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Unseen Microalgal Diversity – Phytotelmata in *Neoregelia* spp. L.B. Smith (Bromeliaceae) from Florists Wholesalers in Laguna, Philippines

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The aquatic microcosms in plant axils of bromeliads are unique and favorable habitat for diverse kinds of new and rare species of microalgae. This study presents the first taxonomic survey in the Philippines to focus on the algal community existing in phytotelmata of *Neoregelia* spp. L.B. Smith (Bromeliaceae) from florist wholesalers in Laguna. A total of 20 microalgal taxa were described and taxonomically identified – of which six species belong to Cyanophyceae, five species both for Bacillariophyceae and Chlorophyceae, two species for Trebouxiophyceae, and one species each for Euglenophyceae and Zygnematophyceae. The study reports an additional of eleven species (*Chlorella vulgaris*, *Dictyochloropsis splendida*, *Stigeoclonium tenue, Chlorococcum infusionum, Pinnularia rumrichiae*, *Cymbella affinis*, *Chroococcus schizodermaticus*, *Pseudanabaena minima*, *Oscillatoria limosa*, *Oscillatoria tenuis*, and *Euglena agilis*) as new records in the global inventory list of phytotelm microalgae and 14 new taxa as additional records of microalgae in phytotelm microhabitat of Asia. Also, the occurrence of a microalga, *Dictyochloropsis splendida* Geitler, is described for the first time in the Philippines. Keys and diagnostic descriptions as well as photomicrographs are presented to differentiate the species of phytotelm microalgae. The survey provided important taxonomic records and information on the species composition of phytotelm microalgae of bromeliad tanks found in the Philippines.

Keywords: biodiversity, bromeliad tanks, bromeliads, microalgae, microhabitat, taxonomy

INTRODUCTION

Neoregelia L.B. Smith is a famous genus of bromeliad plants that are abundant in tropical and subtropical countries (Poniewozik *et al.* 2020; Ramos and Moura 2019). These plants are characterized by having short stems with intricate rosettes of firm and spiny leaves. It is capable of accumulating water in the plant cavities (axils), which then create a natural environment (bromeliad tanks) for the growth of microorganisms (microalgae, bacteria, fungi), insects, and other small vertebrates (Kolicka *et al.* 2016; Richardson 1999; Poniewozik *et al.* 2020; Ramos and Moura 2019). These bromeliad tanks provide a variety of ecological niches and spatial compartments in support to various communities of organisms that inhabit them. Thus, phytotelmata observed in these land plants should be regarded as true microcosms (Kolicka *et al.* 2016; Richardson 1999).

The relationship of the existence of phytotelm microalgae in bromeliads is still a poorly understood research topic (Ramos and Moura 2019). Recent studies suggest that

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microalgal communities present in these microhabitats are considered as an alternative energy source in some predators living in the phytotelmata (Brouard *et al.* 2011). Thus, the abundance of microalgae in phytotelmata of bromeliads can be associated with the occurrence of the complementary nondetrital food web in bromeliad tanks (Brouard *et al.* 2011). Microalgae are considered pioneering microorganisms in this microhabitat and are responsible for nutrient, oxygen, and exudate (polysaccharides) production that is being utilized by trichomes of bromeliads and other organisms (Brouard *et al.* 2011; Ramos and Moura 2019). On the other hand, the abundance of microalgae can cause competition against the bromeliad plant in utilizing dissolved inorganic nutrients as a primary source of nitrogen for growth (Ramos and Moura 2019).

Knowledge on microalgal diversity on Asian aquatic ecosystem focused mainly on permanent and larger water bodies (Pantastico 1977; Rañola *et al.* 1990; Zafaralla 1998; Arguelles 2019a, b, c; Arguelles and Martinez-Goss 2019; Martinez-Goss *et al.* 2019; Arguelles 2020b), casting aside temporary and small aquatic habitats (such as phytotelmata) – to a large extent ignored in terms of taxonomic surveys, biodiversity, and ecological studies of freshwater habitats. To date, floristic survey on phytotelm microalgal flora in Asia was limited to only two studies in bromeliad tanks of the Bromeliaceae plant family found in the Philippines and Thailand, which reported a total of 131 microalgal taxa belonging to nine major groups of microalgae (Arguelles 2020a; Poniewozik *et al.* 2020). The Philippine survey of phytotelm microalgae from pineapple reported an additional of nine species as new records in the global inventory list of phytotelm microalgae and 13 new taxa as additional records of microalgae in phytotelm microhabitat of Asia. In addition, the occurrence of a rare photosynthetic euglenoid [*Phacus monilatus* (Stokes) Lemmerman] and a green microalga – *Monoraphidium lunare* Nygaard, J. Komárek, J. Kristiansen, & O.M. Skulberg – are described as new records in the Philippines (Arguelles 2020a). Phytotelm algal flora have also been reported in phytotelmata of bromeliads in other western countries such as Brazil, French Guiana, New Zealand, Mexico, and Jamaica (Brouard *et al.* 2011; Carrias *et al.* 2014). Habitat characteristics such as having high water temperature (23.0–29.0 °C), low pH, organically rich waters, low water conductivity, and high light intensity favor the occurrence of phytotelm microalgae in this microhabitat (Arguelles 2020a).

Previous studies proved that the diversity of phytotelm algae in this overlooked aquatic microhabitat contain rare and endemic species of microalgae that requires further study to increase our knowledge on the diversity and ecological significance of these microorganisms. The aim of this investigation was to provide additional information to the increasing knowledge of microalgal diversity in terrestrial bromeliad tanks found in the Philippines. Since microalgae of phytotelmata from Philippine bromeliads occurring in natural (forest) and semi-natural (greenhouse, gardens) habitats have not yet been characterized, here the investigator reports the first taxonomic survey of algal community existing in phytotelmata of *Neoregelia* spp. from gardens and greenhouse of floral wholesalers in Laguna, Philippines.

