## Phytolith Assemblages in Sago Palm (Metroxylon sagu Rottb.) Leaflets

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Abstract: Phytolith (biogenic opal silica) assemblages in all organs of the palm family are formed by the uptake of silicon from the soil through the root system. Characteristic Metroxylon sagu phytoliths were counted to evaluate their presence and size distribution in different leaf and leaflet positions at the approaching flowering stage. At the sago field in Pangasugan, Leyte, Philippines, the samples of M. sagu were collected from upper (the 4th leaf from the apex), middle (the 8th), and lower (the 11th or 14th) leaf portion and from upper, middle and lower leaflet position of each leaf. Phytolith assemblages in M. sagu leaflets were extracted by a dry combustion method with an electric furnace and described based on morphology and ornamentation according to International Code for Phytolith Nomenclature 1.0 and 2.0 using a light-transmitting and scanning electron microscope. Spheroid echinate phytoliths, which were highly diagnostic with minor exceptions, were observed in the leaflets of M. sagu (three palms) in Pangasugan. Incinerated leaflet samples amounted to approximately 9% of the oven-dry weight of leaflet. The phytoliths were counted under a light-transmitting microscope. The mean diameter of phytoliths was 13.2  $\mu$ m. Phytoliths were divided into five classes by diameter size; <5 (A), 5– 10 (B), 10–15(C), 15–20 (D), and >20  $\mu$ m (E) accounted for 11.3, 23.4, 23.5, 25.9, and 15.9% of the total phytoliths, respectively. The number of conical projections (spines) in the five classes ranged from 8 to 34 with a mean value of 24.8. The largest number of spines (more than 30) was found in 15–20 µm (D) and >20µm (E) phytoliths. The increasing trend of the mean number of spines was observed in the increasing diameter of phytoliths with some exceptions. The phytolith assemblages supplied by M. sagu will contribute to an useful indicator of *M. sagu* growth for vegetation reconstruction and archaeological study.

Keywords: echinate, Philippines, silicon, spheroid, spine

Silicon (Si) is a beneficial element for plants and is present in soil solution as Si(OH)4 (Currie and Perry, 2007; Nawaz et al., 2019). It alleviates the toxic effects caused by stresses of heavy metals, salt, and drought (Ma, 2004). The vigor of plants is assisted by Si (Luyckx et al., 2017). Both monocotyledons and dicotyledons produce phytoliths (Kealhofer and Piperno, 1998; Huisman et al., 2018). Si deposition increases the abrasiveness of plant tissue and reduces its palatability and digestibility for herbivores (Massey and Hartley, 2009). The uptake of Si significantly increases the phytolith content of rice plants to 5 to 6% (Kondo, 2010; Sun et al., 2019). Si has positive effects on the breaking resistance and bending moment of rice (Fallah, 2012), resulting in a sufficient supply of Si that provides stability for culms and serves to decrease the risk of lodging for rice plants.

Plants can absorb Si with radial and passive transport from external solution (Mitani and Ma, 2005). Si dissolved from rocks and minerals in soil is transported to the surface of the rice root by mass flow and is absorbed through root cells to vessels by three kinds of Si transporters (Lsi1, Lsi2, and Lsi6) (Ma and Yamaji, 2006; Mitani et al., 2009). Silicon in the soil solution is taken up to the inside of exodermis cells by Lsi1. Lsi2 can transport Si from the inside of exodermis cells to outside the cells (the apoplast pathway). In endodermis cells, Si is again transported to the cortex by the working of Lsi1 and Lsi2. Finally, Si flows through vessels to aboveground by transpiration flow. Lsi6 plays a role in the unloading of Si in the xylem parenchyma adjacent to the phloem of the leaf blade and sheath (Yamaji et al., 2008).

