages and disadvantages. However, using a combination method will provide more detailed information about the observed specimens (Pospiech et al. 2021). Both methods were significant in examining the overall feature of pollen grains (Halbritter et al. 2018c). Nevertheless, sculpture or ornamentation of pollens under LM observation was not detailed compared to SEM. LM can be utilized to examine various types of pollens. However, LM cannot view the entire detailed part of pollens' exine (Tuler et al. 2017). An individual feature of the exterior layer of pollen can be examined and calculated with SEM, while LM can also identify all pollen forms. Because of these, SEM analysis was conducted to set the image at higher magnification and to make the viewing more lucid than a light microscope. According to Ulrich et al. (2016), there are ten types of ornamentation identified from the pollen of *Amorphophallus*, namely: psilate, striate, striate with psilate caps, areolate, verrucate, fossulate, echinate, reticulate, scabrate, and striate/scabrate, and the most superior among the types is psilate and striate

ornamentations. The differences in the sculpture or ornamentation of *Amorphophallus* pollens are affected by several factors, like its function in plant reproduction (Ulrich et al. 2016). Pacini and Franchi (2020) stated that grains pollinated by insects have more ornamented exine, whereas those wind pollinated have less ornamented exine. According to Ulrich et al. (2016), striate type of pollen ornamentation was dominant compared to other types of pollen ornamentation. However, in our study, the dominant ornamentation was psilate, 12 out of 14 *Amorphophallus* accessions exhibited psilate ornamentations (unacetolyzed), and two species were striate (both unacetolyzed and acetolyzed) (Table 4). Pollen ornamentations of *A. fontarumii*, *A. urceolatus* (Cavite), *A. urceolatus* (Ilocos), and *Amorphophallus* sp.1 (Aurora) are psilate, *A. adamsensis*, and *Amorphophallus* sp.3 (Samar) are reticulate, *A. paeoniifolius* (Bohol), and *A. paeoniifolius* (Romblon) are scabrate, *A. flammeus* rugulate is hamulate, *A. longispathaceus* is rugulate, *A. rayongii*, *A. rostratus*, and *Amorphophallus* sp.2 (Romblon) are striate, and *A. yaoi* is verrucate (acetolyzed) (Table 5). The result of this study divulged the changes in pollens' ornamentation once the pollens were already acetolyzed. There are great differences between unacetolyzed and acetolyzed pollens in some species of Philippine *Amorphophallus*. Detailed ornamentation will be observed once an acetolyzed mixture treats the pollens. However, the acetolyzed mixture may damage the pollen wall in some instances. Based on our observation, psilate pollens, once acetolyzed, exhibited another type of ornamentation when observed under SEM because the acetolysis mixture already removed the outer covering of pollens (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7). Most of the psilate pollens in LM incline to have different ornamentation under higher magnification of SEM (Halbritter et al. 2018c). *Amorphophallus* with a psilate type of pollen made of a very thin wall (Ulrich et al. 2016). The ectexine of psilate is finer in a layer that might crack during germination. The psilate type of ornamentation has a smooth exterior, while the striate type has parallel ridges, and the extent and forms manifest broad spans with differences in the direction of ridges.

Pollens are released abundantly in the form of strands or powder (Ulrich et al. 2016). The sticky strand-like pollen is released from the male flower of *Amorphophallus*. Pollenkitt was present in 14 Philippine *Amorphophallus* accessions. This yellow sticky substance was observed around the pollen grains that influenced the distribution of pollen grains and sometimes formed a congregated pollen. Pollen dispersal units delineate the pattern of pollens' distribution, like monads (single grain), dyads (2 pollen grains), tetrads (4 pollen grains), and polyads (>4 pollen grains). The distribution of pollens from 14 Philippines *Amorphophallus* accessions was monad, consisting of a single pollen grain. Monad pollen dispersal unit is common during the pollens' development with a spherical shape, and the shape turns oval once the pollens are exposed from opened anther due to dehydration (Pacini and Franchi 2020). Pollenkitt is lipidic, which may cause pollens' clustering (Ulrich et al. 2016). This lipid and adhesive