MATERIALS AND METHODS

Sampling of Phytotelm Algae

The collection was carried out to four florists wholesalers in Laguna: Adela's Garden in Los Baños (situated at 14° 10' 34.14" N, 121° 14' 37.58" E), Sardo's Garden in Calamba (situated at 14° 10' 35.76" N, 121° 11' 47.76" E), Abri's Garden in Nagcarlan (situated at 14° 6' 50.04" N, 121° 25' 5.16" E), and Mhin's Garden at Calauan (situated at 14° 10' 22.44" N, 121° 17' 36.24" E) to examine the diversity of phytotelm algal flora present on *Neoregelia* spp. (Figure 1). The bromeliads were identified using reference taxonomic works of literature and monographs done by Santos-Silva *et al.* (2017), Parkhurst (2000), as well as Leme and Marigo (1993). The water samples were collected from leaf cavities of individual bromeliads (*Neoregelia carolinae* (Beer) L.B. Sm. "Devroe," *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta," and *Neoregelia compacta* (Mez) L.B. Sm.) using a 50 mL syringe with a plastic hose (Figure 2). Sampling was done thrice for each bromeliad tank to ensure the high efficiency of collecting biological specimens. The samples were kept in a conical tube (Tarson) 25 x 50 mm size and kept in a portable cooler. It was then transported immediately in the laboratory for further examination.

A total of 18 water samples from three different species of *Neoregelia* (six samples per plant species) were analyzed. The collected water samples were divided into two: the first group was immediately examined using a binocular research microscope (Olympus CX31 with Infinity X digital camera), after which photomicrographs were taken (at 1000x magnification), in order to identify the microalgae in their living state whenever possible. The second group was fixed with 4% formaldehyde for preservation (Arguelles *et al.* 2014; Arguelles 2020a). A portion (5 mL) of the concentrated preserved samples was used for diatom analysis. The samples for diatom analysis were chemically digested following the standard protocol for the cleaning of diatom (Round *et al.* 1990). Suspensions of cleaned diatom were dried onto glass coverslips and mounted. Five slides were prepared for

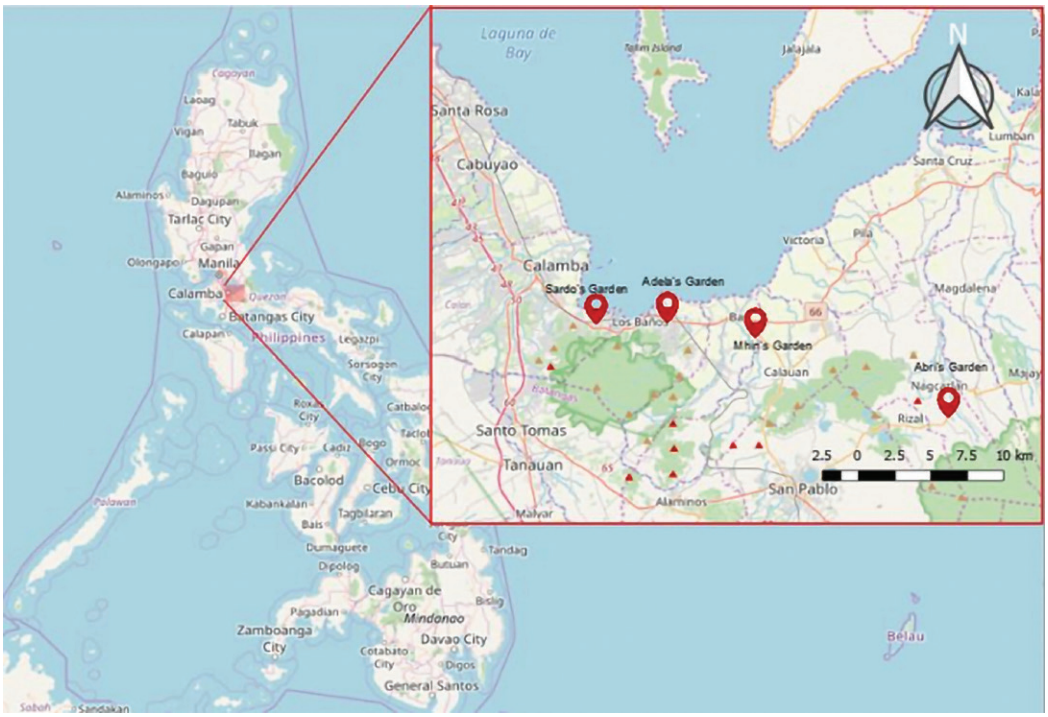

Figure 1. Location map of sampling sites of microalgae in phytotelmata of *Neoregelia* spp. (Bromeliaceae) from florist wholesalers in Laguna, Philippines.

Figure 2. Phytotelmata of bromeliads (A) *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe," (B) *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta," and (C) *Neoregelia compacta* (Mez) L.B. Sm.

each water sample for microscopic observation and enumeration of microalgae. Diatom slides were also prepared in triplicates for each sample and were kept in a slide box. The diatom slides and preserved specimens served as voucher specimens and kept at the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines.

Morphotaxonomic Enumeration and Identification

Taxonomic identification was done up to the algal species level making use of all possible and accessible information and literature. The morphological and metric characteristics important in morphotaxonomic identification such as features of the trichomes and filaments, presence or absence of sheath, and constrictions at the cross wall; appearance and color of the sheath and envelopes; the size and shape of vegetative and specialized (heterocytes and akinetes) cells; features of the protoplasm; and the number of cells in a colony were noted during the taxonomic classification and identification of each phytotelm microalgal species. The microalgal species were identified using reference taxonomic works of literature and monographs done by Desikachary (1959), Prescott (1962), Velasquez (1962), Pantastico (1977), Martinez (1984), Zafaralla (1998), Wehr and Sheath (2003), and Whitton (2002).

RESULTS

Taxonomic characterization and illustrations of the different algal taxa observed in water samples from phytotelmata collected from *Neoregelia* spp. from gardens and greenhouses of floral wholesalers in Laguna are presented below. Altogether, 20 microalgal species belonging to 14 orders, 15 families, and 17 genera were identified from the prepared specimen. The current survey reports Cyanobacteria (30.00%) as the major group of microalgae in phytotelmata, followed by Bacillariophyceae (25.00%), Chlorophyceae (25.00%), Trebouxiophyceae (10.00%), Euglenophyceae (5.00%), and Zygnematophyceae (5.00%). References used in the identification of the different microalgal taxa are listed directly below the description of each taxon. Also, current accepted taxonomic names based on Algaebase (Guiry and Guiry 2020) are presented in the paper.