Morphometric analysis allows researchers to classify the entire palm family (Delhon and Orliac, 2007). Patterer (2014) described phytoliths of the main palm species (Arecaceae) present in the subtropical regions of South America. Satakentia and Arenga phytoliths were found to be 1.84 and 6.87%, respectively, of the total dry weight (Kondo, 2010). Arecaceae produces large amounts of phytoliths and has globular echinate morphotypes in its tissue (Delhon and Orliac, 2007; Kondo, 2010; Benvenuto et al., 2015). The features of globular morphology among Arecaceae, Bromeliaceae, Cannaceae, Marantaceae, Orchidaceae, Strelitziaceae, and Zingiberaceae were distinguished. Arecaceae is a large family of abundant phytolith producers. The small globular bodies (10-15 µm in diameter) in the various phytolith morphologies are of particular use for diagnostic and identification purposes. A continuum of surface ornamentation was displayed on these bodies, varying from psilate to tuberculate, with echinate nodes being the most commonly observed surface feature. Members of Palmae produce two types of phytoliths: hat-shaped or conical forms with flat bases, and irregular spherical forms; both types usually have rough, spinulose surfaces (Tomlinson, 1969). Piperno (1988) noted that Palmae species were common to abundant producers of distinctive spherical phytoliths with echinate to tuberculate surface ornamentation. The ornamented globular bodies produced in the Arecaceae overlap some with those of Bromeliaceae (Tomlinson, 1969; Piperno, 1988). The size differential between families

is remarkable; *Arecaceae* phytoliths were larger than those of *Bromeliaceae*. The ornamented globular phytolith is considered a reliable indicator of palms. Szczepanowska (2018) provided images of rattan (*Calameae* Kunth) phytoliths in parenchyma fibers using a scanning electron microscope and the element in phytoliths using energy-dispersive X-ray spectroscopy (EDX).

However, there are few reports of phytolith formation in M. sagu (Fenwick et al., 2011; Bowdery. 2014). Fenwick et al. (2011) showed that the assemblage-based approach of *M. sagu* phytoliths provided potential value for archaeological analysis. Likewise, Bowdery (2014) found higher percentages of M. sagu phytoliths at a depth of 0.97 m in the Rano Kau (Easter Island, Chile) core sample, which corresponded to AD 1634-1672 based on 14C dating, than those at other layers. Recently, Baba et al. (2020) elucidated *M. sagu* phytolith assemblages in the 6th leaf from the apex in Pangasugan, Leyte, Philippines. However, they did not describe the phytolith assemblages of different leaves and leaflets. This study used the characteristics of M. sagu phytoliths - spheroid echinate shape, 13.2 µm of the mean diameter, 26.0 of the mean number of conical projections (spines), 84.0  $^{\circ}$ of the mean terminal angle, and 0.54  $\mu$ m of the mean length of conical projections (spines) under lighttransmitting microscope presented by Baba et al. (2020) and Okazaki et al. (2020).

The objectives of this study were to describe the morphological characteristics (spheroid echinate) of phytoliths in *M.sagu* leaflets and to show the size distribution, number of spines, and spine length of *M. sagu* phytoliths in leaflets in accordance with the International Code for Phytolith Nomenclature 1.0 (Madella et al., 2005) and 2.0 (Neumann et al., 2019).

#### **Materials and Methods**

#### Phytoliths in sago palm leaflets

Three *M. sagu* (Palms Y, R, and W) were felled for leaf sampling; palm heights were 11.9, 11.7, and 11.1 m, respectively, just before the flowering stage in

Palm	Palm height (m)	Leaf length (m)	Total number of leaves	Leaf position	Number of leaflets
Y	11.9	7.1	16	Upper Middle Lower	144 128 128
R	11.7	6.4	13	Upper Middle Lower	128 126 122
W	11.1	7.1	16	Upper Middle Lower	144 136 136

Table 1. Growth factors of three palms in Pangasugan, Leyte, Philippines

Pangasugan, Leyte, Philippines (Table 1). Of the three palms, Palm W was the most severely damaged by Typhoon Yolanda in 2015 (Okazaki et al., 2013). *M. sagu* leaf samples (Fig. 1) of the 4th, 8th, and 11th or



Fig 1. Metroxylonsagu leaf inPangasugan, Leyte, Philippines (2019)

14th leaf from the apex were collected from the experimental sago field of Pangasugan in 2019. Leaf samples were divided into three positions: upper, middle, and lower. Furthermore, leaflet samples were divided into three positions of different leaves: upper, middle, and lower and air-dried after washing with distilled water (Fig. 2). About 21 g of fresh leaflet samples was oven-dried at 105 °C and incinerated in an electric furnace at 500°C for 4 hours. Incinerated leaflet samples were weighed. Parts of the incinerated samples were rinsed in 0.01 mol/L HCl and washed



