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Phytoliths in the Sago Palm (*Metroxylon sagu* Rottb.) from Pangasugan, Leyte, Philippines

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Abstract: The sago palm (*Metroxylon sagu* Rottb.) forms phytoliths (biogenic opal silica) in all organs as a result of silicon uptake from soil. The phytolith assemblages in the sago palm were extracted using a dry combustion method with a muffle and described based on morphology and ornamentation according to the International Code for Phytolith Nomenclature 1.0 and 2.0. Using an optical microscope, spheroid (globular) echinate phytoliths, of which the morphology is highly diagnostic with minor exceptions, were observed in a leaflet of *M. sagu* from the Philippines. Studies of the *M. sagu* phytolith, which showed spheroid, echinate grains from 2.5 to >22.5 μm in diameter, with a peak range of 10 to 12.5 μm, could provide a tool for documenting the biosilicification of phytoliths and archeological evidence of sago cultivation before rice in coastal areas of the Philippines.

Keywords: echinate, globular, phytolith, sago palm, silicon, spheroid

Introduction

Silicon uptake and biogenic opal silica (biogenic silica plant microfossil, phytolith) formation have been found in a vast number of plant species. Silicon polymerization, however, is not known in plants (Currie and Perry, 2007). Several morphometric analyses have been undertaken on phytoliths of the palm family. These phytoliths are silt size in general and have a refractive index of 1.41–1.47 and a specific gravity between 1.5 and 2.3 (Ryan, 2014). The sago palm (*Metroxylon sagu* Rottb.) takes up silicon through the roots and accumulates phytoliths in all of its organs. Fenwick et al. (2011) identified the phytolith extracted from a sago palm specimen in comparison with *Areca catechu L., Calamus aruensis* Becc., and *Cocos nucifera* L. and reported that the globular echinate (Madella et al., 2005) shape was from 6 to 15 μm in diameter, and the mean number of spines was 20.412. In addition, Bowdery (2014) extracted a palm phytolith from a soil core sample named 'Rano Kao2,' collected in 1983 in Easter Island (Rapa Nui), and detected the M. sagu phytolith image under microscope, compared it to the palm reference image, and described it according to the International Code for Phytolith Nomenclature 1.0 (Madella et al., 2005). Recently, the International Code for Phytolith Nomenclature 2.0 was published by Neumann et al. (2019). *Metroxylon sagu* contains

globular echinate phytoliths (Madella et al., 2005) and spheroid echinate (Code: SPH_ECH) (Neumann et al., 2019). However, there are no reports on *M. sagu* phytoliths in the sago palm growing area in the Philippines.

It is deduced that *Homo sapiens* migrated from Africa to Southeast Asia by 73,000 to 63,000 years ago, based on the finding of bones at Calao Cave in the Philippines (Mijares et al., 2010). According to the results of genome analysis by Lipton et al. (2018) and McColl et al. (2018), a group spread eastward from Africa and then divided into two groups: people heading north and people heading south. Furthermore, the group heading south was subdivided into three groups: the Papuan people, the Hoabinhian people, and the Onge people. The Hoabinhian people formed a technocomplex in Southeast Asia in the later hunter-gatherer period (Ji et al., 2015) and formed basic Southeast Asian people. Agricultural, Austroasiatic-language people came from northern regions and mixed with the Hoabinhian people. However, there were few genetic effects between the Hoabinhian and Austroasiatic-language people. The question is when and where hunter-gatherers encountered the sago palm in the sago growing area before the introduction of the rice plant. Phytoliths show stable and definitive distribution in soil horizons, as compared with pollen, which become a useful tool to elucidate the indistinct cultivation history of *M. sagu* in the Philippines, accompanied by carbon 14 dating.

In this study, we attempt to show the presence and size distribution of phytoliths in a *M. sagu* phytolith leaflet taken from Pangasugan, Leyte, Philippines.

