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Phytotelm Algae of Pandan [*Pandanus amaryllifolius* Roxb.] (Pandanaceae) Leaf Axil Tanks from Laguna (Philippines)

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ABSTRACT.– Phytotelmata of pandan leaf axils represent a unique microhabitat for microalgae since this plant provides suitable environmental conditions for their growth and proliferation. The current study is the first taxonomic survey to focus on the taxonomy of phytotelm algae of *Pandanus amaryllifolius* (Pandanaceae). A total of 15 microalgal species were taxonomically identified and described from the collected water samples, of which six species (*Lyngbya taylorii*, *Cyanothece aeruginosa*, *Eudorina elegans*, *Staurastrum avicula* var. *lunatum*, *Ulnaria ulna*, and *Gomphonema sphaerophorum*) are new additional records of microalgae in the global inventory list of phytotelm microalgae. Also, the survey reported the occurrence of *Staurastrum avicula* var. *lunatum* (Ralfs) Coesel & Meesters for the first time in the Philippines. Taxonomic keys and descriptions as well as photomicrographs are presented to differentiate the species of phytotelm microalgae. The survey provided important taxonomic records on the species composition of microalgae of leaf axil tanks of plants found in the Philippines.

KEY WORDS: Cyanobacteria, microhabitat, microalgae, phytotelmata, tropical

INTRODUCTION

Phytotelmata is a term used to describe water impounded by plants. It is generally small and usually exists in leaf axils, tree holes, modified leaves, fallen fruit husks and fallen vegetative parts (such as bracts), and flowers (Yanoviak et al., 2001). Phytotelmata are present in several regions of the world, but they are most commonly observed in the neotropical region, usually because of the high rainfall and diverse number of plants capable of impounding water (Ramos et al., 2018). These aquatic environments are inhabited by diverse kinds of organisms such as microorganisms (bacteria and microalgae), annelids, and arthropods. Among these organisms, microalgae play an essential role for the ecological dynamics of the phytotelmata, mainly in plant areas that are subjected to high light irradiance (Ramos et al., 2017).

Pandanus amaryllifolius Roxb. (Pandan) is a famous tropical plant belonging to the family Pandanaceae known for its fragrant leaves and therapeutic properties. The leaves of this plant are considered as screw pine, since it is similar to spiral arrangement of pineapple with green, narrow, long and strap-shaped leaves (Ghasemzadeh and Jaafar, 2003). The spiral arrangement of the leaves is capable of holding water and detritus in cistern tanks. This water reserve contains nutrients in various concentrations, produced by the decomposition of detritus from external sources, such as animal fecal material, twigs, leaves and other living organisms present in the phytotelmata (Wittman, 2000). The formation of aquatic microhabitat (phytotelmata) in *P. amaryllifolius* is capable of supporting the existence organisms such as of arthropods, small

animals (amphibians) and other microorganism such as microalgae. Microalgae in phytotelmata produce oxygen and other organic compounds that are rapidly being consumed by bacteria in the cistern tank. The growth of bacteria favors decomposition that is beneficial to the *P. amaryllifolius* as an additional source of food via absorption of nutrients using the plant trichomes (Gebühr et al., 2006).

Studies concerning algal taxonomy and community development in phytotelmata are still limited. To date, majority of the reports about microalgae inhabiting phytotelmata are derived from the natural environment from countries such as Brazil, Congo, Puerto Rico, Ecuador, Jamaica, Guiana, Mexico, Thailand, and Philippines (Arguelles, 2021; Poniewozik et al., 2020; Ramos and Moura, 2019). Taxonomic groups of microalgae observed in these studies were cyanobacteria (Arguelles, 2021; Arguelles, 2020a; Ramos et al. 2018b, 2019), diatoms (Lyra, 1971), zygnematophytes (Sophia, 1999; Ramos et al. 2017b, 2018c), chlorophytes (Arguelles, 2021; Arguelles, 2020a; Poniewozik et al., 2020; Ramos et al. 2018a), xanthophyceans (Sophia 1999; Brouard *et al.* 2012), euglenophytes (Arguelles, 2021; Poniewozik et al., 2020; Ramos et al., 2017a), cryptophyceans (Duarte et al., 2013), synurophyceans (Ramos et al., 2018b) and dinoflagellates (Ramos et al., 2016). In comparison with other aquatic habitats (such as lakes, ponds, and streams) that have been well studied regarding the ecological function and occurrence of microalgae within the aquatic environment, to the best of our knowledge nothing is reported regarding species composition of microalgae existing in leaf axil tanks of *P. amaryllifolius*. This taxonomic survey is the first report of microalgae observed in leaf axil tasks of pandan. The aim of this investigation is to provide a taxonomic account of the algal species present in *P. amaryllifolius* phytotelmata in order to contribute important information regarding diversity and distribution pattern of microalgae in this microhabitat. This paper will focus on the morphotaxonomic characterization and identification of dominant and rare species of microalgae observed from water samples taken from leaf axil tanks of *P. amaryllifolius*.

MATERIALS AND METHODS

Sampling of Phytotelm Algae

In December 2019, a total of 45 water samples from 20 leaf axil tanks of *P. amaryllifolius* were collected from two sampling sites in Laguna: Adela's Garden in Los Baños (situated at 14° 10' 34.14" N, 121° 14' 37.58" E) and Javier Eco Farm in Calauan (situated at 14° 7' 47.28" N, 121° 18' 59.04" E) (Fig. 1 and 2). The total volume of water samples ranged from 4 to 8 ml for each leaf axil tanks and was gathered using a sterile Pasteur pipette. The sampling procedure was repeated three times in each leaf axil tank to ensure that all the biological material present was collected. The liquid samples were then placed in a sterile conical specimen tube (Tarson) of 25 mm x 50 mm size and was transported (using a portable cooler) immediately in the laboratory for *in vivo* analysis (Arguelles and Martinez-Goss, 2019; Arguelles, 2016; Arguelles et al., 2014). The water samples were divided into two lot: the first lot was examined using an Olympus CX31 research microscope with Infinity X digital camera to identify the microalgae in their living state. The second lot was preserved using 4% formaldehyde where a portion (5 ml) of the concentrated