Fig 2. Simplified diagram of *Metroxylon sagu* leaves and leaflets
1: upper leaf; 2: middle leaf; 3: lower leaf;
4: upper leaflet; 5: middle leaflet; 6: lower leaflet

with distilled water three times. Eight hours after shaking, the top 10 cm of supernatant was removed from the water in a test tube. The phytolith samples in the remaining solution were centrifuged and dried in a drying oven at 105 °C. Matsunami MGK-S embedding agent (polystyrene) was used for mounting and covered with a cover glass for observation under a light-transmitting microscope (LTM) (Meiji Techno MT5000) at 100x and 400x magnification in order to count the small to large phytoliths, although Albert et al. (2009) and Katz et al. (2010) proposed a method of solution separation involving a sequential procedure of sieving, acid digestion, and sodium polytungstate (Na6(H2W12O40)·H2O). The phytolith samples were stored individually in small vials. The presence of phytoliths was recorded according to the description of

the International Code for Phytolith Nomenclature 1.0 (Madella et al., 2005) and 2.0 (Neumann et al., 2019): 3D shape, 2D shape, texture, and ornamentation. The maximum diameter from spine (conical projection) tip to spine tip (the phytolith's largest visible dimension) and the number of spines were determined. A total of 20 to 70 phytoliths was counted per sample to quantify the relative abundances of morphotypes (Albert et al., 2009). Around 30 phytoliths were measured to obtain the size distribution of 5 classes by diameter size: <5, 5–10, 10–15, 15–20, and >20  $\mu$ m. The percentage of different sizes of *M. sagu* phytoliths was calculated as a reference from the results of Yong et al. (2010). The total numbers of *M. sagu* leaflet spines were counted under LTM (Fig. 3) and multiplied by two. A scanning



Fig 3. Spines on the surface of an *M. sagu* phytolith Open circles show spines. The total number of spines is calculated by doubling the spines on the front.



WMm



Fig 4. *Metroxylon sagu* phytoliths in incinerated leaflet samples from Pangasugan, Leyte, Philippines in 2019 YMm: Palm Y, middle leaf, middle leaflet RMm: Palm R, middle leaf, middle leaflet WMm: Palm W, middle leaf, middle leaflet

Table 2.	Dry weight percent of incinerated leaflet samples
	in different leaves and leaflets of three Metroxylon
	SAQU

		Dry weight %	SD
Palm	Y	8.8	3.9
	R	7.7	3.3
	W	10.7	6.7
Leaf position	Upper	11.7	1.8
	Middle	10.4	6.6
	Lower	4.9	1.0
Leaflet position	upper	10.7	7.0
	middle	8.7	3.7
	lower	7.7	2.9
Mean of three pa	an of three pairns 9.0		4.9

Dry weight %: (Incinerated weight / dry leaflet weight) x 100 SD: Standard deviation

electron microscope (SEM) (Hitachi Miniscope TM-1000) was used to confirm the morphological features of dried samples, angles, and lengths of spines without any special pretreatment.

### Results

# 1. Phytoliths in different leaf positions of *Metroxylon sagu*

Silicon uptake by M. sagu resulted in the formation of spheroid echinate phytoliths. The mean incinerated

leaflet samples amounted to  $9.0 \pm 4.9\%$  of the total leaflet dry weight (Table 2). The phytoliths in the leaflet epidermis of M. sagu were observed under microscope (Figs. 4 to 6). With several exceptions, they presented 20 to 30 spines symmetrically arranged at the periphery, which agreed with the observation of Okazaki et al. (2020). Phytoliths serve as one diagnostic feature that distinguishes





0 10 µm

Fig 5. *Metroxylon sagu* phytoliths in an incinerated leaflet sample from Pangasugan, Leyte, Philippines in 2019 RMu: Palm R, middle leaf, upper leaflet RMm: Palm R, middle leaf, middle leaflet RMI: Palm R, middle leaf, lower leaflet

0\_10 μm



Fig 6. LTM (A) and SEM (B) images of phytoliths in *M. sagu* 

palms at the genus level (Kealhofer and Piperno, 1998; Prychid et al., 2003; Piperno, 2006). In Figs. 4 and 5, the phytoliths in the leaflet tissue were distributed in rows, and slightly larger phytoliths than those observed in the field of view were located in the peripheral part of the stomata guard cells. Figure 6 shows diagnostic globular echinate types (Madella et al., 2005; Osterrieth et al., 2009) or spheroid echinate types (Neumann et al., 2019) of

different sizes. LTM and SEM images of phytoliths in Fig. 6 clearly show a spheroid echinate morphotype, which corresponds to the results of Fenwick et al. (2011) and Bowdery (2014).

