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Species Composition of Algal Epiphyton of Pink Lotus (*Nymphaea pubescens* Willd) Found in Laguna de Bay (Philippines)

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Abstract

In spite of the fact that epiphytic algae are considered an important component of freshwater ecosystems, our knowledge of their diversity and distribution is still rather poor. Taxonomic study on the composition of epiphytic algae living on submerged leaf and root tissues of macrophyte *Nymphaea pubescens* Willd, found at Laguna de Bay (Philippines), was conducted. In total, 21 algal taxa were identified: 10 Cyanophyceae, 6 Trebouxiophyceae, and 5 Bacillariophyceae. The taxa described in this study represent 13 orders, 16 families, 18 genera, and 21 species based on the recent combined taxonomical approach. Of these taxa, the occurrence of a rare cyanobacteria, *Chroococcus schizodermaticus* West, is reported for the first time in the Philippines. One species is also reported here for the first time in the Philippines, based on current taxonomic nomenclature, and this is *Cyanothece aeruginosa* (Nägeli) Komárek, which is based on the former name of *Synechococcus aeruginosus* Nägeli. These taxonomic records are considered important basal information in enriching the knowledge about the diversity and habitat distribution of cyanobacteria and microalgae in macrophytes found in freshwater habitats in the Philippines.

Keywords: Microalgae, epiphytic algae, macrophyte, taxonomy, Laguna de Bay

Introduction

The pink lotus (*Nymphaea pubescens* Willd) is an aquatic macrophyte of the family *Nymphaeaceae*. This plant is characterized by having short, erect, roundish, tuberous perennial rhizomes; leaves floating, peltate, sharply sinuate-toothed; flowers large, floating, solitary, variable in color from pure white to deep red [1]. It is found in freshwater habitats throughout temperate and tropical Asia. This floating macrophyte provides a number of important ecological functions in an aquatic ecosystem. In wetlands, a macrophyte community of pink lotus gives shelter in opposition to predation for susceptible prey species, such as small zooplankton and fishes. Also, this aquatic plant serves as a habitat for epiphytes that are fed upon by a number of invertebrates that themselves serve as a significant portion of the diet of many fishes and birds [2]. In addition, aquatic macrophytes (e.g., pink lotus) constitute a vast substrate for the growth of periphytic communities, such as epiphytic microalgae and cyanobacteria, that play important ecological roles in many water bodies.

Epiphytic microalgae and cyanobacteria are considered as important components of the autotrophic community of a macrophyte-oriented aquatic ecosystem [3]. These organisms are considered primary producers, capable of carbon fixation and uptake of nutrients from the water column, thereby providing these essential nutrients accessible at higher trophic levels [4]. Also, epiphytons can influence the nutrient turnover and transfer between the pelagic and the benthic zone in an aquatic ecosystem. In addition, epiphytic algae are considered useful indicator groups for pollution bioassessment, due to their sensitivity to pollution [5]. Even though epiphytic algae are considered important components of the tropical

freshwater ecosystem, their biodiversity and ecology has been the focus to relatively few investigations in the world [2]. In the Philippines, only few studies were conducted regarding taxonomy and ecology of algal epiphytes in different aquatic macrophytes found in shallow lakes. Previously, abundance and taxonomic studies of algal epiphytes associated with water hyacinth (*Eichhornia crassipes*) found in Laguna de Bay were studied [6,7], but no documented studies about the taxonomic composition of epiphytic algae on other macrophytes found in Laguna de Bay were reported. This paper will serve as a source of baseline information on the species composition of epiphytic algae inhabiting the submerged roots and rear portion of the leaves of pink lotus (*Nymphaea pubescens* Willd) found in Laguna de Bay (Philippines).