Fig 1. Map of Leyte, Philippines

Fig 2. Leaflet samples collected from Pangasugan, Leyte, Philippnesin 2016

Materials and Methods

1. *Metroxylon sagu* leaflet sample and phytolith extraction

Middle leaflet samples of the 6th and lower position leaves from the top were taken in 2016 from an experimental sago field (Inceptisols) in Pangasugan, Leyte, Philippines (Figs. 1 and 2), air-dried and then dried at 70 °C for 24 hours, and ignited at 500 °C for 4 hours according to the result of Wu et al. (2012). They were washed with distilled water and collected via decantation. After extraction, phytolith assemblages were weighed for total dry weight, mounted on clean

28 x 48 mm glass slides with distilled water, and covered with a 22 x 24 mm cover slide to obtain the whole picture of the phytolith assemblage. Finally, phytolith samples after air-drying were mounted onto slides using a suitable permanent fixative (polystyrene) (Matsunami, MGK-S).

2. Phytolith identification

The phytoliths in *M. sagu* leaflet tissue and those released from the tissue were examined under transmitted light using a Meiji Tech MT-5000 light microscope at 400x magnification, and their photographs were taken using a Cannon EOS Kiss X5. The *M. sagu* phytoliths were clearly distinguishable, being globular (spheroid) and having spines (echinate) (Fenwick et al., 2011; Bowdery, 2014; Neumann et al., 2019). A scanning electron microscope (Hitachi Miniscope TM-1000) was also used for the identification of *M. sagu* phytoliths.

3. Quantitative variables of phytoliths

Quantitative measurement of the maximum diameter of phytoliths, measured from spine tip to spine tip, was performed using an eyepiece graticule with a 1.25 μm tolerance. One hundred fifty phytoliths were measured to obtain the mean diameter data. The total phytolith weight in oven-dried leaflet samples was determined. The analysis was performed in duplicate.

Results

1. Phytoliths in incinerated leaflet tissue of *Metroxylon sagu*

The dry weight of incinerated *M. sagu* leaflet samples ranged from 11.8 to 13.8% of the oven-dried leaflet samples (Table 1). These incinerated leaflet samples contain characteristic and diagnostic palm family phytoliths (Fig. 3). A large number of globular (spheroid) echinate phytoliths, which were aligned to the inside of cells (Fig. 4) and along leaf veins (Fig. 5), while avoiding stomatal guard cells (Figs. 6–8).

Table 1. Phytolith percentage in *Metroxylon sagu* leaflet

Sample	Dry weight of leaflet (A) g	Dry weight of incinerated leaflet (B)	(B/A) × 100 %
1	4.42	0.61	13.8
2	3.81	0.52	13.7
3	3.74	0.44	11.8

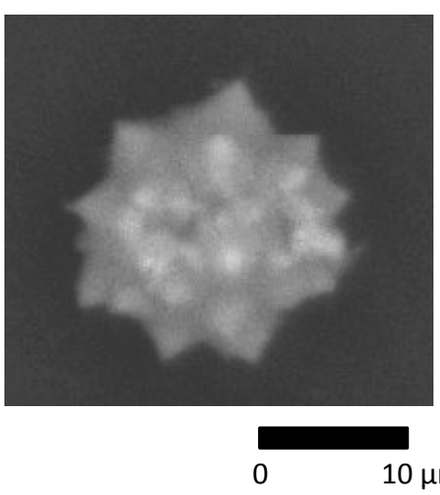

Fig 3. Scanning electron microscope image of phytolith in leaflet.

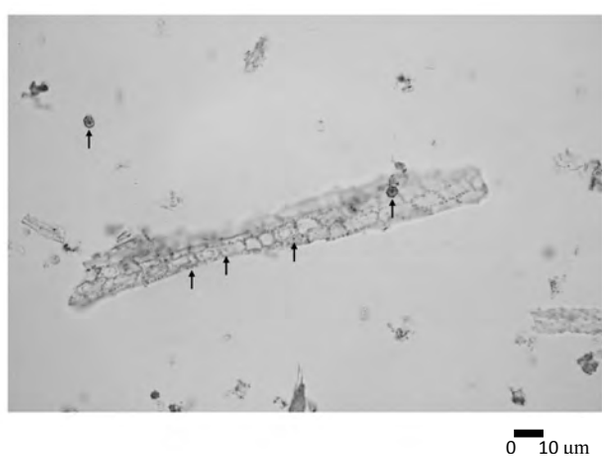

Fig 4. Microscope image of phytoliths (almost less than 5 μm in diameter) in abaxial epidermal cells of leaflet. Arrowed lines indicate phytoliths.