The mean maximum diameter of *M. sagu* leaflet phytoliths was  $13.2 \pm 1.8 \mu m$  (Table 3). The distribution of different sizes of *M. sagu* phytoliths in leaflets is shown in Fig. 7, indicating 11.3, 23.4, 23.5, 25.9, and 15.9% for <5  $\mu m$  (A), 5–10  $\mu m$  (B), 10–15  $\mu m$  (C),

 Table 3. Mean maximum diameter, number, angle, and length of spines of Metroxylon sague phytolith assemblage (LTM)

	Mean	SD	
Mean maximum diameter (µm)	13.2	1.8	
Number of spines	24.8	3.7	
Angle of spines (degree)	87.1	10.7	
Length of spines (µm)	1.1	0.4	

SD: Standard deviation

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**Fig 7.** Different size distributions of phytoliths in *M. sagu* leaflets from Pangasugan, Leyte, Philippines (three palms) Leaflet samples taken from the upper, middle, and lower positions of the upper (4th), middle (8th), and lower (11th and 14th) leaves Figures show %. 15–20  $\mu$ m (D), and >20  $\mu$ m (E), respectively. Upper leaf samples of three palms contained (D) + (E) size phytolith assemblages as 40 to 50% of the total number of phytoliths with some exceptions (Fig. 8). Meanwhile, (D) + (E) phytolith assemblages of middle and lower leaf samples provided 20 to less than 50% with large variations (Figs. 9 and 10). Among three *M. sagu*, the percentages of incinerated leaflet weight to oven-dried leaflet weight are shown in Table 2. The mean incinerated leaflet weight was 9.0%, ranging from 7.7 to 10.7% among the three different palms, 4.9



Fig 8. Percentage of different sizes of phytoliths in the upper leaf

YUu: Palm Y, upper leaf, upper leaflet; YUm: Palm Y, upper leaf, middle leaflet; YUI: Palm Y, upper leaf, lower leaflet; RUu: Palm R, upper leaf, upper leaflet; RUm: Palm R, upper leaf, middle leaflet; RUI: Palm R, upper leaf, lower leaflet; WUu: Palm W, upper leaf, upper leaflet; WUm: Palm W, upper leaf, middle leaflet; WUI: Palm W, upper leaf, lower leaflet



Fig 9. Percentage of different sizes of phytoliths in the middle leaf YMu: Palm Y, middle leaf, upper leaflet; YMm: Palm Y, middle leaf, middle leaflet; YMI: Palm Y, middle leaf, lower leaflet; RMu: Palm R, middle leaf, upper leaflet; RMm: Palm R, middle leaf, middle leaflet; RMI: Palm R, middle leaf, lower leaflet; WMu: Palm W, middle leaf, upper leaflet; WMm: Palm W, middle leaf, middle leaflet; WMI: Palm W, middle leaf, lower leaflet

to 11.7% among different leaf positions, and 7.7 to 10.7% among different leaflet positions. The lower leaf position provided the lowest percentage of phytolith content in M. sagu. The accumulation of silicon actively proceeds in the early growth stage, and phytoliths may gradually grow larger.

### 2. Phytoliths in different leaflet positions of

#### Metroxylon sagu

The (D) + (E) phytolith assemblages of upper leaflet samples ranged from 20 to 50% (Fig. 11). In

the case of middle leaflet samples, the (D) + (E) phytolith assemblages revealed 40 to 50% of the total phytolith assemblages, decreasing in order of upper, middle, and lower leaves except for Palm R (Fig. 12). On the other hand, lower leaflet samples ranged from 30 to 50% with large fluctuations (Fig. 13). The phytolith assemblages in *M. sagu* leaves were not formed uniformly. The mean oven-dried weight percentage of phytoliths varied from 7.7 to 10.7% for different leaflet positions (Table 2).