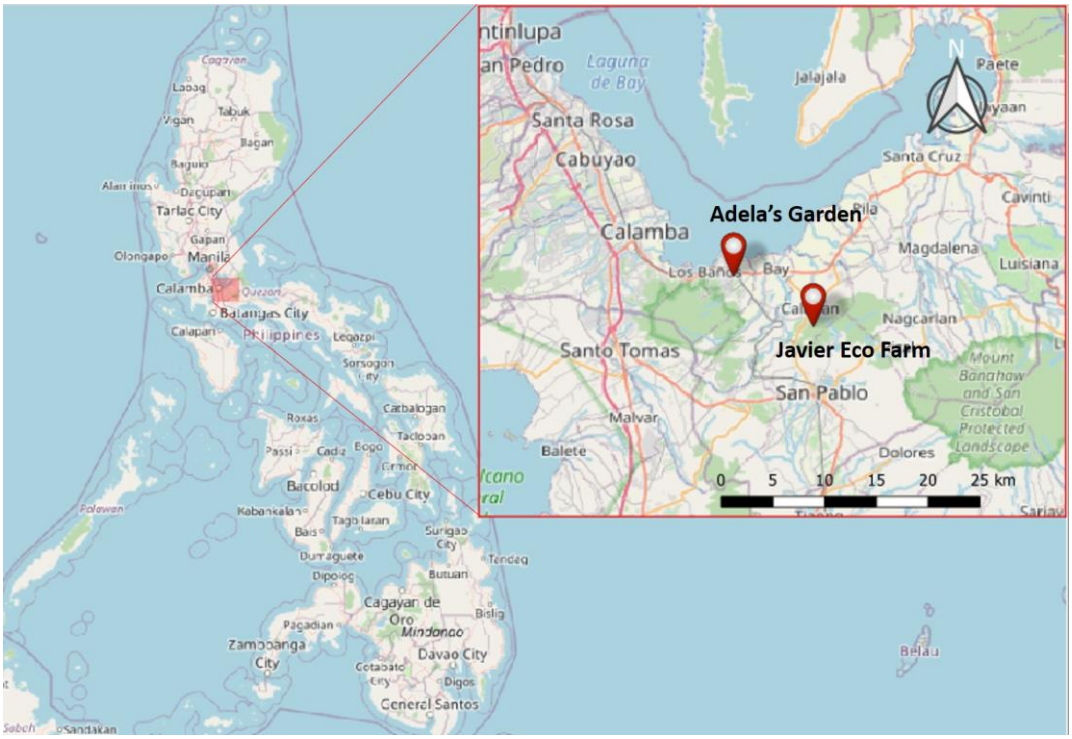

FIGURE 1. Location map of the sampling sites in Laguna (Philippines)

samples were used for diatom cleaning and analysis following the standard protocol of Round et al. (1990). Photomicrographs of each prepared slides were taken using the microscope at 1000x magnification.

Determination of Water Quality Parameters

The water quality parameters were taken at 1200–1300 h of the sampling day. Water temperature, pH, and dissolved oxygen were measured using Xplorer GLX (PASCO). All measured values were expressed as degrees Celsius for temperature and mg L^{-1} for dissolved oxygen. In addition, water samples were also tested for nitrate-nitrogen and orthophosphate concentration photometrically using Hach DR/2010 portable spectrophotometer following the methods of Villaroman et al. (2010).

Taxonomic Enumeration and Identification

The microalgae were identified to the species level via morphotaxonomic characterization using monographs and taxonomic literatures done by Desikachary (1959), Prescott (1962), Velasquez (1962), Pantastico (1977) Martinez (1984), Wehr and Sheath (2003), and Whitton (2002). Morphological and metric characteristics such as cell (specialized and vegetative) shape and size, features of the filaments and trichomes, pigmentation, filament cross wall constrictions, characteristics of the mucilaginous sheath; chloroplasts and pyrenoid features; cell wall characteristics and presence of specialized cells (heterocyst and akinetes) were used in the identification and

FIGURE 2. Pandan leaf axil tanks from sampling areas in Laguna (Philippines)

classification for each phytotelm microalgal taxa (Arguelles, 2019b).

RESULTS

The habitat of phytotelm microalgae in leaf axil tank of *P. amaryllifolius* was characterized by assessing different water quality parameters. Analysis of the collected water samples showed high nitrate (6.97 ± 0.93 mg L^{-1}) and phosphate (0.15 ± 0.07 mg L^{-1}) concentration. In addition, low pH (5.90 ± 0.31), high temperature (24.0 ± 0.73 ^{o}C) and dissolved oxygen (6.01 ± 0.16 mg L^{-1}) were also observed in this habitat. The presence of particulate matter, dark coloration, high temperature and nitrate concentration as well as acidic pH is indicative that organic matter decomposition occurs in the leaf axil tank during the study period. The availability of high nutrients in this microhabitat is mainly derived from windborne organic particles and detritus materials present in the leaf axil tanks (Gebühr et al., 2006). The presence of these substances favors the existence of phytotelm algae, which are responsible for production nutrients and other substances that can be utilized by the host plant and other invertebrates present in the habitat (Arguelles, 2021).

In total, 15 microalgal taxa were observed and identified in water samples collected from phytotelmata of Pandan in Laguna. The microalgal taxa found are subdivided into six classes: Bacillario-phyceae, Mediophyceae, Zygnematophyceae, Trebouxiophyceae, Chlorophyceae, and Cyanophyceae. The richest class is Cyano-phyceae and Bacillariophyceae, which included 26.67% of the taxa followed by Chlorophyceae with 20.0%. The other

classes were represented by only one taxon and constituted 6.67% of the total taxa. The observed taxa were classified into 12 orders, 14 families, 15 genera and 15 species based on recent taxonomic classification system. Taxonomic references used in the identification of the microalgal taxa are included directly below the description of each taxon. Moreover, current accepted taxonomic names of each algal taxa based on Algaebase (Guiry and Guiry 2020) are presented in the paper.