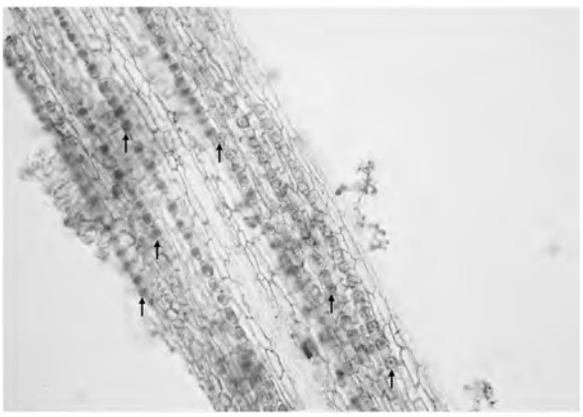

Fig 5. Microscope image of phytoliths (almost less than 5 µm in diameter) in abaxial epidermal cells of leaflet. Arrowed lines indicate phytoliths.

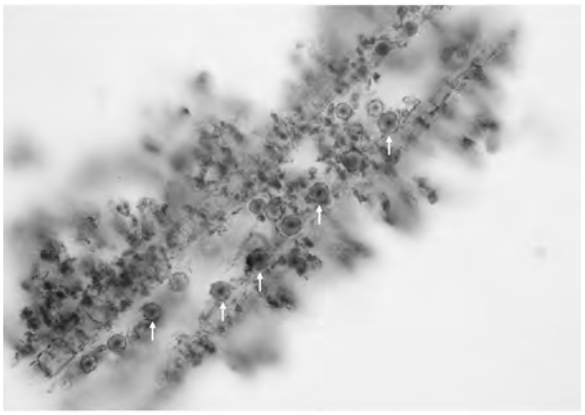

Fig 8. Microscope image of phytoliths (almost 5-10 µm in diameter) in abaxial epidermal cells of leaflet. Arrowed lines indicate phytoliths.

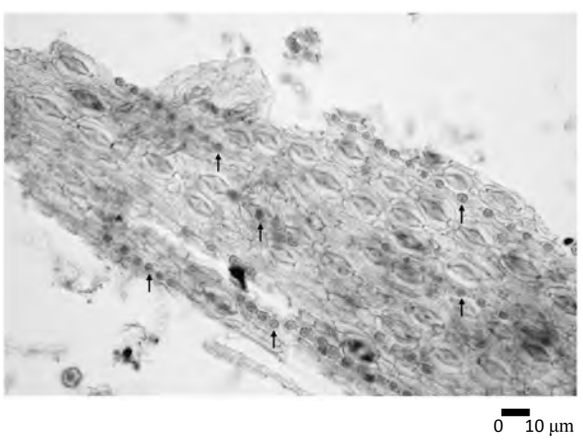

Fig 6. Microscope image of phytoliths (almost less than 5 µm in diameter) in abaxial epidermal cells ofleaflet. Arrowed lines indicate phytoliths.

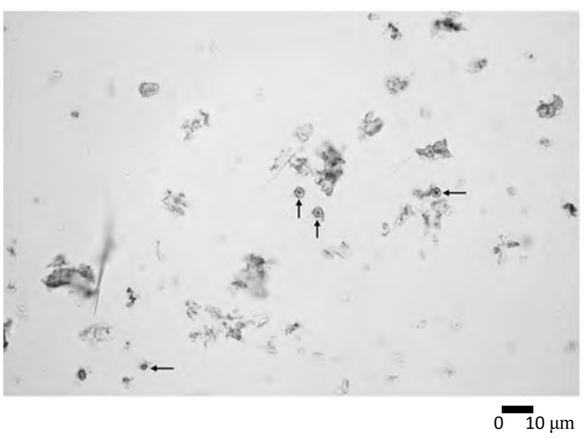

Fig 9. Microscope image of phytoliths (less than 5 µm in diameter, extracted from leaflet) in Pangasugan, Leyte, Philippines
Arrowed lines indicate phytoliths.