Fig 10. Percentage of different sizes of phytoliths in the lower leaf YLu: Palm Y, lower leaf, upper leaflet; YLm: Palm Y, lower leaf, middle leaflet; YLl: Palm Y, lower leaf, lower leaflet; RLu: Palm R, lower leaf, upper leaflet; RLm: Palm R, lower leaf, middle leaflet; RLl: Palm R, lower leaf, lower leaflet; WLu: Palm, lower leaf, upper leaflet; WLm: Palm W, lower leaf, middle leaflet; WLL: Palm W, lower leaf, lower leaflet



Fig 11. Percentage of different sizes of phytoliths in the upper leaflet YUu: Palm Y, upper leaf, upper leaflet; YMu: Palm Y, middle leaf, upper leaflet; YLu: Palm Y, lower leaf, upper leaflet; RUu: Palm R, upper leaf, upper leaflet; RMu: Palm R, middle leaf, upper leaflet; RLu: Palm R, lower leaf, upper leaflet; WUu: Palm, upper leaf, upper leaflet; WMu: Palm W, middle leaf, upper leaflet; WLu: Palm W, lower leaf, upper leaflet



Fig 12. Percentage of different sizes of phytoliths in the middle leaflet YUm: Palm Y, upper leaf, middle leaflet; YMm: Palm Y, middle leaf, middle leaflet; YLm: Palm Y, lower leaf, middle leaflet; RUm: Palm R, upper leaf, middle leaflet; RMm: Palm R, middle leaf, middle leaflet; RLm: Palm R, lower leaf, middle leaflet; WUm: Palm, upper leaf, middle leaflet; WMm: Palm W, middle leaf, middle leaflet; WLm: Palm W, lower leaf, middle leaflet



Fig 13. Percentage of different sizes of phytoliths in the lower leaflet YUI: Palm Y, upper leaf, lower leaflet; YMI: Palm Y, middle leaf, lower leaflet; YLI: Palm Y, lower leaf, lower leaflet; RUI: Palm R, upper leaf, lower leaflet; RMI: Palm R, middle leaf, lower leaflet; RLI: Palm R, lower leaf, lower leaflet; WUI: Palm, upper leaf, lower leaflet; WMI: Palm W, middle leaf, lower leaflet; WLI: Palm W, lower leaf, lower leaflet

## 3. Mean maximum diameter, number, angle, and

### length of spines on Metroxylon sagu phytoliths

The mean maximum diameter of phytoliths is  $13.2 \pm 1.8 \mu m$  (Table 3), which is slightly smaller than that reported by Fenwick et al. (2011), who noted the maximum diameter of 14.1  $\mu m$  for 50 phytoliths of a *M. sagu* reference specimen. The number of spines on *M. sagu* phytolith (Palm Y) varied from 8 to 34, with a mean value of 24.8 $\pm$  3.7 (Figs. 14 and 15). The 15–20 $\mu m$  (D) and >20  $\mu m$  (E) phytolith assemblages have more spines than the <5 (A) and 5–10 $\mu m$  (B)

ones. The angle and length of spines were  $87.1 \pm 10.7^{\circ}$  and  $1.1 \pm 0.4 \mu m$ , respectively, which showed the intermediate values between the TLM and SEM measurements of Okazaki et al. (2020).

### Discussion

#### 1. Phytolith sizes in Metroxylon sagu

The Si content in the soil varied from 53.5 to 86.8% (mean: 70.6%) as SiO<sub>2</sub> (Bowen, 1979). Generally, the Si concentration in a soil solution ranges from 0.1 to 0.6 mmol/L (Epstein, 1994). Si



Fig 14. Mean number of spines on the surface of phytoliths with different positions of the leaf (Palm Y) in Pangasugan, Leyte, Philippines The bar shows the standard deviation.Y: Palm Y; U: upper leaf; M: middle leaf; L: lower leaf;





Fig 15. Mean number of spines on phytoliths (Palm Y) with different leaflet positions in Pangasugan, Leyte, Philippines The bar shows the standard deviation.