Dichotomous Key

1. Cells with typical nucleus, pigments bounded by membranes on specific organelle……....**2**
1. Cells without typical nucleus, pigments dispersed in the peripheral portion of the cell……..…**12**
2. Cells with red eyespot and proteinacious pellicle……...***Phacus* sp.**
2. Cells without red eyespot and proteinacious pellicle……..**3**
3. Cells with siliceous cell wall……..…….…..…..**4**
3. Cells with cell walls made of cellulose……..…….…...**8**
4. Valves are linear and elongated……..……....…….……..**5**
4. Valves rounded (disc-shaped) and small with a narrow mantle……...***Cyclotella meneghiniana***
5. Valves are linearly clavate with a capitate headpole and narrow basal pole……..***Gomphonema shaerophorum***
5. Valves are linear - lanceolate……...……...……**6**
6. Valves are linear-lanceolate with a tapered sternum…….……...…….***Fragillaria* sp.**
6. Valves are linear lanceolate, tapering rapidly at the pole with bluntly rostrate apices……**7**
7. Valves are long, linear-lanceolate; 97.0-140.0 μm in length and 6.0-7.0 μm in width…***Ulnaria ulna***
7. Valves are linear or lanceolate; 15.0 – 21.0 μm in length and 3.5 – 4.0 μm in width……... ……..***Nitzschia palea***
8. Cells are capable of semicell formation……***Staurastrum avicula* var. *lunatum***
8. Cells are not capable of semicell formation……**9**
9. Cells are cylindrical existing as elongated filaments…….***Oedogonium* sp.**
9. Cells are unicellular, solitary and spherical…….……..…**10**
10. Cells are spherical and solitary with cup –shaped chloroplasts…….…**11**
10. Cells are spherical and are capable of forming colonies…….……***Eudorina elegans***
11. Cells 2.0 - 3.5 μm in diameter…….……***Chlorella vulgaris***
11. Cells 9.5 – 17.0 μm in diameter……...…….***Chlorococcum infusionum***
12. Cells are unicellular, spherical, sub-cylindrical or oval occurring as singly or in cluster…….……**13**
12. Filamentous-type, existing as solitary or in groups of trichomes……..…**14**
13. Cells are spherical and occurring in clusters of 2-4 cells usually enclosed in a colorless mucilaginous sheath……..***Chroococcus minutus***
13. Cells are solitary, sub-cylindrical or oval with rounded apical ends and homogenous protoplast…… ……..…***Cyanothece aeruginosa***
14. Filaments are straight, non-branching and without constrictions at the crosswalls; apical cells are rounded, without calyptra and not capitate……..……..***Oscillatoria subbrevis***
14. Filaments are not attenuated at the apical ends, cylindrical, slightly constricted at crosswalls with distinct thin and colorless sheaths……..……...…….***Lyngbya taylorii***

Phylum: BACILLARIOPHYTA
Class: Bacillariophyceae
Order: Cymbellales
Family: Gomphonemataceae
Genus: *Gomphonema* Ehrenberg
***Gomphonema sphaerophorum* Ehrenberg**
Figs. 3A, 4A

Valves are linearly clavate with a capitate headpole and narrow basal pole. Length is 44.5 – 49.5 µm, width 13.0 -13.5 µm. Central area is incomplete and transversally elliptic characterized by having a stigma opposite of the single shortened stria. The raphe is undulate and lateral. Striae are 13-15 in 10 µm, parallel at the central valve and slightly radiate at the apices.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: Vouilloud et al., 2010, Revista de Biologia Tropical, 57p., Fig. 40-41.

Class: Bacillariophyceae
Order: Licmophorales
Family: Ulnariaceae
Genus: *Ulnaria* (Cleve) Mereschkowsky
***Ulnaria ulna* (Nitzsch) Compère**
Figs. 3B, 4B

Basionym: *Bacillaria ulna* Nitzsch

Valves are long, linear-lanceolate with parallel margins narrowing at both poles with rostrate ends. Length is 97.0-140.0 µm, width 6.0-7.0 µm. Striae are uniform, uniseriate and parallel arranged opposite each other and radiating at the valve ends; usually 7-9 striae for every 10 µm. Central area is round to oval.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: Angel et al., 2018, Advances in Microbiology, 224p., Fig. 8x; Bahls and Luna, 2018, Phytokeys, 42p., Pl. 2, Fig. 27.

Class: Bacillariophyceae
Order: Bacillariales
Family: Bacillariaceae
Genus: *Nitzschia* Hassall
***Nitzschia palea* (Kützing) W. Smith**
Figs. 3C, 4C

Basionym: *Synedra palea* Kützing

Valves are linear or lanceolate, tapering rapidly at the poles with bluntly rounded or rostrate apices. Fibulae are distinct and equidistant with visible striae (11 - 14 rows in 10 µm). Areolae are indistinct. Valves are 15.0 – 21.0 µm in length and 3.5 – 4.0 µm in width.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles, 2020b, Walailak Journal of Science and Technology, 253p., Pl. III, Fig. 3; Bahls and Luna, 2018, PhytoKeys, 51p., Pl. II, Fig. 15; Bahls et al., 2018, PhytoKeys, 53p., Pl.16, Fig. 11.

Class: Bacillariophyceae
Order: Fragilariales
Family: Fragilariaceae
Genus: *Fragilaria* Lyngbye
***Fragilaria* sp. Figs. 3D, 4D**

Frustules are in colonies, rectangular in girdle view, normally forming a linear, band-shaped colonies connected by several interlocking spines. Valves are usually linear-lanceolate with a tapered sternum, 14.0 – 22.0 µm in length and 3.0 – 4.5 µm in width.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: Kociolek et al., 2015, Centric and Araphid Diatoms. In Freshwater Algae of North America: Ecology and Classification, 682p. Fig. 1C-D and 13D, G, M.