Key to the Species of Chlorophyta

1.	Cells are capable of forming coenobia existing either in straight chains or spherical composed of several cells joined by several processes.....	2
1.	Cells are unicellular and occurring as elongated filaments, spherical, or cylindrical in shape not capable of forming coenobium.....	4
2.	Coenobium are spherical composed of 8, 16, or 32 cells joined by 5–6 processes; cells are ovoid or spherical with a single parietal chloroplast and a pyrenoid.....	*Coelastrum* sp.
2.	Coenobium exist in a single straight chain of 2–8 cells with or without spiny projections.....	3
3.	Coenobium exists in 2 or 4 (up to 6) cells attached linearly and parallel to each other; cells are ellipsoidal in shape; terminal cells with two long, straight spiny projections.....	*Scenedesmus quadricauda*
3.	Coenobium is flat and straight-chain composed of 2–4 cells; cells are spindle-shaped; terminal cells are curved and arcuate without spiny projections.....	*Tetradesmus obliquus*
4.	Cells are cylindrical with visible constrictions occurring as elongated filaments with laminar parietal chloroplasts; thalli are thin and irregularly branched with alternating origin.....	*Stigeoclonium tenue*
4.	Cells are unicellular, solitary, spherical, or globose with parietal or cup-shaped chloroplasts.....	5
5.	Cells are solitary, spherical, or globose with smooth, compact cell wall and parietal chloroplasts.....	*Dictyochloropsis splendida*
5.	Cells are solitary and spherical with thin cell wall and cup-shaped chloroplasts.....	6

6. Cells are spherical to ovoid sometimes occurring in several clusters of cells; 10.5–19.0 µm in diameter with chloroplast nearly filling the cell with a single pyrenoid..... *Chlorococcum infusionum*

6. Cells are solitary, spherical with a smooth, thin cell wall; 1.0–3.0 µm in diameter with cup-shaped and parietal chloroplasts..... *Chlorella vulgaris*

Phylum: CHLOROPHYTA

Class: Trebouxiophyceae
Order: Chlorellales
Family: Chlorellaceae
Genus: *Chlorella* Beyerinck [Beijerinck]

1. *Chlorella vulgaris*, Beyerinck [Beijerinck], Bot Zeitung. 4: 758, Pl. VII: Fig. 2a–d, 1890. **Pl. I, Fig. 1**

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

Cells are solitary, spherical with a smooth, thin cell wall; 1.0–3.0 µm in diameter; chloroplast is cup-shaped and parietal, solitary, and with a single pyrenoid.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC2-1-a-c, PNCM).

References: Arguelles 2019a, Pertanika J Trop Agric Sci, 819p., Fig. 1a; Arguelles and Monsalud 2017, Philipp J System Biol, 31p., Pl. II Fig. 4; Ortega-Calvo *et al.* 1993, Nova Hedwigia, 246p., Pl. 2, Figs. 16 and 17; Prescott 1962, Algae of the Western Great Lakes Area, 237, Pl. 53, Fig. 13.

Class: Trebouxiophyceae
Order: Trebouxiales
Family: Trebouxiaceae
Genus: *Dictyochloropsis* Geitler

1. *Dictyochloropsis splendida* Geitler, Österr Bot Z, 162–163, Figs. 1–4. **Pl. I, Fig. 2**

Cells are globose, spherical, and solitary, 20.5–29.0 µm in diameter. Cell wall is thin, smooth, and compact. Chloroplasts are parietal, single appearing as a homogenous mass covering almost the entire cell membrane.

A new record for the Philippines

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC2-1-a-c, PNCM).

References: Škaloud *et al.* 2016, J Phycol 608p., Figs. 4A–C; Shubert and Gärtner 2015, Nonmotile Coccoid and Colonial Green Algae, In: Freshwater Algae of North America. Ecology and Classification, 342p., Fig. 12A.

Class: Chlorophyceae
Order: Chaetophorales
Family: Chaetophoraceae
Genus: *Stigeoclonium* Kützing

1. *Stigeoclonium tenue* (C. Agardh) Kützing, Phycol Gen Anat Phys System Tange, 253. 1843. **Pl. I, Fig. 3**

Basionym: *Draparnaldia tenuis* C. Agardh

Elongated filaments and light green in color, thalli are thin, irregularly branched with alternating origin; cylindrical cells with visible constrictions at the cross-walls, 8.5–12.0 µm long and 6.5–7.0 µm in diameter, sharply tapered terminal end cells, rounded or sometimes blunt end cells; hairs occurring at the terminal cells, infrequent; chloroplasts are laminar parietal usually found at the central part of the cell, flat, round, or sometimes disk shape.

Specimen: LUZON, Laguna, Calamba (Brgy. Bagong Kalsada, Sardo's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC1-1-a-c, PNCM).

Reference: Skinner and Entwisle 2004, Telopea, 625p., Fig. 6; John 2002, Phylum Chlorophyta: Orders Chaetophorales, Klebsormidiales, Microsporales, Ulotrichales, In: Freshwater Algal Flora of British Isles: An Identification Guide to Freshwater and Terrestrial Algae, 465p., Pl. 111E.

Class: Chlorophyceae
Order: Chlamydomonodales
Family: Chlorococcaceae
Genus: *Chlorococcum* Meneghini

1. *Chlorococcum infusionum* (Schrank) Meneghini, Mem Reale Accad Sci Torino. 25: 27, Pl. 2, Fig. 3, 1842 **Pl. I, Fig. 4**

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

Cells are spherical to ovoid, greenish in color; solitary or in flat irregular colonies; sometimes occurring in several clusters of cells; chloroplast nearly filling the cell with

a single pyrenoid; cells are 10.5–19.0 µm in diameter.

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

References: Arguelles 2019a, Pertanika J Trop Agric Sci, 820p., Fig. 1b; Arguelles and Monsalud 2017, Philipp J System Biol, 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.

Class: Chlorophyceae
Order: Sphaeropleales
Family: Scenedesmaceae
Genus: *Scenedesmus* Meyen

1. *Scenedesmus quadricauda* (Turpin) Brébisson, Mém Soc Acad des Sci Art Bel-Lett de Falaise. 66. 1835. **Pl. I, Fig. 5**

Basionym: *Achnanthes quadricauda* Turpin

Coenobium exists in 2 or 4 (up to 6) cells attached linearly and parallel to each other; cells are elongated and ellipsoidal in shape, 4.0–6.0 µm in width and 8.0–10.5 µm in length; inner cells are without projections (spines) while terminal (end) cells with two straight, long, spiny projections.