Materials and methods

Sampling of epiphytic algae

A single preliminary collection of submerged roots and leaves of pink lotus (*Nymphaea pubescens* Willd) was made on 20 May 2017 to study the diversity and species composition of epiphytic algal flora occurring on the macrophyte. The plant parts were placed in polyethylene bags and kept wet for laboratory examination. The epiphytic algae population was separated from its host plant by scraping and manual shaking for 30 min [8]. A total of 11 macrophyte samples were analyzed throughout the study period. Samples were rinsed with sterile distilled water, producing a combined sample of 100 mL fixed with 4 % formaldehyde. The combined sample was mixed thoroughly and a portion of 50 mL was kept for diatom analysis. The sample for diatom analysis was digested following the standard protocol for cleaning of diatom [9,10]. Suspensions of cleaned diatom were dried onto glass coverslips and mounted. The remaining 50 mL was transferred into a sterile graduated cylinder and allowed to subside for 24 hours. At the end of the settling period, 45 mL of water was removed from the graduated cylinder and the residual 5 mL of water was poured into a sterile glass vial for microscopic observation and enumeration [9,10].

Micrometry, photomicrography, and identification

Enumeration and identification of algae were done using an AO Spencer microscope and Olympus CX31 binocular research microscope [11]. The morphological features pertinent to morphotaxonomic identification, such as the size and shape of vegetative cells, as well as specialized cells (heterocytes and akinetes); characteristics of the filaments and trichomes, presence or absence of constriction at the cross wall; presence or absence of sheath color and appearance of the sheath; and absence or presence of specialized cells such as heterocytes and akinete were recorded during the enumeration, identification, and classification of each algal species. The algal species were identified using the monographs and standard works of literature [12-17]. Morphotaxonomic identification was done up to the species level possible using all available information. In the present study, the orthographs 'hormogonia' and 'heterocytes', instead of 'hormogones' and 'heterocytes', respectively were used, as suggested by the International Association for Cyanophyte Research (IAC) [18].

Results and discussion

A total of 21 algal epiphytes (belonging to Bacillariophyceae, Cyanophyceae, and Trebouxiophyceae) were identified (**Table 1**). Thirty-eight percent of the taxa identified were present to the 11 macrophyte samples analyzed throughout the study period, and included the diatoms *Nitzschia palea, Cyclotella meneghiniana,* the cholorophytes *Scenedesmus quadricauda, Chlorella vulgaris,* and the cyanobacteria *Chroococcus minutus, Oscillatoria tenuis, Pseudanabaena catenata,* and *Leptolyngbya lagerheimii.* Cyanobacteria, chlorophytes, and diatoms that are represented by 10 (or 48 %), 6 (or 28 %), and 5 taxa (or 24 %) respectively of the total species identified have *Chroococcus, Oscillatoria, Pseudanabaena, Leptolyngbya, Scenedesmus, Chlorella, Nitzschia,* and *Cyclotella* present at all the macrophyte samples observed during the sampling period. In addition, cosmopolitan taxa of algal epiphytes, such as *Phormidium, Lyngbya, Botryococcus, Leptolyngbya, Hapalosiphon, Chlorococcum,*

Eunotia, Tetradesmus, Rhopalodia, and Pinnularia, were also observed in the survey. The specimens were described and photographed for the first time in the studied area in order to fill the gap of information of epiphytic algae associated with pink lotus found in Laguna de Bay (Philippines) (Figure 1). Taxonomy based on morphological characterization of each of the isolates is presented, together with a short description of the place of collection and habitat of their occurrence. Current taxonomic names were used, based on AlgaeBase: An On-line Resource for Algae [19].

Table 1 Epiphytic algae on plant tissues of pink lotus (Nymphaea pubescens Willd) from Laguna de Bay

C	Sampling Sites	
Species	Mayondon, Station 1	Bayan, Station 2
Cyanobacteria Class Cyanophyceae Order: Chroococcales Family: Chroococcaceae		
Chroococcus schizodermaticus West	+	
Chroococcus minutus (Kützing) Nägeli		+
Order: Oscillatoriales Family: Cyanothecaceae		
Cyanothece aeruginosa (Nägeli) Komárek		+
Family: Oscillatoriaceae		
<i>Oscillatoria tenuis</i> C. Agardh <i>ex</i> Gomont	+	+
Phormidium autumnale Gomont		+
<i>Lyngbya martensiana</i> Meneghini ex Gomont		+
Order: Synechococcales Family: Leptolyngbyaceae		
<i>Leptolyngbya lagerheimii</i> (Gomont ex Gomont) Anagnostidis & Komárek Family: Pseudoanabaenaceae	+	+
Pseudanabaena minima (G.S. An) Anagnostidis	+	
Pseudanabaena catenata Lauterborn		+
Order: Nostocales Family: Hapalosiphonaceae <i>Hapalosiphon welwitschii</i> West & G.S.West		+
Chlorophyta Class: Trebouxiophyceae Order: Chlorellales Family: Chlorellaceae		
Chlorella vulgaris Beyerinck [Beijerinck]	+	+