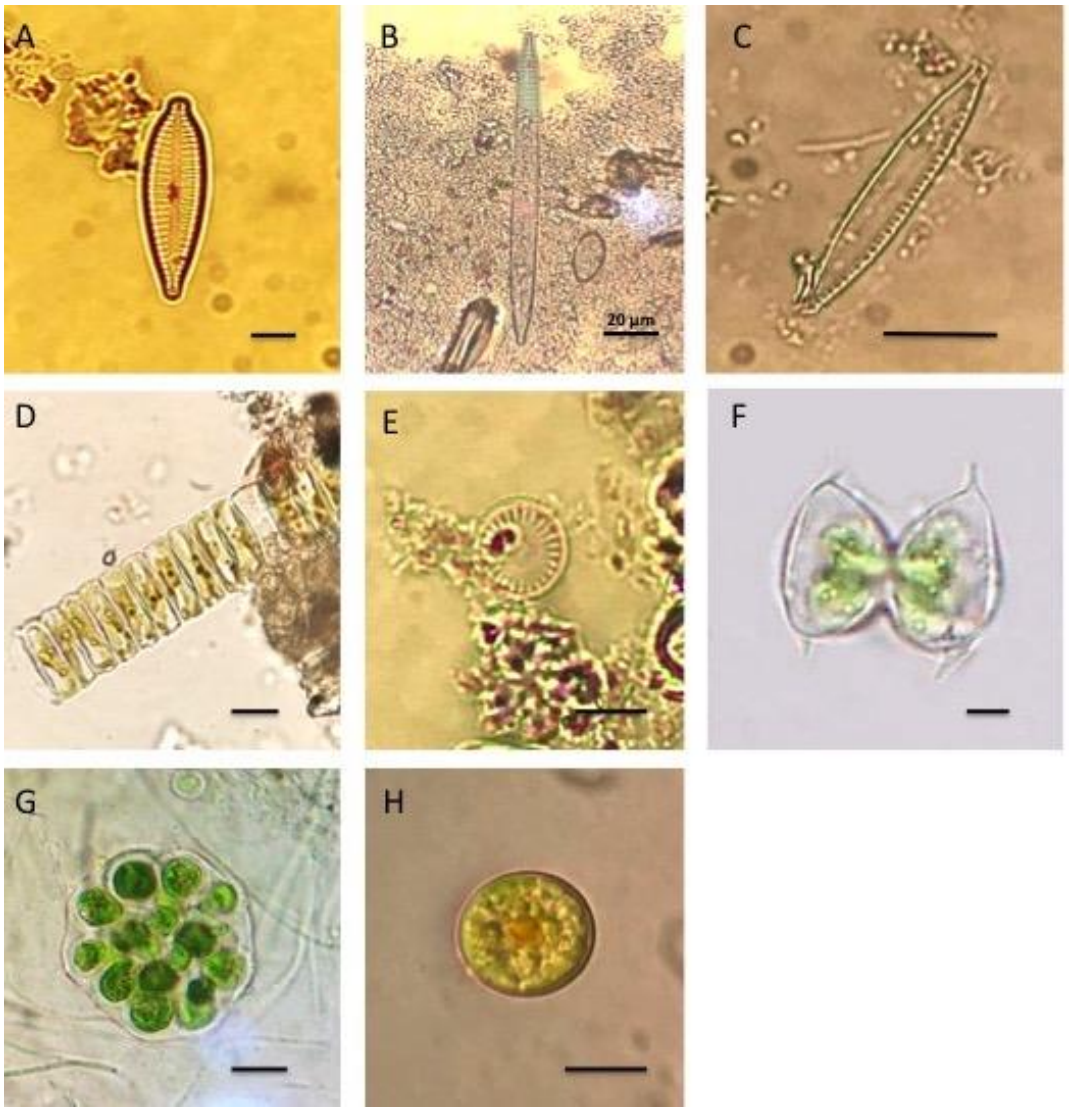

FIGURE 3. Photomicrographs of (A) *Gomphonema sphaerophorum* Ehrenberg, (B) *Ulnaria ulna* (Nitzsch) Compère, (C) *Nitzschia palea* (Kützing) W. Smith, (D) *Fragilaria* sp., (E) *Cyclotella meneghiniana* Kützing, (F) *Staurastrum avicula* var. *lunatum* (Ralfs) Coesel & Meesters, (G) *Eudorina elegans* Ehrenberg, (H) *Chlorococcum infusionum* (Schrank) Meneghini. All scale bars = 10 µm unless specified otherwise.

Class: Mediophyceae
Order: Stephanodiscales
Family: Stephanodiscaceae
Genus: *Cyclotella* (Kützing) Brébisson
***Cyclotella meneghiniana* Kützing**
Figs. 3E, 4E

Valves are circular, small with a narrow mantle; surface of the valve is smooth and flat; central area is distinct and smooth occupying 1/2 of the valve surface and are radially striated. Cell is 8.0-12.0 µm in diameter, striae is 6-8 in 10 µm.