Specimen: LUZON, Laguna, Nagcarlan (Brgy. Banago, Abri's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC5-1-a-c, PNCM).

References: Zafaralla 1998, Microalgae of Taal Lake, 39p., Pl. 9g–j; Pantastico 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 119p., Pl. IX, Fig. 8; Prescott 1962, Algae of the Western Great Lakes Area, 280p., Pl. 64, Fig. 2.

Class: Chlorophyceae
Order: Sphaeropleales
Family: Scenedesmaceae
Genus: *Tetradesmus* G.M. Smith

1. *Tetradesmus obliquus* (Turpin) M.J. Wynne, Feddes Repert 126: 84, 2016. **Pl. I, Fig. 6**

Basionym: *Achnanthes obliqua* Turpin

Cells are spindle-shaped existing in a single, flat and straight chain of 2–4 cells forming a coenobia; 12.0–13.0 µm in length and 6.50–7.50 µm in width; terminal cells are curved and arcuate (bow-shaped) while the inner cells with sharp apices and straight. Both cells have a smooth cell wall with parietal chloroplasts and a single pyrenoid.

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

Reference: Arguelles 2019b, Trop Life Sci Res, 9p., Pl. II, Fig. 2.

Class: Chlorophyceae
Order: Sphaeropleales
Family: Scenedesmaceae
Genus: *Coelastrum* Nágeli

1. *Coelastrum* sp. **Pl. I, Fig. 7**

Coenobium is spherical composed of 8, 16, or 32 cells joined by 5–6 processes. Cells are ovoid (in lateral view) or spherical in (apical view); cell wall is smooth, single parietal chloroplast with a pyrenoid. Cell has a diameter of 11.0–14.0 µm while the coenobium is 36.0–55.0 µm in diameter.

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

Reference: Shubert and Gärtner 2015, Non-motile Coccoid and Colonial Green Algae, In: Freshwater Algae of North America: Ecology and Classification, 336p. Figs. 7C, D, and 8E.

Key to the Species of Charophyta and Euglenophyta

1. Cells are cylindrical and highly metabolic with proteinacious pellicle and elongated chloroplasts; flagellum is long with length that is almost equal to body length ..... *Euglena agilis*
1. Cells are capable of semicell formation without proteinacious pellicle and are usually longer than broad with a deep sinus; semicells are pyramidate in outline with rounded angles and slightly convex lateral margins ..... *Cosmarium* sp.

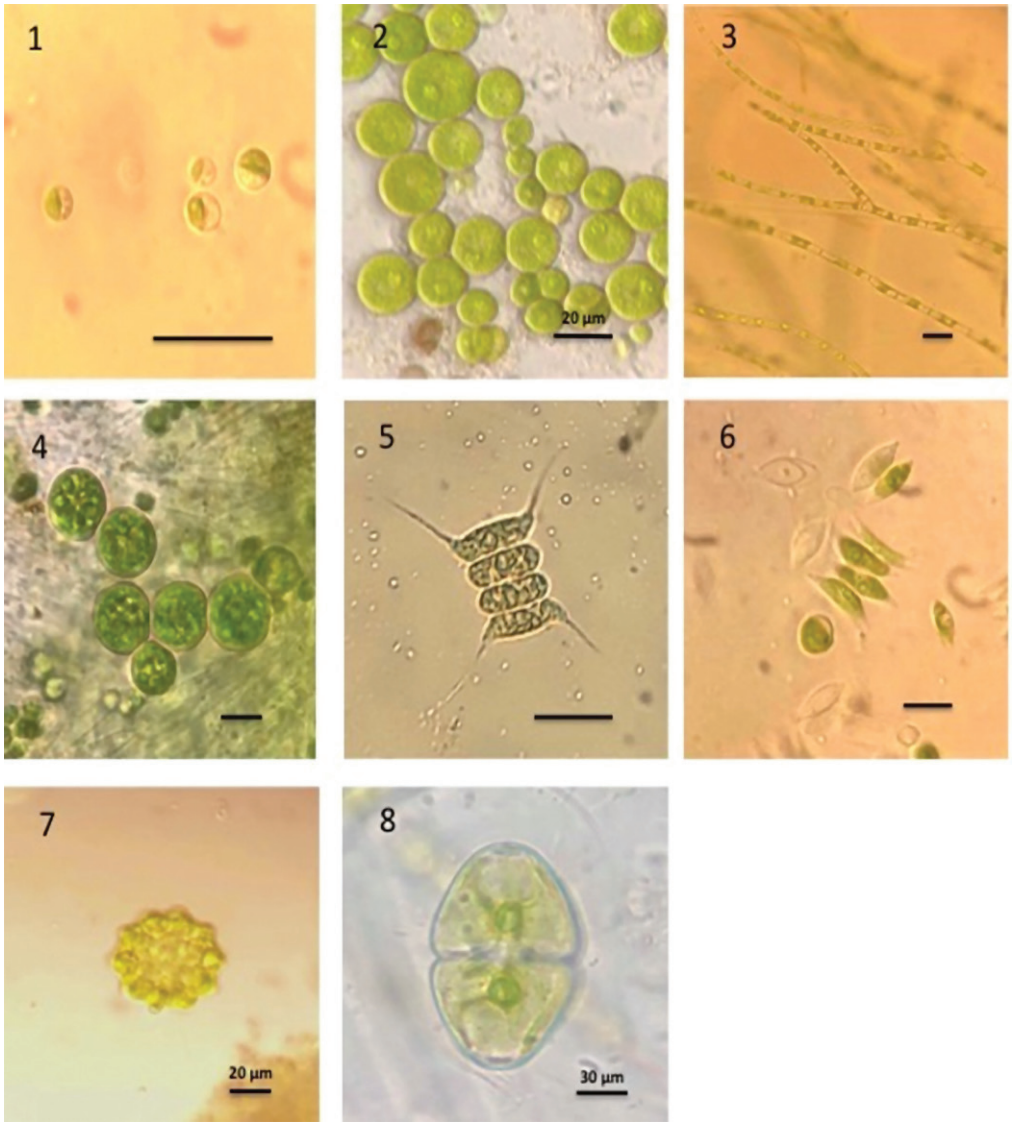

Plate I. Photomicrographs of (1) *Chlorella vulgaris* Beyerinck [Beijerinck], (2) *Dictyochloropsis splendida* Geitler, (3) *Stigeoclonium tenue* (C. Agardh) Kützing, (4) *Chlorococcum infusionum* (Schrank) Meneghini, (5) *Scenedesmus quadricauda* (Turpin) Brébisson, (6) *Tetradesmus obliquus* (Turpin) M.J. Wynne, (7) *Coelastrum* sp., and (8) *Cosmarium* sp. All scale bars = 10 µm unless specified otherwise.