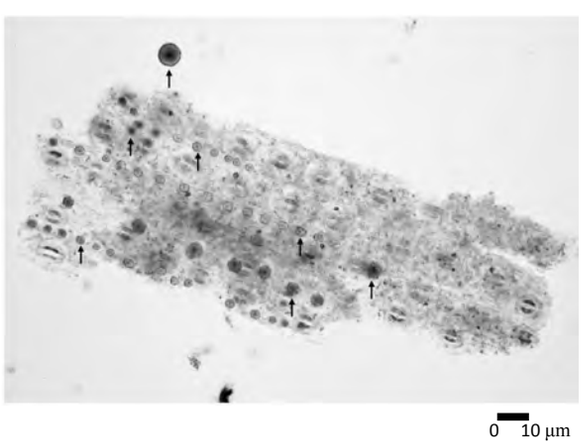

Fig 7. Microscope image of phytoliths (less than 5 µm and 5-10 µm in diameter) in abaxial cells of leaflet. Arrowed lines indicate phytoliths.

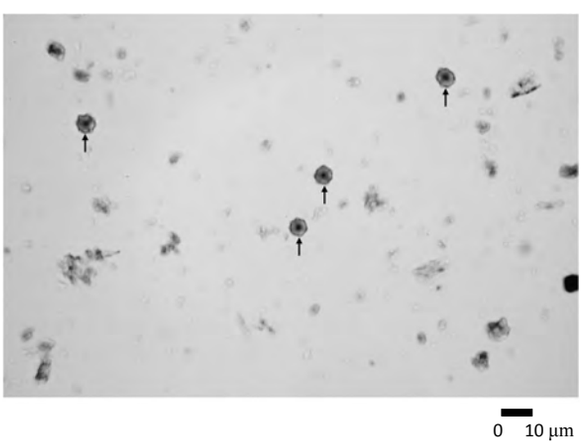

Fig10. Microscope image of phytoliths (5-10 µm in diameter, extracted from leaflet) in Pangasugan, Leyte, Philippines
Arrowed lines indicate phytoliths.

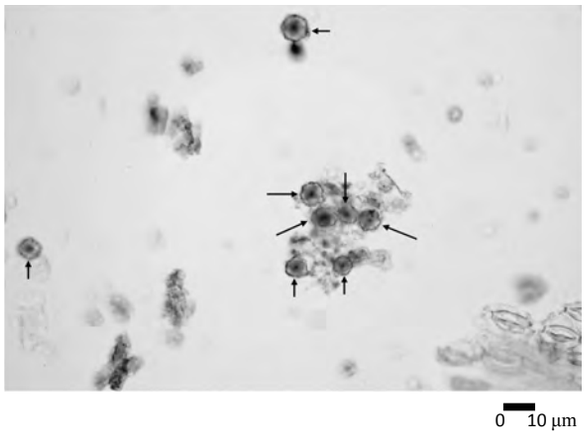

Fig11. Microscope image of phytolith (10-15 µm in diameter, extracted from leaflet) in Pangasugan, Leyte, Philippines
Arrowed lines indicate phytoliths.

Figure 4 clearly shows the distribution regions of small phytoliths, less than 2.5 µm in diameter, which are intracellularly deposited. The phytolith morphology of *M. sagu* was spheroid echinate, with conical projections distributed over the entire surface (Figs. 9–11). Conical ornamentation occurred singly on the surface. The diameter of incinerated *M. sagu* phytoliths was in a range from less than 2.5 µm to more than 22.5 µm. Conical spine lengths were more or less 1 µm and numbered about 20, which corresponded to the result of Benvenuto et al. (2015). From the microscope observation of phytoliths in leaves from an apical to a lower position leaf, it is deduced that the size of phytoliths gradually increases with *M. sagu* growth. Under a microscope, the center portion of the spheroid echinate phytolith was darker than the marginal portion, and this variation was caused by amorphous organic substances that occupied the thick longitudinal part of the sphere.

2. Phytoliths collected from the leaflet tissue of *M. sagu* after incineration

Figures 9–11 show spheroid echinate phytoliths extracted from incinerated leaflet samples of *M. sagu*. After incineration, they were released from leaflet tissues as a result of the disappearance of small phytoliths via decantation treatment. The phytolith sizes ranged from 2.5 µm to more than 22.5 µm in diameter.