accumulation has been found to a great extent, but not exclusively, in monocotyledonous plants (Currie and Perry, 2007). Plants can accumulate, store, and process Si to create mesoporous silica nanoparticles (Sun et al., 2014) and phytoliths (Huisman et al., 2018). Palms form characteristic phytoliths (Kondo, 1977; Kondo and Sase, 1986; Barboni et al., 2007; Mercader et al., 2009; Kondo, 2010; Morcote-Rios et al., 2016). *M. sagu* is also capable of taking up Si as silicic acid in pH ranges up to 9.8, finally forming phytoliths in leaves, petioles, and seeds. Fenwick et al. (2011) reported that *M. sagu* phytoliths were more clearly distinguishable than those of *Arecacatechu*, *Cocos nucifera*, and *Calamusaruensis*, in order to

have a large mean diameter of 14.09  $\mu$ m, a strong tendency to be spherical (93.6% of the assemblage), and a weak tendency toward having right-angled spines (58.8% of the assemblage). However, the results with respect to diameter in this study were smaller than those of Fenwick et al. (2011) because of different portions of leaves and leaflets and different extraction methods used.

# 2. Description of *Metroxylon sagu* phytoliths for further study

The morphometrical analysis of palm phytoliths shows important variations of size and shape parameters from one species to another and from one organ to another (Delhon and Orliac, 2007). The International Working Group on Phytolith Nomenclature (IWGPN) developed a description of phytoliths because standardizing and harmonizing the names and descriptions of phytoliths would improve communication between researchers and facilitate the comparison of phytolith types and analyses (Madella et al., 2005). The International Code for Phytolith Nomenclature 1.0 presented by Madella et al. (2005) and the International Code for Phytolith Nomenclature 2.0 (Neumann et al., 2019) are appropriately accepted worldwide. The characteristic information of phytoliths should be supplied in the order of shape, texture/ornamentation, symmetric features, morphometric data, illustrations, and anatomical origin. Palms produce two types of diagnostic phytoliths. One type is a globular (spheroid) echinate morph. The other type is a hemispherical or conical hat-shaped rimmed morph, whose distribution is restricted to Caryota, Orania, and Arenga. M. sagu phytoliths are composed of globular or ellipsoid morphs with a mean diameter of 14.086 µm, and they exhibit regular echinate to nodulose surface decorations (Fenwick et al., 2011). Almost all M. sagu phytoliths are spheroid echinates, which are diagnostic and can be definitively distinguished from other Arecaceae phytoliths.

# 3. Relationship between phytoliths and the breaking resistance/bending moment in *Metroxylon sagu*

Silicified cells in rice provide the much-needed strength to resist culm breaking (Dorairaj and Ismail, 2017). Fallah (2012) showed that the breaking resistance of rice plants increased with increasing Si content. Hossain and Choudhury (1975) also revealed that added silicon under hydroponic culture significantly increased the rigidity of rice stalks. Okazaki et al. (2013) reported the breaking resistance of M. sagu leaflets. However, there has not yet been a report on the relationship between phytolith content in cells of M. sagu and breaking resistance. The strong breaking resistance of M. sagu might be dependent on the array of phytoliths.

# 4. *Metroxylon sagu* phytoliths for past vegetation reconstruction

Plants accumulate Si up to 0.1 to 10% on a dryweight base as biogenic silica phytoliths and provide phytolith assemblage to soil (Currie and Perry, 2007). Morcote-Rios et al. (2016) used phytoliths as a tool for archaeobotany, palaeobotany, and palaeoecology, reconstructing ancient floras and landscapes and interpreting events in plant evolution and documenting plant use by ancient peoples. Huisman et al. (2018) also concluded that the analysis of phytoliths could track local-scale vegetation dynamics, whereas pollen, which was commonly used in palaeoecological reconstructions, reflected regional-scale vegetation change. Lu and Liu (2005) reveled phytolith assemblages as indicators of coastal environmental changes and hurricane overwash deposition.

Silicon uptake by M. sagu resulted in the accumulation and formation of phytoliths in leaves, roots, and seeds. Bowdery (2014) reported the spherical echinate (globular echinate: International Code for Phytolith Nomenclature) form of phytolith in M. sagu to compare and classify the vegetation on the remote southern oceanic island of Rapa Nui (Easter Island) and, based on <sup>14</sup>C dating, concluded that M. sagu was present from AD 1425 to 1634 on Easter

Island in the southeast Pacific Ocean. This makes *M*. *sagu* a relative newcomer, as compared to *Metroxylon vitiense*.