Phylum: CHAROPHYTA

Class: Zygnematophyceae
Order: Desmidiales
Family: Desmidiaceae
Genus: *Cosmarium* Corda ex Ralfs

1. *Cosmarium* sp. **Pl. I, Fig. 8**

Cells are longer than broad (usually 1.3–1.5 times) with a deep sinus (median constriction), 145.0–216.0 µm in length and 89.0–128.0 µm in width; semicells are pyramidate in outline with rounded angles and slightly convex lateral margins, both the apex of the semicells are rounded and smooth; each semicells have chloroplasts with a single pyrenoid.

Specimen: LUZON, Laguna, Calamba (Brgy. Bagong Kalsada, Sardo's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC1-1-a-c, PNCM).

Reference: Gerrath 2003, Conjugating Green Algae and Desmids, In: Freshwater Algae of North America: Ecology and Classification, 374p., Figs. 22–26, 32, and 71–75.

Phylum: EUGLENOPHYTA

Class: Euglenophyceae
Order: Euglenida
Family: Euglenidae
Genus: *Euglena* Ehrenberg

1. *Euglena agilis* H.J. Carter, Annal Mag Nat Hist 2(18): 240, Pl. 6, Fig. 62. **Pl. II, Fig. 6**

Cells are highly metabolic and cylindrical; 26.0–28.0 µm in length and 8.0–10.0 µm in width; pellicle is moderately striated (may or may not be visible); flagellum is long with length that is almost equal to the body length. Elongated chloroplasts are present per cell. Anterior end is oval when fully expanded; posterior end is short with blunt point.

Specimen: LUZON, Laguna, Nagcarlan (Brgy. Banago, Abri's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC6-1-a-c, PNCM).

References: Arguelles 2019b, Trop Life Sci Res, 9p., Pl. II, Fig. 5; Arguelles *et al.* 2014, Philipp Sci, 12p. Pl. I, Fig. 4; Wolowski *et al.* 2013, Polish Botan J, 666p., Fig. 18 & 80; Boonmee *et al.* 2011, Asia Life Sci, 119p.

Phylum: BACILLARIOPHYTA

Class: Bacillariophyceae
Order: Naviculales
Family: Pinnulariaceae
Genus: *Pinnularia* Ehrenberg

1. *Pinnularia rumrichiae* Krammer, Diat Eur, 1: 110 and 223, Pl. 84, Figs. 9–12 (as "rumrichae"), 2000. **Pl. II, Fig. 1**

The valves are linear with capitate apices; central area is rhombic and wide covering nearly all the valves' margin; raphe is straight and filiform; striae are convergent near the apices and radiate on the central area of the cell. Striae are 11–14 rows in 10 µm. Valves are 31.0–40.0 µm in length and 5.50–9.50 µm in width.

Key to the Species of Bacillariophyta

1.	Valves are linear and elongated with bluntly rounded (rostrate) or capitate apices	2
1.	Valves not linear, usually cymbelloid or rounded with uniseriate striae marginally present or radiate on the way to the apical end.....	3
2.	Valves are lanceolate tapering rapidly at the apices with distinct fibulae equidistant to the striae (11–14 rows in 10 µm).....	*Nitzschia* sp.
2.	Valves are linear with capitate apices; striae are convergent near the apices and radiate on the central area of the cell.....	*Pinnularia rumrichiae*
3.	Valves are lanceolate (cymbelloid) with rounded or subrostrate ends; striae radiate on the dorsal side and are parallel on the ventral side.....	*Cymbella affinis*
3.	Valves are rounded or oval in shape with rounded ends and uniseriate striae	4
4.	Valves are small, rounded (disc-shaped) with a narrow mantle and striae that are marginally present along the surface.....	*Cyclotella meneghiniana*
4.	Valves are oval-shaped with curved margin and rounded terminal apices with uniseriate striae that are strongly radiate on the way to the apical end.....	*Diploneis* sp.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC3-1-a-c, PNCM).

References: da Silva *et al.* 2016, Biota Neotropica, 18p., Fig. 26.

Class: Bacillariophyceae
Order: Cymbellales
Family: Cymbellaceae
Genus: *Cymbella* C. Agardh

1. *Cymbella affinis* Kützing, Die Kieselschal Bacill oder Diatom, 80, Pl. 6, Fig. 15, 1844. **Pl. II, Fig. 2**

Cells are solitary and cymbelloid, 20.5–27.0 µm in length and 5.6–8.0 µm in breadth; axial area is tapered while the central area is linear-arched. Valves are lanceolate with concave dorsal margin, ventral area with slightly convex margin, valve ends are slightly subrostrate or rounded. Striae radiate on the dorsal side and are parallel on the ventral side; 8–10 rows for every 10 µm.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC3-1-a-c, PNCM).

Reference: Lee and Chung 1991, Algae 109p., Figs. 1–11.

Class: Bacillariophyceae
Order: Stephanodiscales
Family: Stephanodiscaceae
Genus: *Cyclotella* (Kützing) Brébisson

1. *Cyclotella meneghiniana* Kützing, Die Kieselschal Bacill oder Diatom, 50, Pl. 30, Fig. 68, 1844. **Pl. II, Fig. 3**

Valves rounded (disc-shaped) and small with a narrow mantle; 7.0–18.0 µm in diameter; central portion is smooth and flat (approximately 1/3 of the total surface of the valve) with striae marginally present along the surface. Striae are 5–7 rows in 10 µm.

Specimen: LUZON, Laguna, Calamba (Brgy. Bagong Kalsada, Sardo's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC1-1-a-c, PNCM).