Species	Sampling Sites	
	Mayondon, Station 1	Bayan, Station 2
<i>Chlorella ellipsoidea</i> Gerneck	+	
Family: Chlorococcaceae		
Chlorococcum infusionum (Schrank) Meneghini		+
Order Trebouxiales Family Botryococcaceae <i>Botryococcus braunii</i> Kützing		+
Order: Sphaeropleales Family: Scenedesmaceae Scenedesmus quadricauda (Turpin) Brébisson	+	+
Tetradesmus dimorphus (Turpin) M.J. Wynne	+	
Bacillariophyta Class: Bacillariophyceae Order: Bacillariales Family: Bacillariaceae Nitzschia palea (Kützing) W. Smith	+	+
Order: Naviculales Family: Pinnulariaceae <i>Pinnularia grunowii</i> Krammer	+	
Order: Eunotiales Family: Eunotiaceae <i>Eunotia pectinalis</i> (Kützing) Rabenhorst		+
Order: Rhopalodiales Family: Rhophalodiaceae <i>Rhopalodia operculata</i> (C. Agardh) Håkanasson	+	
Order: Stephanodiscales Family: Stephanodiscaceae Cyclotella meneghiniana Kützing	+	+

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Figure 1 Location map of Laguna de Bay and its vicinities

Cyanobacteria

Class Cyanophyceae

Order: Chroococcales

Family: Chroococcaceae

Genus: Chroococcus Nägeli

1. Chroococcus schizodermaticus West {Plate I(1)}

McGregor, Phytotaxa, 133(1): 53, pl. 37, Figure C, D, 2013; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta) In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 54; Tavera and Komárek, Algological Studies/Archiv für Hydrobiologie, 83: 527, Figure 12; Desikachary, 1959, Cyanophyta, 103, pl. 26, Figure 17.

Colonies are microscopic, usually occurring 2 - 4 celled, seldom with more cells, lacking diffluent mucilage, 22 - 43 μ m in diameter. Envelopes concentrically lamellate around cells, yellowish or yellowish brown in color, with outer layer often enlarged. Cells are 3.0 - 5.0 μ m wide and 8.0 - 10.0 μ m long; blue-green in color, usually spherical in shape but sometimes occurring as hemispherical or irregularly-rounded.

A new record for the Philippines

Found occurring as a slimy, blue-green film attached on the submerged roots and leaves of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

2. Chroococcus minutus (Kützing) Nägeli {Plate I(2)}

Basionym: Protococcus minutus Kützing

Arguelles, IAMURE International Journal of Ecology and Conservation, 17:30, pl. I. g. 6, 2016; Singh, *et al.*, Tropical Plant Research. 1(1): 28, Figure 2C, 2014; Komárek, Czech Polar Reports, 3 (2): 130, Figure 7. 2013; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta) In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 54, pl. 11F; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 31;Desikachary, 1959, Cyanophyta, 104-105, pl. 24, g. 4 and pl. 26, g. 4 & 15.

Cells spherical or irregularly spherical usually occurring as single or in groups of 2 - 4 cells, bluegreen in color; colonies enclosed in an amorphous, colorless, homogenous mucilage diffluent at the margin; 5 - 7 μ m in diameter with sheath and 3 - 4 μ m in diameter without sheath; protoplast is slightly granulated.

Found occurring as a slimy, blue-green crust attached on the roots of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order: Oscillatoriales

Family: Cyanothecaceae

Genus: Synechococcus Nägeli

1. Cyanothece aeruginosa (Nägeli) Komárek {Plate I(3)}

Basionym: Synechococcus aeruginosus Nägeli

McGregor, Phytotaxa, 133(1): 21, pl. 26, Figure C, D, E, F, 2013; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta) In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 58, pl. 14E; Zafaralla, 1998, Microalgae of Taal Lake, 12, pl. 2f; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 17, pl. 1, Figure 6; Desikachary, 1959, Cyanophyta, 143, pl. 25, Figure 6, 12.