The Si-stable isotope composition of phytoliths was a good tool for elucidating paleoenvironmental information. During the uptake and conversion of silicic acid into particulate hydrous silica, the lighter <sup>28</sup>Si is preferentially incorporated into the silica over the heavier <sup>29</sup>Si and <sup>30</sup>Si (Leng et al., 2009), resulting in phytoliths with lighter specific density than those of quartz and opal. The element composition of rice phytoliths was determined by Li et al. (2014). Phytoliths have carbon of 1 to 5 wt% (Jones and Beavers, 1963), which was occluded within the micrometric internal cavities (Alexandre et al., 2015). The  ${}^{13}C/{}^{12}C$  ( $\delta^{13}C$ ) ratios of carbon occluded in phytoliths express the paleoclimatic condition and vegetation (Kelly et al., 1991). Alexandre et al. (2015) showed the phytolith structure and occluded the carbon location using 3-D X-ray microscopy and nanoscale secondary ion mass spectrometry (NanoSIMS), concluding that two groups of organic carbon were presented in the cavities of phytoliths; one was susceptible to rapid oxidation, and the other was continuously distributed in and protected by the silica structure.

Phytolith study is a common tool used by many researchers to document vegetation changes and disturbance patterns related to human settlement and plant exploitation (Horrocks and Wozniak, 2008; Benvenuto et al., 2015), accompanied by phytolith <sup>14</sup>C dating (Piperno and Stothert, 2003). Wilding (1967) and Wilding et al. (1967) showed that occluded carbon was a suitable substrate for <sup>14</sup>C dating, although the <sup>14</sup>C age of phytolith-occluded carbon increased directly with the combustion temperature (up to 1100°C), resulting in age overestimations of hundreds of years (Yin et al., 2014). Piperno and Stothert (2003) reported that Cucurbita (squash and gourd) phytolith carbon in southwest Ecuador was identified as dating to 10,130 to 9320 <sup>14</sup>C years ago (about 12,000 to 10,000 calendar years ago).

Recently, multiple studies have revealed that the occluded carbon in phytoliths may play a role in atmospheric carbon dioxide sequestration and climate change mitigation (Parr et al., 2010; Song, et al., 2014; Zuo et al., 2014). However, the fluxes of carbon in phytoliths from vegetation to soil and the residence time of carbon in phytoliths in soil are not clear (Alexandre et al., 2015). We hope that we are able to discover when Leyte Island started to cultivate *M. sagu* and expanded to the east and west coastal sides of Leyte and why the east Leyte areas still have *M. sagu* and the west coastal side of Leyte has lost *M. sagu* in the present time.

# 5. Contribution of *Metroxylon sagu* phytoliths to archaeological research

Phytoliths are used in archaeology to study ancient plant remains and can provide insight into ancient diet, non-food uses of plants, the spatial arrangements of plant use and discard across settlements, agricultural practices, and the seasonality of pre-agrarian site occupations (Ryan, 2014). Taxonomically identifiable phytoliths are produced by many plants. Diagnostic spheroid echinate forms can be distinguished (Madella et al., 2005; Morcote-Rios et al., 2016; Huisman et al., 2018; Neumann et al., 2019; Yin et al., 2014). M. sagu phytolith assemblages from soil layers in Southeast Asia and South Pacific Islands and radiocarbon dating provide the distribution ages when transported from the center of sago growing areas, as well as its growing and environmental conditions.

#### Conclusion

*M. sagu* can take up and accumulate silicon to form phytolith (biogenic opal silica) assemblage, which amounted to 9.0% of dry leaflet weight. The phytoliths of *M. sagu* are spheroid echinate forms, indicating a characteristic feature of the palm family. The formation of phytoliths might be determined by genetics and, accidentally, by the silicon concentration in the xylem and phloem fluid. The mean diameter of *M. sagu* phytoliths was  $13.2\pm1.8$  µm. From this result, it is concluded that *M. sagu* phytoliths have sufficient qualifications for vegetation reconstruction and are a stable archaeological indicator.

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