References: Arguelles 2020b, Walailak J Sci & Tech, 252p., Pl. III, Fig. 7; Arguelles 2019a, Pertanika J Trop Agric Sci, 822p., Fig. 2a; Leira *et al.* 2017, Anal del Jard Bot de Madrid, 7p., Fig. 2e; Marra *et al*. 2016, Biota Neotrop 8p., Fig. 2.

Class: Bacillariophyceae
Order: Naviculales
Family: Diploneidaceae
Genus: *Diploneis* Ehrenberg ex Cleve

1. *Diploneis* sp. **Pl. II, Fig. 4**

Cells are longer than wide, 31.0–57.0 µm in length and 17.0–28.0 µm in width. Valves are oval-shaped with curved margin and rounded terminal apices. The central area of the valve is rounded and wide. Striae are uniseriate (with round and complex areolae) radiate in the middle of the valve and strongly radiate on the way to the apical end.

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

Reference: Kociolek and Spoulding 2003, Symmetrical Naviculoid Diatom, In: Freshwater Algae of North America: Ecology and Classification, 648p., Figs. 2K–N.

Class: Bacillariophyceae
Order: Bacillariales
Family: Bacillariaceae
Genus: *Nitzschia* Hassall

1. *Nitzschia* sp. **Pl. II, Fig. 5**

Valves are lanceolate, tapering rapidly at the poles. Fibulae are distinct and equidistant with visible striae (11–14 rows in 10 µm). Valves are 15.0–21.0 µm in length and 3.5–4.0 µm in width.

Specimen: LUZON, Laguna, Nagcarlan (Brgy. Banago, Abri's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC5-1-a-c, PNCM).

Reference: Lowe 2003, Keeled and Canalled Diatoms, In: Freshwater Algae of North America: Ecology and Classification, 648p., Figs. 15–20.

Key to the Species of Cyanobacteria

1.	Unicellular, spherical (or oval), or hemispherical occurring as singly or in clusters of 2–4 cells, with a smooth protoplasm (without granular content) and enclosed by a clear or colorless gelatinous sheath.....	2
1.	Filamentous-type, existing as straight, solitary, or in groups of trichomes, may or may not be attenuated and with or without visible constrictions at the cross walls.....	3
2.	Cells are spherical or hemispherical with wide, delimited, and lamellate envelopes covering the entire cells and with enlarged outer layers.....	*Chroococcus schizodermaticus*
2.	Cells are spherical (or oval) with wide and distinct margin and smooth granular content enclosed in a thin, colorless, and non-lamellate mucilage.....	*Chroococcus minutus*
3.	Trichomes are straight and cylindrical; cells in a filament have distinct constrictions at the cross walls with homogenous protoplasm.....	4
3.	Trichomes are straight and scattered; cells in a filament have slight constrictions at the cross walls with granulated protoplasm.....	5

Plate II. Photomicrographs of 1) *Pinnularia rumrichiae* Krammer, 2) *Cymbella affinis* Kützing, 3) *Cyclotella meneghiniana* Kützing, 4) *Diploneis* sp., 5) *Nitzschia* sp., and 6) *Euglena agilis* H.J. Carter. All scale bars = 10 µm unless specified otherwise.

4.	Cells are 2.0–3.5 µm in length and 1.0–2.0 µm in width, homogenous protoplasm; posterior and anterior apices are rounded.....	*Pseudanabaena catenata*
4.	Cells are cylindrical, 1.8–3.5 µm in length and 0.5–1.0 µm in width, homogenous protoplasm, and with blunt and rounded apices.....	*Pseudanabaena minima*
5.	Cells are blue-green in color with slightly granulated protoplasm (not homogenous); apices are flattened or rounded, not attenuated, and without calyptra.....	*Oscillatoria limosa*
5.	Cells are dark blue-green in color with finely granulated protoplasm; apical cells are rounded, slightly bend and without calyptra.....	*Oscillatoria tenuis*

Phylum: CYANOBACTERIA

Class Cyanophyceae
Order: Chroococcales
Family: Chroococcaceae
Genus: *Chroococcus* Nägeli

1. *Chroococcus schizodermaticus* West, J R Microsc Soc London, 742, Pl. 10, Figs. 61–63, 1892.
Pl. III, Fig. 1

Cells are hemispherical or spherical, bluish-green in color, 3.5–8.5 µm in diameter. Colonies are composed of 2–4 cells, 25.0–39.0 µm in diameter, common diffluent mucilage is lacking. Envelopes are wide and delimited, lamellate covering the entire cells, with enlarged outer layers.

Specimen: LUZON, Laguna, Nagcarlan (Brgy. Banago, Abri's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC5-1-a-c, PNCM).

References: McGregor 2013, Phytotaxa, 53p., Pl. 37, Figs. C & D; Tavera and Komárek 1996, Algol Stud, 527p., Fig. 12; Desikachary 1959, Cyanophyta, 103p., Pl. 26, Fig. 17.

2. *Chroococcus minutus* (Kützing) Nägeli, Neue Denks Allg Schweiz Gesells Gesam Natur 10(7): 46, 1849.
Pl. III, Fig. 2

Basionym: *Protococcus minutus* Kützing

Cells are 3.5–5.0 µm in diameter; bluish-green in color,

spherical (or oval) cells enclosed in a thin, colorless and non-lamellate mucilage; solitary or in groups (2–4 cells) forming a colony; protoplasm is smooth without granular content, enclosed by a wide and distinct margin and granular content.

Specimen: LUZON, Laguna, Calamba (Brgy. Bagong Kalsada, Sardo's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC1-1-a-c, PNCM).

References: Arguelles 2020a, Philipp J Sci 149(3): 596, Pl. II, Fig. 1; 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: Synechococcales
Family: Pseudanabaenaceae
Genus: *Pseudanabaena* Lauterborn

1. *Pseudanabaena catenata* Lauterborn Verhandl Natur-Med Ver Heidelberg 2(13): 437, Pl. 3, Fig. 27, 1915. **Pl. III, Fig. 3**

Trichomes are cylindrical, solitary, straight, and isopolar with filaments that have distinct constrictions at the cross walls; 3.0–5.0 µm in diameter. Cells are bluish-green with protoplasm that are homogenous, posterior and anterior apices are rounded, 2.0–3.5 µm in length and 1.0–2.0 µm in width. Cells are longer than wide (usually 1.0–2.0 times).