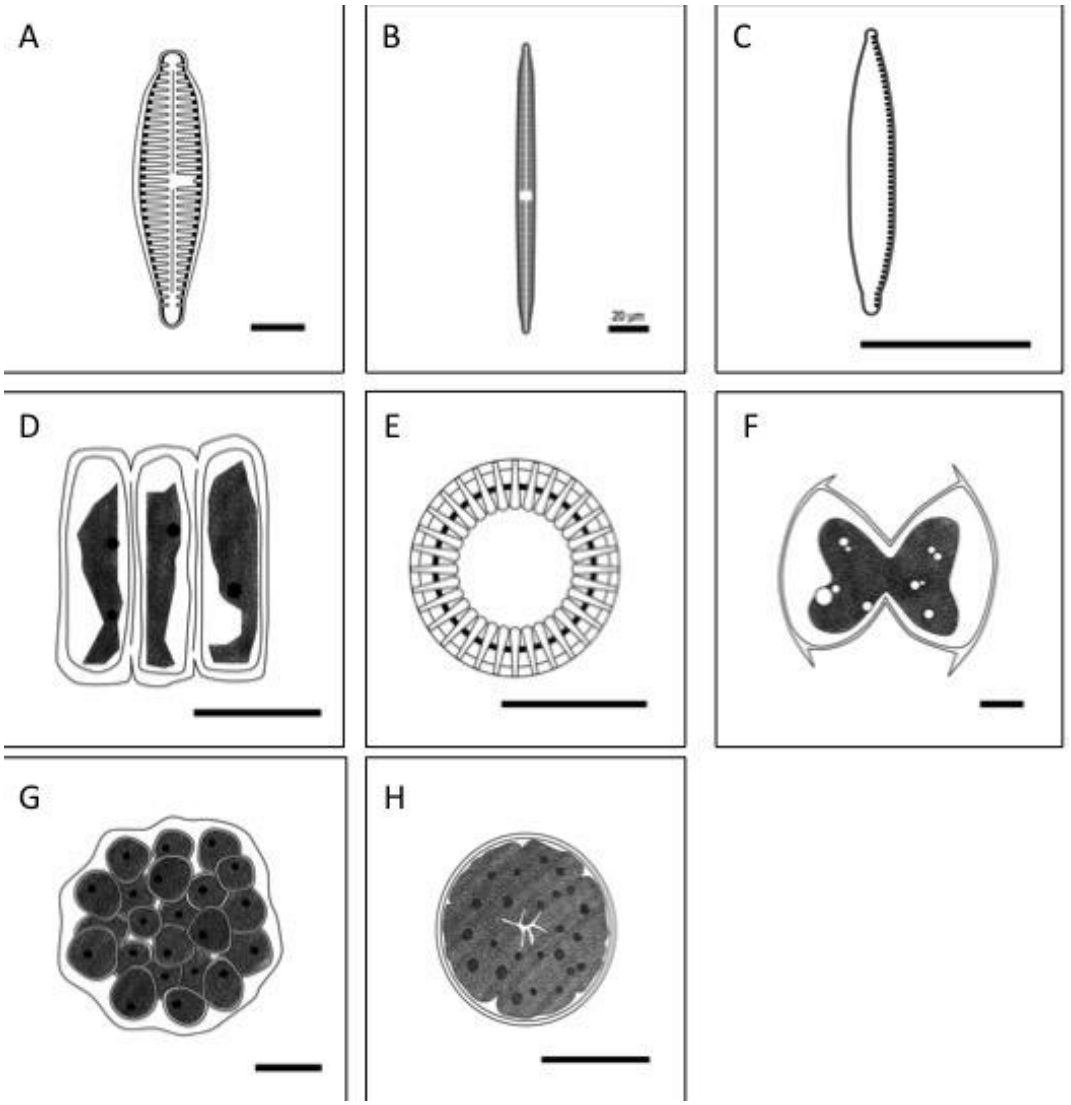

FIGURE 4. Drawings of (A) *Gomphonema sphaerophorum* Ehrenberg, (B) *Ulnaria ulna* (Nitzsch) Compère, (C) *Nitzschia palea* (Kützing) W. Smith, (D) *Fragilaria* sp., (E) *Cyclotella meneghiniana* Kützing, (F) *Staurastrum avicula* var. *lunatum* (Ralfs) Coesel & Meesters, (G) *Eudorina elegans* Ehrenberg, (H) *Chlorococcum infusionum* (Schrank) Meneghini. All scale bars = 10 μm unless specified otherwise.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles, 2019a, Pertanika Journal of Tropical Agricultural Science, 822p., Fig. 2a; Marra et al., 2016, Biota Neotropica 8p., Fig. 2.

Phylum: CHAROPHYTA
Class: Zygnematophyceae
Order: Desmidiales
Family: Desmidiaceae
Genus: *Staurastrum* Meyen ex Ralfs
***Staurastrum avicula* var. *lunatum* (Ralfs) Coesel & Meesters Figs. 3F, 4F**

Basionym: *Staurastrum lunatum* Ralfs

Cells are almost as broad as long and deeply constricted, 39.0–40.5 μm in length and 35.5–37.0 μm in width without spines (with spines 43.0–45.0 μm wide). Sinus open and acute angled. Semicells are bowl or cup-shaped in outline with one upwardly projected spine at each apical angle. Isthmus is 13.5–14.0 μm wide.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

A new record for the Philippines.

References: Shakmatov et al., 2018, Folia Cryptogamica Estonica, 11p., Fig. 4, 28; Coesel and Meesters, 2007, Desmids of the Lowlands: Mesotaeniaceae and Desmidiaceae of the European Lowlands, 68p., Pl. 68, Fig. 6-14.

Phylum: CHLOROPHYTA
Class: Chlorophyceae
Order: Chlamydomonadales
Family: Volvocaceae
Genus: *Eudorina* Ehrenberg
***Eudorina elegans* Ehrenberg Figs. 3G, 4G**

Colonies spherical or cylindrical, 63.0 – 70.0 μm in diameter, composed of 16, 32, or 64 cells surrounded by a mucilaginous sheath. Cells are spherical, 5.0-7.0 μm diameter, covered by a colorless mucilaginous sheath. Chloroplast is smooth and cup-shaped with 1-4 pyrenoids. Flagella are usually 1.5-2 times the total cell length and homodynamic.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Menezes and Bicudo, 2008, Hoehnea, 454p., Fig. 89-94; John and Tsarenko, 2011, Phylum Chlorophyta (Green Algae) In: The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae, 398p., pl. 102G.

Class: Chlorophyceae
Order: Chlamydomonodales
Family: Chlorococcaceae
Genus: *Chlorococcum* Meneghini
***Chlorococcum infusionum* (Schrank) Meneghini Figs. 3H, 4H**

Basionym: *Cystococcus humicola* Nägeli *Lepra infusionum* Schrank

Cells are spherical sometimes ovoid, solitary but sometimes occurring in cluster of cells, 9.5 – 17.0 μm in diameter. The cell has a single parietal chloroplast (with a pyrenoid) covering the cell surface of the microalga.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles and Monsalud, 2017, Philippine Journal of Systematic Biology, 30p., Pl. I Fig. 9; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 76p., Pl. VII, Fig 1; Zafaralla, 1998, Microalgae of Taal Lake, 33p., Pl 8e.f; Prescott, 1962, Algae of the Western Great Lakes Area, 280p., Pl. 45, Fig. 1.

Class: Chlorophyceae
Order: Oedogoniales
Family: Oedogoniaceae
Genus: *Oedogonium* Link ex Hirn
***Oedogonium* sp. Figs. 5A, 6A**