Cells solitary or in irregular groups, slightly motile, oval, ellipsoidal or sometimes sub-cylindrical with widely rounded ends, mostly longer than 3 μ m (up to 10 μ m long) and 2 - 3 μ m wide, without mucilage or with a colorless, thin gelatinous layer around the cells. Cells are blue-green or olive-green in color with finely granular protoplasm, often with visible keritomized chromatoplasma. Reproduction is through cell division, perpendicular to the longitudinal axis of the cell forming two morphologically similar semi-globular to globular daughter cells, which grow to the earlier shape prior to the succeeding division.

Found occurring as a bluish green crust on submerged roots and leaves of pink lotus associated with other filamentous cyanobacteria and green microalgae.

Order: Oscillatoriales

Family: Oscillatoriaceae

Genus: Oscillatoria Vaucher ex Gomont

1. Oscillatoria tenuis C. Agardh ex Gomont {Plate I(4)}

Kesarwani, Tandon, and Tiwari, Phykos, 45(1): 25, pl. 1, Figure 9; pl. 3, Figure 40, 2015; Martins, *et al.*, Brazilian Journal of Botany, 35(4): 336, Figure 24, 2012; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta). In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 101, pl. 20K; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 54, pl. IV, Figure 11; Prescott, 1962, Algae of the Western Great Lakes Area, 491, pl. 110, Figure 8, 9, 14; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 66; Velasquez, Philippine Journal of Science, 91(3): 289, pl. 1. Figure 20, 1962; Desikachary, 1959, Cyanophyta, p. 222, pl. 42, Figure 15.

Cells bluish-green in color, $0.9 - 2.5 \ \mu m$ long and $3 - 5 \ \mu m$ wide, crosswalls are narrowed, protoplasm is finely granular; crosswalls often granulated but sometimes slightly granulated; posterior end cell is more or less hemispherical. Anterior end cells are rounded and sometimes flattened, not attenuated and without a calyptra; specialized cells such as heterocytes and akinetes are not present. Trichomes are usually scattered, straight or sometimes slightly bend in the apical end cells, $4 - 5 \ \mu m$ broad and slightly constricted to crosswalls.

Found occurring as a brownish to bluish-green crust on leaves of pink lotus slightly submerged in water associated with other cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1; Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Genus: Phormidium Kützing ex Gomont

1. *Phormidium autumnale* Gomont {Plate I(5)}

Synonym: Microcoleus autumnalis (Gomont) Strunecky, Komárek & J.R. Johansen

Sing, *et al.*, Journal of Biosciences, 39(4): Figure 3b, 3c, 2014; Park, 2012; Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales, 5(2): 61, Figure 29B; Martins, *et l.*, Brazilian Journal of Botany, 35(4): 332, Figure 12, 2012; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta). In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 101, pl. 22B,C; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 68; Velasquez, Philippine Journal of Science, 91(3): 308, pl. 3. Figure 3, 1962; Desikachary, 1959, Cyanophyta, p. 276, pl. 44, Figure 24, 25.

Trichomes are solitary, straight, entangled, bent and attenuated at the anterior end, not constricted (sometimes slightly constricted) at the cross-walls, $3.5 - 4.5 \mu m$ wide; cells 0.5 - 1.0 times shorter than wide (isodiametric), $3.0 - 4.5 \mu m$ long; cells are blue-green in color and not granulated (homogenous); anterior cells a bit elongated, capitated, with rounded calyptra. Heterocytes and akinetes are absent; cell division is by transverse fission; reproduction by separation of the trichome into motile hormogonia with the aid of necridic cells.

Found occurring as a bluish green crust attached to submerged roots of pink lotus associated with other filamentous cyanobacteria.