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

References: Arguelles 2020a, Philipp J Sci 149(3): 596, Pl. II, Fig. 2; Arguelles 2019d, J Microb, Biotech Food Sci 9(1): 3, Fig. 6; Park 2012, Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales 5(1): 48, Fig. 16G, 17A, B; McGregor 2007, Freshwater Cyanoprokaryota of North-Eastern Australia 1: Oscillatoriales, 36p., Fig. 2f.

2. *Pseudanabaena minima* (G.S. An) Anagnostidis, Preslia, Praha 73: 360, 2001. **Pl. III, Fig. 4**

Basionym: *Achroonema minimum* G.S. An

Cells are blue-green, usually longer than wide (1.5 times), cylindrical and long, 1.8–3.5 µm in length and 0.5–1.0 µm in width; homogenous with blunt and rounded apices. Trichomes are solitary, straight, not attenuated, and with visible constrictions at the cross walls.

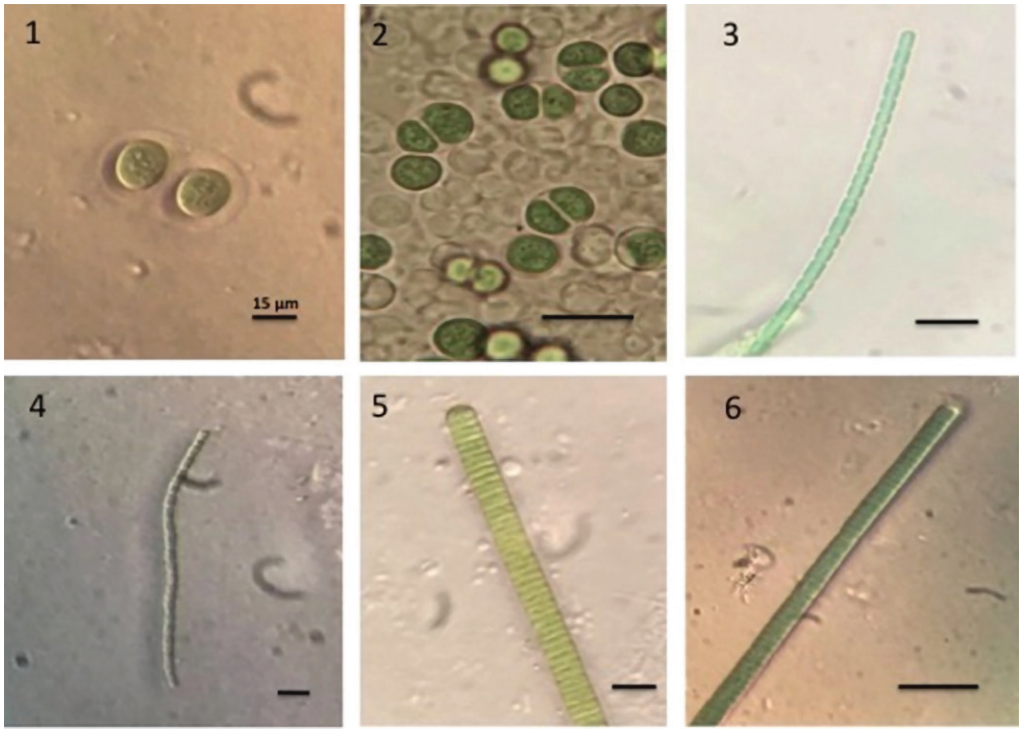

Plate III. Photomicrographs of 1) *Chroococcus schizodermaticus* West, 2) *Chroococcus minutus* (Kützing) Nägeli, 3) *Pseudanabaena catenata* Lauterborn, 4) *Pseudanabaena minima* (G.S. An) Anagnostidis, 5) *Oscillatoria limosa* C. Agardh ex Gomont, and 6) *Oscillatoria tenuis* C. Agardh ex Gomont. All scale bars = 10 µm unless specified otherwise.

Specimen: LUZON, Laguna, Calamba (Brgy. Bagong Kalsada, Sardo's Garden, *Neoregelia carolinae* (Beer) L.B. Sm. "Devroe"), E.DLR. Arguelles *s.n.* (Herb. No. AC1-1-a-c, PNCM).

Reference: Park 2012. Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales 5(1): 49, Figs. 17D–G.

Class Cyanophyceae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: *Oscillatoria* Vaucher ex Gomont

1. *Oscillatoria limosa* C. Agardh ex Gomont, Ann Sci Nat Bot 7(16): 210, Pl. 6: Fig. 13, 1892. **Pl. III, Fig. 5**

Trichomes are filamentous and straight with crosswalls that are slightly constricted and blue green in color. Cells 3.0–4.0 µm long and 10.0–15.0 µm wide, slightly granulated protoplasm (not homogenous); apices are flattened or rounded, not attenuated, and without calyptra.

Specimen: LUZON, Laguna, Calauan (Brgy. Masiit, Mhin's Garden, *Neoregelia compacta* (Mez) L.B. Sm), E.DLR. Arguelles *s.n.* (Herb. No. AC4-1-a-c, PNCM).

References: Martinez 1984, A Checklist of Blue-Green Algae of the Philippines, 61p.; Pantastico 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 46p., Pl. IV, Fig. 1; Velasquez 1962, Philipp J Sci 91(3): 342, Pl. 2, Fig. 22; Prescott 1962, Algae of the Western Great Lakes Area, 489p., Pl. 109, Fig. 17; Desikachary 1959, Cyanophyta 206p., Pl. 42, Fig. 11.

2. *Oscillatoria tenuis* C. Agardh ex Gomont, Ann Sci Nat Bot 7(16): 210, Pl. 7: Figs. 2-3, 1892. **Pl. III, Fig. 6**

Trichomes are straight and scattered, occurring occasionally with apical end cells that are slightly bend, 4.0–5.5 µm broad. Cells are bluish-green with finely granulated protoplasm, 0.9–2.0 µm in length and 1.0–2.5 µm in width. Anterior cells without calyptra and are rounded; posterior end cell is rounded or hemispherical.

Specimen: LUZON, Laguna, Los Baños (Brgy. San Antonio, Adela's Garden, *Neoregelia cruenta* (R. Graham) L.B. Sm. "Selecta"), E.DLR. Arguelles *s.n.* (Herb. No. AC3-1-a-c, PNCM).