Filaments are unbranched and uniseriate

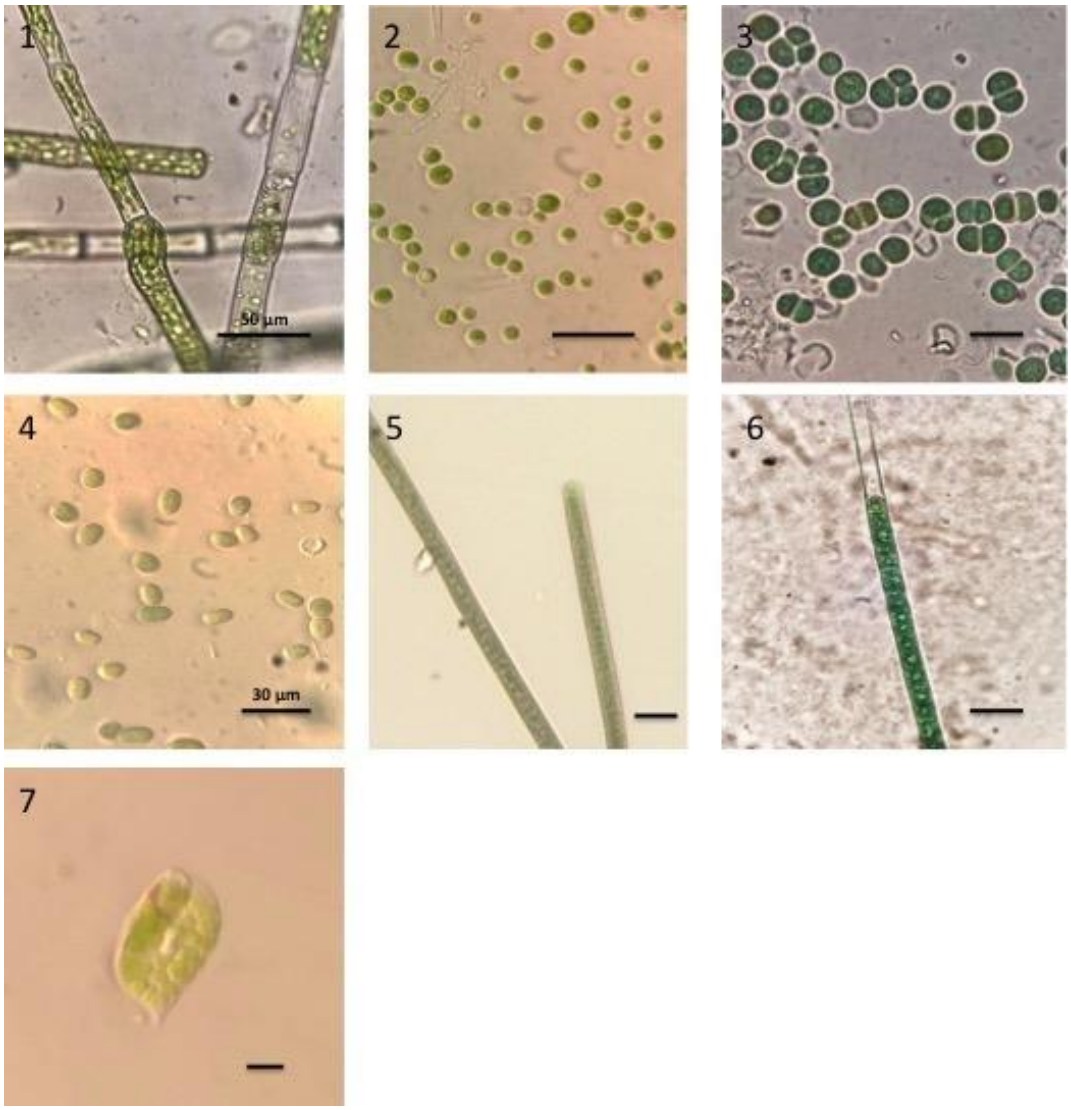

FIGURE 5. Photomicrographs of (A) *Oedogonium* sp., (B) *Chlorella vulgaris* Beyerinck [Beijerinck], (C) *Chroococcus minutus* (Kützing) Nägeli, (D) *Cyanothece aeruginosa* (Nägeli) Komárek, (E) *Oscillatoria subbrevis* Schmidle, (F) *Lyngbya taylorii* Drouet & Strickland, (G) *Phacus* sp. All scale bars = 10 µm unless specified otherwise.

attached in a substratum using basal holdfast cells. Vegetative cells are 7.0-8.0 µm in width and 29.0-50.0 µm in length, uniform in size and shape usually cylindrical or nodulate. Cells are highly vacuolate and uninucleate with reticulate, parietal chloroplast containing several pyrenoids.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: John and Rindi, 2015,