pollenkitt is very useful during pollination. Pollen coating protects the pollen from drying and serves as an insulating layer. The amount of pollen coat or pollenkitt on the surface of the pollen wall is also dependent on the pollen surface or ornamentation pattern that may influence pollen distribution and adhesion of pollen to the stigma (Wang and Dobritsa 2018). Based on our study, pollenkitt was abundant in psilate pollens compared to striate pollens such as *A. rayongii* and *Amorphophallus* sp.2 (Romblon). Also, the present investigation concluded that pollenkitt is profused in *A. yaoi*, the one with verrucate ornamentation in 14 pollens of Philippine *Amorphophallus* accessions. Pollenkitt is less in striate and echinate pollens, but it is abundant in psilate and verrucate pollens (Ulrich et al. 2016).

Wang and Dobritsa (2018) reported that from exine, the various pattern of pollens can be observed, while the part where exine is not fully developed or absent are the apertures of the pollen. The aperture is a significant part of the pollen. It is the opening in which the water enters and exits during dehydration and the site of pollen tube germination. However, not all species possess an aperture. Araceae is one of the families with pollens without apertures (Pacini and Franchi 2020). Pollen tubes germinate in any part of the pollen for those inaperturate pollens. However, in some Angiosperms like *Amorphophallus*, exine shed to develop the pollen tube (Halbritter et al. 2018c). Inaperturate pollens are present among monocotyledons and many in cotyledons. It is considered the most prominent type of aperture present in the family Araceae. According to Ulrich et al. (2016), the pollen tube develops without the prior formation of an exit zone in pollens without an aperture. Inaperturate pollen of *Amorphophallus* is commonly present in psilate pollen grains. All examined pollens of 14 selected Philippine *Amorphophallus* accessions were inaperturate. Pores were not observed from the exine of the pollens. Therefore, *Amorphophallus* pollen grains lack an opening or aperture and are frequently full of amyloplasts.

Similarities of 14 Philippine *Amorphophallus* accessions in shapes, sizes, sculpture or ornamentations, pollen distribution, and apertures manifested the closeness of each species. The ectexine structure of *Amorphophallus* is comparable to *Arisaema* (Ulrich et al. 2016). It shows that pollen morphological characters are useful for explicitly identifying genera and species (Attar et al. 2018). Palynology could identify related species using pollen morphology (Tuler et al. 2017). Different kinds of pollen grains are present in Aroids, and their features are significant for categorizing genera or higher taxonomic groups. *Amorphophallus* was considered the most diverse genus of Araceae for being inaperturate, having a varied size and sculpturing or ornamentation. Inaperturate, thin ectexine with very few sporopollenins and the presence or absence of polysaccharides at the external wall of the pollens are the characteristics of pollens under subfamily Aroideae which are evident in *Amorphophallus* pollens (Ulrich et al. 2016). The interconnection of several taxa and their rank in classification, such as subspecies, species, genera, tribes, subfamilies, and families were identified

using pollen characters. Through palynological descriptions from several palynological investigations, the connection between species and subspecies level has been recognized, and several phylogenetic problems were resolved (Gokcen et al. 2018).

Based on cluster analysis using pollen characters, 14 *Amorphophallus* accessions were grouped in 2 clusters. Cluster 1 has spheroidal to subspheroidal in shape with a value of 40-60 μm (polar axis), 40-58 μm (equatorial axis), and a medium size, striate pollen. On the other hand, species grouped in Cluster 2 have 30-50 μm in size, the P/E ratio is 1.04 -1.18, and the size ranges from very small to medium size. The finding conforms to the reports of van der Ham et al. (2005) that three sizes occur in this taxa, namely average, minima and maxima. Most of the pollen ornamentation in *Amorphophallus* is psilate and striate.

In conclusion, the study of palynology and morphometry of 14 accessions indicated the outward appearance of both acetolyzed and unacetolyzed pollens. The shapes of pollens from 14 accessions are varied, and the sizes are very small to medium. There are great differences between unacetolyzed and acetolyzed pollens in some species of Philippine *Amorphophallus*. The study divulged the changes in pollens' ornamentation once the pollens were already acetolyzed. Examination of pollens both under LM and SEM is significant to prevent the misinterpretation of pollen ornamentation. Therefore, the pollen characters are significant as additional characters in the delineation of Philippine *Amorphophallus* identification.

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