References: Arguelles 2019c, Egypt J Aquat Biol and Fish 23(2): 19p., Pl. II. Fig. 2; Martinez 1984, A Checklist of Blue-Green Algae of the Philippines 66p.; Pantastico 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity 54p., Pl. IV, Fig. 11; Prescott 1962, Algae of the Western Great Lakes Area, 491p., Pl. 110, Figs. 8 & 9, 14; Velasquez 1962, Philipp J Sci 91(3): 289p., Pl. 1. Fig. 20.

DISCUSSION

The current study is the first taxonomic survey in the Philippines to focus on the taxonomy of phytotelm algae of *Neoregelia* spp. (Bromeliaceae) from florists wholesalers in Laguna (Arguelles 2020a). A total of 20 microalgal taxa were taxonomically identified and described from the collected samples, of which eleven species are new additional records of microalgae in the global inventory list of phytotelm microalgae (Ramos and Moura 2019; Poniewozik *et al.* 2020; Arguelles 2020a). Also, the survey reported fourteen new taxa as additional records of microalgae in phytotelm microcosm of Asia, which includes: *S. quadricauda*, *S. tenue*, *C. infusionum*, *C. vulgaris*, *D. splendida*, *E. agilis*, *O. limosa*, *O. tenuis*, *P. minima*, *C. schizodermaticus*, *C. affinis*, *C. meneghiniana*, *Coelastrum* sp., and *Diploneis* sp. (Poniewozik *et al.* 2020; Arguelles 2020a). In addition, this study presented the occurrence of microalga, *Dictyochloropsis splendida* Geitler, described for the first time in the Philippines. The results prove that gardens and greenhouses (from florist wholesalers) provide favorable microhabitat conditions for the existence of microalgae and play a crucial role in terrestrial ecological systems and biodiversity.

Phytotelmata of bromeliads found in semi-natural environments (garden and greenhouse) are considered an important refuge for the existence of microalgae. The species richness of microalgae observed in this survey was greater in *N. carolinae* (eight species), followed by *N. compacta* (six) and *N. cruenta* (six). In relation to the seasons, the species richness of the algal taxa was observed to be highest during dry periods (March 2019), with notable microalgal taxa such as *E. agilis*, *S. quadricauda*, *P. minima*, *D. splendida*, *C. affinis*, and *Diploneis* sp. observed only to this period. A comparison of the phytotelm microalgae identified in the current survey with those from previous studies indicated *Chroococcus minutus*, *Pseudanabaena catenata*, *Scenedesmus quadricauda*, *Tetradesmus obliquus*, *Cyclotella meneghiniana*, *Cosmarium* sp., *Diploneis* sp., and *Nitzschia* sp. as the only eight algal taxa in common. In terms of microalgal diversity in bromeliad tanks observed from the natural environment (forests), this study showed greater species diversity as compared to the taxonomic study of phytotelm algae from other bromeliads done by Killick *et al.* (2014), Carrias *et al.* (2014), Hernández-Rodriguez *et al.* (2014),

and Arguelles (2020a), where they reported not more than 16 microalgal taxa from their survey. The genera *Chlorella*, *Cosmarium*, *Chroococcus*, *Oscillatoria*, and *Pseudanabaena* were present most frequently in the collected samples. *Chlorella vulgaris* and *Oscillatoria* spp. largely dominated the algal density and biomass in *N. cruenta* and *N. compacta* while *Cosmarium* sp., *Chroococcus* spp., and *Psuedanabaena* spp. were the dominant algal taxa in the bromeliad tank of *N. carolinae*. In addition, some microalgal taxa (*E. agilis* and *D. splendida*) are found exclusively in *N. cruenta* and not on other *Neoregelia* species. Thus, microalgal taxa that were observed in each *Neoregelia* spp. contain specific and distinct algal communities. Also, these algal taxa are regarded as widespread and specialists' species notable for their broad habitat tolerance (Kolicka *et al.* 2016).

The diversity and richness of microalgae present in *Neoregelia* species can be associated with its tank morphology, which shows widely exposed and shallow pools containing warm water. This morphology is considered more receptive to algae inoculum transported by means of biotic factors (insects and invertebrates), as well as abiotic (rain and air) factors (Sophia *et al.* 2004; Poniewozik *et al.* 2020). It is important to take note that these factors are considered of minor significance in reference to semi-natural environments. It is probable that the anthropogenic means of dispersal (people visiting the garden and greenhouse) are the main disperser of microalgae in these bromeliads. Several studies were already reported indicating that people unintentionally contributed to algal dispersal in this environment (Cofey and Miller 1988; Poniewozik *et al.* 2020). Based on interviews from floral wholesalers about 1000–1500 people visit their shop every year, thus suggesting the potential of these people as algal dispersers. These potential dispersers can bear resting spores and airborne cells on their clothing, which can be distributed to bromeliad tanks of *Neoregelia* plant species while visiting the gardens and greenhouses (Poniewozik *et al.* 2020). Also, other anthropogenic means of dispersal via transport of these ornamental bromeliads may affect the spread as well as microalgal species diversification outside their initial habitats, and may even become more dominant in other algal species from other bromeliad tanks from the different geographic area (Kolicka *et al.* 2016).

Generally, there are only a few published surveys showing the diversity of microalgae in phytotelmata of bromeliads. Thus, additional taxonomic studies that will expand our understanding of diversity and ecological significance of phytotelm microalgae in bromeliads found in a semi-natural and natural environment (such as tropical rain forest) are needed. Also, an integrated analysis of the abiotic factors of aquatic microcosm and bromeliads plant architecture and position is recommended to elucidate the pattern of algal community succession in tanks of bromeliads in this semi-natural environment.

CONCLUSION

The study presents the first taxonomic survey in the Philippines to focus on the algal community existing in phytotelmata of *Neoregelia* spp. (Bromeliaceae) from gardens and greenhouses of florist wholesalers in Laguna. The survey reports an additional of eleven species as new records in the global inventory list of phytotelm microalgae and 14 new taxa as additional records of microalgae in phytotelm microhabitat of Asia. Also, the occurrence of a microalga – *Dictyochloropsis splendida* Geitler – is described for the first time in the Philippines. This study highlighted the importance of bromeliad tanks (phytotelmata) as a habitat for the existence of diverse species of microalgae.

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