Genus: Lyngbya C. Agardh ex Gomont

1. Lyngbya martensiana Meneghini ex Gomont {Plate I(6)}

Park, 2012. Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales, 5(2): 48, Figure 52A-D; Martins, *et al.*, Brazilian Journal of Botany, 35(4): 335, Figure 18, 2012; Whitton, 2011, Phylum Cyanobacteria (Cyanophyta). In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 92, pl. 19G; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 43.

Filaments solitary, long, straight or flexuous, 7.0 - 10.5 μ m wide; hyaline sheaths are thick, colorless (sometimes yellowish) and lamellate; trichomes are not constricted at the crosswalls, not attenuated, 5.5 - 8.0 μ m wide; cells 0.3 - 0.5 times longer than wide, 1.5 - 3.5 μ m long; cells blue-green in color, homogenous, later granular; apical cell rounded, not capitate, without calyptra or thickened outer cell wall; Cell reproduction by more or less short, motile hormogonia by the help of necridic cells.

Found occurring as a slimy, blue-green film attached on the roots of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Bayan, Station 2), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Order: Synechococcales

Family: Leptolyngbyaceae

Genus: Leptolyngbya K. Anagnostidis & J. Komárek

1. *Leptolyngbya lagerheimii* (Gomont ex Gomont) Anagnostidis & Komárek {Plate I(7)} Basionym: *Lyngbya lagerheimii* Gomont ex Gomont

Park, 2012. Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales, 5(2): 39, Figure 19A-D; Whitton, 2011, Phylum Cyanophyta. In: The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 91, pl. 11D,M; Prescott, 1962, Algae of the Western Great Lakes Area, 501, pl. 112, Figure 5, 6; Desikachary, 1959, Cyanophyta, 290, pl. 48, Figure 6 and pl. 53, Figure 2.

Trichomes are usually clumped and slightly curled up. Filaments are blue green in color, $2 \mu m \log and 2 - 3 \mu m$ wide, solitary or rarely loosely entangled, sometimes irregularly spiral, protoplasm not granular, septa not granulated, apical cells rounded without calyptra; end cells rounded; sheaths 2 um wide, thin, colorless, attached to trichomes. Reproduction is by crosswise binary fission, often cells must reach the original size before entering the next division.

Found occurring as a bluish green crust attached to submerged roots of pink lotus associated with other green microalgae.



Plate I (1) Chroococcus schizodermaticus West, (2) Chroococcus minutus (Kützing) Nägeli, (3) Cyanothece aeruginosa (Nägeli) Komárek, (4) Oscillatoria tenuis C. Agardh ex Gomont, (5) Phormidium autumnale Gomont, (6) Lyngbya martensiana Meneghini ex Gomont, (7) Leptolyngbya lagerheimii (Gomont ex Gomont) Anagnostidis & Komárek. All scale bars = 10 μm.

Family: Pseudoanabaenaceae

Genus: Pseudoanabaena Lauterborn

1. Pseudanabaena minima (G.S. An) Anagnostidis {Plate II(1)}

Basionym: *Achroonema minimum* Park, 2012, Algal Flora of Korea (Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales) 49, Figure 17D-G.

Cells usually longer than wide, $1.5.0 - 3.5 \mu m$ long, blue-green in color; cell content is smooth and homogenous lacking aerotopes. Anterior end cells are usually widely rounded and without aerotopes. Trichomes are usually 1.5 - 2.0 μm wide, occurring as solitary or crowded in clusters, straight, highly constricted at the cross-walls, posterior end cells are not attenuated.

Found occurring as a slimy, blue-green film attached on the roots of the pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

2. Pseudanabaena catenata Lauterborn {Plate II(2)}

Park, 2012. Algal Flora of Korea: Cyanophyta: Cyanophyceae: Chroococcales, Oscillatoriales, 5(1): 48, Figure 16G,17A,B; McGregor, 2007. Freshwater Cyanoprokaryota of North-Eastern Australia 1: Oscillatoriales, 36: Figure 2f; Komárek and Anagnostidis, 2005. Cyanoprokaryota.
2. Teil: Oscillatoriales. Süßwasserflora von Mitteleuropa, 83: Figure 59.