3. Diameter analysis

Spheroid echinate phytoliths from *M. sagu* show a range of mean values, from 2.5 µm to 22.5 µm, in increments of 1.25 µm with simple normal distribution (Fig. 12). The peak of distribution was found in the diameter range of 10.0 to 12.5 µm, which was smaller than that in Fenwick et al. (2011).

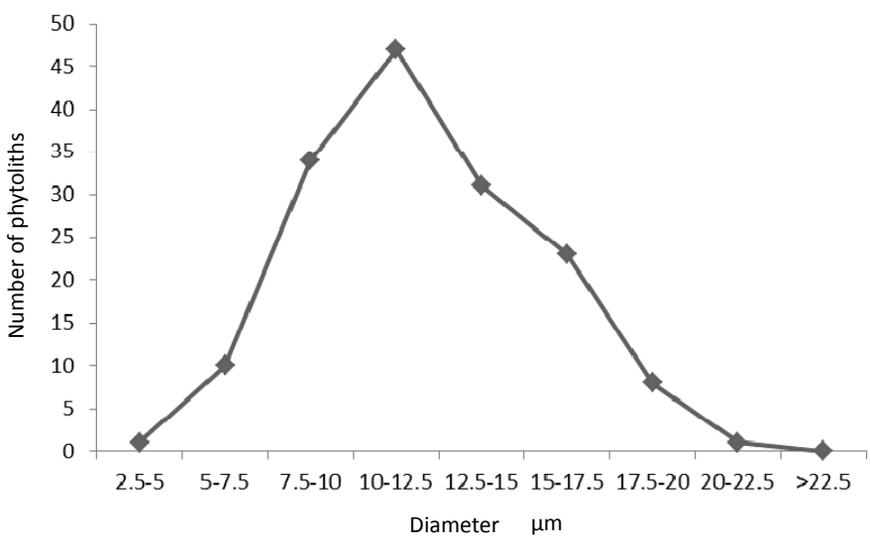

Fig 12. Distribution of number of phytoliths in sago palm (*Metroxylon sagu* Rottb.) leaflet from Pangasugan, Leyte, Philippines

Discussion

1. Silicon uptake and phytolith formation in *Metroxylon sagu*

Many researchers are interested in the formation of biogenic opal silica in plants (Nawaz et al., 2019).

Plants accumulate silicon up to 10% of leaf on a dry weight basis (Currie and Perry, 2007). Silicon is present in soil solutions as $Si(OH)_4$ or $Si(OH)_3O^-$ and is a beneficial element for plants. Ma (2004) reported that silicon alleviated the toxic effects caused by stress from heavy metals, salt, and drought. Plants can absorb silicon via radial and passive transport from the external solution (Mitani and Ma, 2005). In the case of rice plants, silicon is transported to the root surface by mass flow and reaches the xylem through two kinds of transporters (Ma and Yamaji, 2006). Sahebi et al. (2015) showed that the complex of silicon and organic substances might be moved to plant organs and create polymers for biogenic opal silica (phytolith) formation in rice plants (Currie and Perry, 2007). Although Patterer (2014) described phytoliths in the main palm species present in subtropical regions of South America, there is no report on the silicon polymerization process for phytoliths in *M. sagu*. Since the center portion of spheroid echinate was the thick central part of the sphere and partly occupied by organic substances, such as amino acids, peptides, and proteins (Kumar et al., 2010), it was darker than the marginal portion under a microscope. The positively charged amino acid side chains and negatively charged silica species via electrostatic interaction show the largest effect on the formation of phytoliths. The phytolith formation mechanisms in *M. sagu* will be studied in the near future.

2. Importance of *Metroxylon sagu* phytolith study for crop cultivation in the Philippines

Metroxylon sagu produces diagnostic phytoliths. However, the information on *M. sagu* phytoliths is quite limited. Stable *M. sagu* phytoliths definitely show traces of their growth in the soil of Southeast Asia. The accumulation of a large amount of phytoliths in the soil profile is considered to indicate the semicultivation or cultivation of *M. sagu*. This is of interest with regard to the long-term transition of people from hunters and gatherers to rice cultivators. There is a shortage of phytolith information extracted from stones and earthenware in the Philippines. Our study would give the cultivation history of *M. sagu* in Philippines prior to rice cultivation.

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