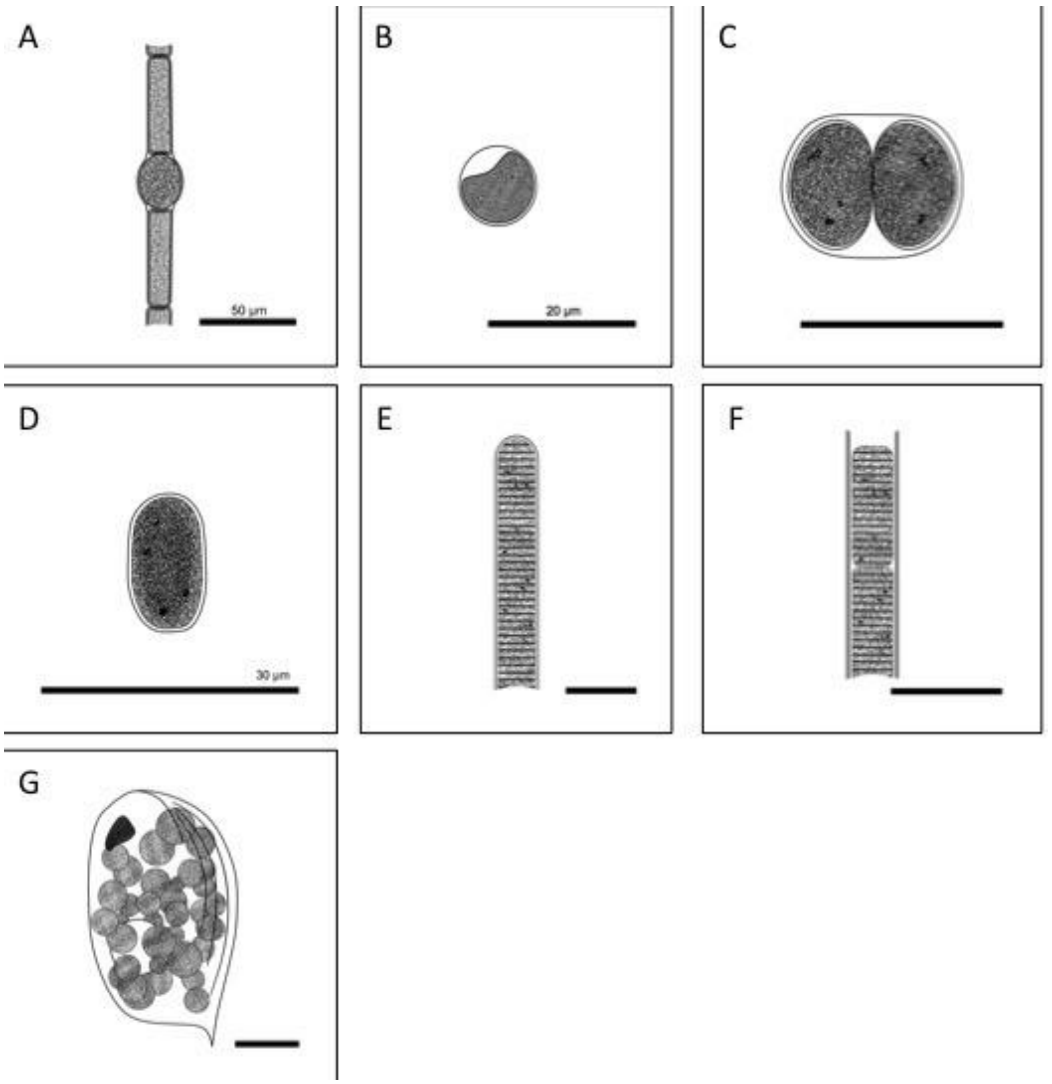

FIGURE 6. Drawings of (A) *Oedogonium* sp., (B) *Chlorella vulgaris* Beyerinck [Beijerinck], (C) *Chroococcus minutus* (Kützing) Nägeli, (D) *Cyanothece aeruginosa* (Nägeli) Komárek, (E) *Oscillatoria subbrevis* Schmidle, (F) *Lyngbya taylorii* Drouet & Strickland, (G) *Phacus* sp. All scale bars = 10 µm unless specified otherwise.

Filamentous (Nonconjugating) and Plantlike Green Algae. In Freshwater Algae of North America: Ecology and Classification, 406p. Fig. 7B,C and 8C.

Class: Trebouxiophyceae
Order: Chlorellales
Family: Chlorellaceae
Genus: *Chlorella* Beyerinck [Beijerinck]
***Chlorella vulgaris* Beyerinck [Beijerinck]**
Figs. 5B, 6B

Basionym: *Chlorella pyrenoidosa* var. *duplex* (Kützing)

Cells are solitary and coccoidal, 2.5 - 3.5 μm in diameter. The cells have cup-shaped and parietal chloroplast with a single pyrenoid. Cells are capable of forming 2-4 hemispherical spores that are released by rupture of the mother cell as a means of reproduction.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles, 2019c, Egyptian Journal of Aquatic Biology and Fisheries, 19 p., Pl. II, Fig. 3; Arguelles, 2019a, Pertanika Journal of Tropical Agricultural Science, 819p., Fig. 1a; Prescott 1962, Algae of the Western Great Lakes Area, 237, Pl. 53, Fig. 13.

Phylum: CYANOBACTERIA
Class Cyanophyceae
Order: Chroococcales
Family: Chroococcaceae
Genus: *Chroococcus* Nägeli
***Chroococcus minutus* (Kützing) Nägeli**
Figs. 5C, 6C

Basionym: *Protococcus minutus* Kützing

Cells are blue-green, small, oval or spherical usually enclosed in a colorless mucilaginous sheath. The cells may exist in groups of 2-4 cells sometime solitary with homogenous protoplasts; cells are 4.0–5.0 μm in diameter without mucilaginous sheath and 7.0–8.0 μm with mucilaginous sheath.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles, 2021, Philippine Journal of Science, 133 p., Pl. III, Fig. 2; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 31p.; Desikachary, 1959, Cyanophyta, p. 104–105, Pl. 24, G.4 and Pl. 26, G. 4 & 15.

Class Cyanophyceae
Order: Oscillatoriales
Family: Cyanothecaceae
Genus: *Cyanothece* Komárek
***Cyanothece aeruginosa* (Nägeli) Komárek**
Figs. 5D, 6D

Basionym: *Synechococcus aeruginosus* Nägeli

Cells are solitary, blue–green in color, sub-cylindrical or oval with rounded apical ends and homogenous protoplast. Cells are 11.0 – 27.0 μm in length and 9.0 – 15.0 μm in width, sometimes occurring with colourless mucilage or envelope.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: McGregor, 2013, Phytotaxa, 21p., Pl. 4A, 26C–F.

Class Cyanophyceae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: *Oscillatoria* Vaucher ex Gomont
***Oscillatoria subbrevis* Schmidle**
Figs. 5E, 6E

Filaments are straight, non-branching and without constrictions at the crosswalls; apical cells are without calyptra, rounded, and not capitate. Cells are blue-green in color, 5.5- 8.5 μm in width and 1.5-2.0 μm in length with smooth, homogenous protoplasm.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

References: Arguelles, 2020a, Philippine Journal of Science, 597 p., Pl. II, Fig. 4; Martinez 1984, A Checklist of Blue-Green Algae of the Philippines, 65p.; Prescott 1962, Algae of the Western Great Lakes Area, 491p., Pl. 107, G. 23; Desikachary

1959, Cyanophyta, p. 204–214, Pl. 37, Fig. 2 and Pl. 40, Fig.1.

Class Cyanophyceae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: *Lyngbya* C. Agardh ex Gomont
***Lyngbya taylorii* Drouet & Strickland Figs. 5F, 6F**

Basionym: *Phormidium taylorii* Drouet & Strickland

Filaments are dark blue-green or blue-green, generally with long filaments forming tufts. Sheaths are thin, firm and colorless (for young filaments), lamellated and thickened (for mature filaments), opened at the ends, containing a single trichome. Trichomes are not attenuated at the apical ends, cylindrical, 4.5-6.5 μm wide and slightly constricted at cross-walls. Cells are isodiametric, shorter than wide, 2.0-5.5 μm in length. Apical cells are broadly convex, with or without thickened cell wall. Specialized cells such as akinetes and heterocytes are absent.

Specimen: LUZON, Laguna, Calauan (Brgy. Balayhangin, Javier Eco Farm), E. DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: Park, 2012, Algal Flora of Korea (Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales) 74, Figs. 38A and B.

Phylum: EUGLENOPHYTA
Class: Euglenophyceae
Order: Euglenales
Family: Phacaceae
Genus: *Phacus* Dujardin
***Phacus* sp. Figs. 5G, 6G**

Cells are suborbicular and flattened (56.5 μm in length and 46 μm in width) in outline with conspicuous eyespot; anterior end is narrowly rounded while the posterior end have a short tail-piece; one paramylon body in the median part of the cell; pellicle is striated longitudinally; parietal chloroplasts are scattered in the protoplasm; flagellum is usually equal to or longer than the cell length.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden), E.DLR. Arguelles *s.n.* Photomicrograph prepared from the mounted specimen.

Reference: Triemer and Zakrys 2015, Photosynthetic Euglenoids. In Freshwater Algae of North America: Ecology and Classification, 474p. Fig. 14A-D and 15A-I.

DISCUSSION

Phytotelmata of pandan leaf axils represent a unique microhabitat for microalgae since this plant provides suitable environmental conditions for their growth and proliferation. However, the limited number of reported floristic studies that focuses on microalgae living in phytotelmata has a negative impact on our understanding on algal diversity on this overlooked habitat. To date, there have been only three reports in Asia of the occurrence of microalgae in bromeliad tanks, which was documented in Thailand and the Philippines (Arguelles, 2021; Poniewozik et al., 2020; Arguelles, 2020a). The current study is the first taxonomic survey to focus on the taxonomy of phytotelm algae of *Pandanus amaryllifolius* (Pandanaceae) from Laguna. A total of 15 microalgal taxa were identified and described from the collected samples, of which six species (*Lyngbya taylorii, Cyanothece aeruginosa, Eudorina elegans, Staurastrum avicula* var. *lunatum, Ulnaria ulna,* and *Gomphonema sphaerophorum*) are new additional records of microalgae in the global inventory list of phytotelm microalgae. In addition, this study presented the occurrence of *Staurastrum avicula* var. *lunatum* (Ralfs)

Coesel & Meesters for the first time in the Philippines. Comparison of the phytotelm microalgae identified in the current study with those from previous studies indicated *O. subbrevis, C. minutus, C. vulgaris, C. infusionum, C. meneghiniana, N. palea, Oedogonium* sp., *Fragillaria* sp. and *Phacus* sp. as the only six algal taxa in common. Variations in the species composition of microalgal communities in phytotelmata are quite common, with some usual taxa and others considered as temporary. The diversity of microalgae in leaf axil tanks of pandan is comparable from other taxonomic survey of phytotelm algae from bromeliads done by Arguelles (2020a) and Carrias et al. (2014) where they reported 15 and 16 microalgal taxa, respectively. When comparing the algal flora in *P. amaryllifolius* leaf axil tanks observed in this survey with the 35 taxa previously reported in the same sampling area (Arguelles, 2021; Arguelles, 2020a), nine taxa are constantly present and are widely distributed among phytotelmata of other plants (such as pineapple and *Neoregelia* spp.) in the area. These microalgae are considered well-adapted species to the local environmental conditions since these taxa are capable of forming dense populations even when exposed at varying environmental conditions (such as heavy rain and extreme dryness).

Microalgae in phytotelmata are dispersed in this aquatic microcosm by means of air and rain droplets (Gebühr et al., 2006). Also, other species phytotelm algae can be transported in phytotelmata by means of insects, small animals, and other invertebrates (such as crustaceans and mites) that regularly visit this microhabitat (Płachno and Wolowski, 2008). In the case of *Pandanus amaryllifolius,* majority of the microalgae recorded inside the leaf axils are mobile (diatoms and cyanobacteria) moving freely inside the axil tanks by means of a mucilage allowing colonization of the microhabitat (Płachno and Wolowski, 2008). The plant axils of pandan are arranged spirally forming a tank or vase that holds detritus and nutrient rich water from decomposition of confined detritus (animal fecal material, leaves and small twigs) from outside sources (Wittman, 2000). The rich nutrient water (high nitrate and phosphate concentration) in the plant axil tanks of pandan can favors the growth of certain group of microalgae such as *Chlorella vulgaris, Chroococcus minutus, Cyclotella meneghiniana, Lyngbya taylorii,* and *Oscillatoria subbrevis.* Ecological interactions between phytotelm microalgae and plants are common phenomenon in this microhabitat. Microalgae in phytotelmata are beneficial to plants since they are responsible in production of some essential nutrients, exudate (polysaccharides) and oxygen, which are being absorbed by plant trichomes and other small organisms (Brouard et al., 2011; Ramos and Moura, 2019). However, high population density of microalgae in phytotelmata can cause competition against the plant in absorbing inorganic nutrients as principal source of nitrogen for growth (Ramos and Moura, 2019). Microalgae in phytotelmata of some carnivorous plants (such as *Genlisea* sp.) contend with the host plant for utilization of organic phosphate from prey digestion. A study made by Płachno and Wolowski (2008) showed that two presiding groups of microalgae observed in phytotelmata of *Genlisea* sp. shows organic phosphate (phosphomonoesters) hydrolyzing ability similar to *Genlisea* sp. trichomes that have surface bound phosphomonoesterase activity. It is also crucial to take note that microcosms from bromeliads and leaf axils are

considered as ephemeral waters in cavities that are regularly exposed to sunlight that may lead to periods of drought. Ecological adaptation of microalgae against dry condition includes formation of autospores (*Rhopalosolen cylindricum*), cysts (*Euglena agilis*), zygospores (*Cosmarium bahianum*), mucilaginous sheath (*Xanthidium mamillosum* var. *borgei),* and akinete formation (*Cylindrospermum licheniform*). These adaptations allow phytotelm microalgae to survive long period of desiccation in regions with low rainfall index.

The survey presented the diversity of microalgae inhabiting a peculiar aquatic microhabitat and will act as a baseline information for further studies (a) in ecological interactions of organisms in this environment as well as (b) comparative study on the taxonomic composition of microalgal species on other sources of phytotelmata (such as bromeliads). To date, there are only few published surveys showing the diversity of microalgae in phytotelmata. Thus, additional taxonomic survey and ecological studies (analyses of the effect of plant morphology and limnological properties) that will deepen our knowledge of diversity and distributional pattern of phytotelm microalgae in other plants species found in other unexplored natural habitats are needed.

CONCLUSION

The current study enriches the biological knowledge regarding phytotelm algal flora of the Philippines. To date, this survey is the first taxonomic study done on species composition of microalgae present in leaf axil tanks of Pandan. The survey reports an additional of six species as new records in the global inventory list of microalgae in phytotelmata. Also, the survey reports the first record of a microalgae, *Staurastrum avicula* var. *lunatum* (Ralfs) Coesel & Meesters for the Philippines. This study provided baseline information of some of the rare and dominant species of phytotelm microalgae in leaf axil tanks of Pandan found in the Philippines.

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