Trichomes are usually solitary or sometimes in a mass forming a tiny tangle, straight, cylindrical and isopolar, with distinct constrictions at the cross walls, $1.0 - 2.0 \,\mu\text{m}$ wide. Cells are 1.5 - 2.0 times longer than wide, cells are blue-green in color, smooth, homogeneous protoplasm. Anterior end cells are usually rounded or sometimes somewhat conical.

Found occurring as a bluish green crust on leaves slightly submerged in water associated with other cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order: Nostocales

Family: Hapalosiphonaceae

Genus: Hapalosiphon Nägeli ex É. Bornet & C. Flahault

1. Hapalosiphon welwitschii West & G.S.West {Plate II(3)}

Arguelles, IAMURE International Journal of Ecology and Conservation, 17:30, pl. I. Figure 5, 2016; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 39; Desikachary, 1959, Cyanophyta, 588, pl. 137, Figure 5.

Cells are sub-spherical and elongated; bluish green in color, $1.5.0 - 2.5 \mu m$ long and $3.0 - 4.0 \mu m$ wide, smooth protoplasm, septa somewhat granulated, end cells usually rounded; trichomes are arranged consistently in one series and unevenly arcuate displaying true branches, $3.5 - 4.0 \mu m$ broad, slightly constricted at the crosswalls, anterior end cell is not attenuated and capitated; gelatinous sheaths are thin and colorless; lateral branches are usually shorter than the main filament, with similar cellular characteristics and dimensions as the cells in the main filament.

Found occurring as a brownish to bluish-green crust on leaves of pink lotus slightly submerged in water associated with other cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Chlorophyta

Class: Trebouxiophyceae

Order: Chlorellales

Family: Chlorellaceae

Genus: Chlorella Beyerinck [Beijerinck]

1. Chlorella vulgaris Beyerinck [Beijerinck] {Plate II(4)}

Basionym: *Chlorella pyrenoidosa var. duplex* (Ku tzing)

Satpati, *et al.*, Journal Algal Biomass Utilization, 4 (1): 30, pl. 1, Figure 1 and pl. 5, Figure 2, 2013; Ortega-Calvo, *et al.*, Nova Hedwigia. 57: 246, pl. 2, Figure 16 and 17, 1993; Zafaralla, 1998, Microalgae of Taal Lake, 36, pl. 7j; Prescott, 1962, Algae of the Western Great Lakes Area, 237, pl. 53, Figure 13.

Cells are spherical characterized by having a thin cell wall; chloroplast is solitary and is cup-shaped containing a single spherical pyrenoid occupying the basal zone of the cell; immature young cells either ellipsoidal or spherical, $1.5 \times 2.5 \ \mu m$ or $3.0 \ \mu m$ in diameter; cell propagation is by formation of 2 or 4 autospores of the similar sizes, released by bursting of the mother cell wall.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1; Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

2. Chlorella ellipsoidea Gerneck {Plate II(5)}

Synonym: Chloroidium ellipsoideum (Gerneck) Darienko, Gustavs, Mudiumu,

Menendez, Schumann, Karsten, Friedl & Proschold

John, 2011, Phylum Chlorophyta (Green Algae) In: The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae, 476, pl. 103F; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 98, pl. VIII, Figure 3.

Cells are 1.0 - 6.0 um wide and 2.0 - 8.0 um long, symmetrical; chloroplast band-shaped, becoming more lobed as the cell matures; pyrenoid is solitary associated with starch grains; cell propagation is by autospore formation (2, 4, or 8 in each sporangium), autospores are unequal in sizes released by rupture of the mother cell wall.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.

Family: Chlorococcaceae Genus: Chlorococcum Meneghini

1. Chlorococcum infusionum (Schrank) Meneghini {Plate II(6)}

Synonym: *Chlorococcum humicola* (Nägeli) Rabenhorst 1868 Basionym: *Cystococcus humicola* Nägeli

Prescott, 1962, Algae of the Western Great Lakes Area, 280, pl. 45, Figure 1; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 76, pl. VII, Figure 1; Zafaralla, Microalgae of Taal Lake, 33, pl 8e.f, 1998; Samad and Adhikary, Algae, 23(2): 91, pl. 1 Figure 1, 2008; Arguelles, IAMURE International Journal of Ecology and Conservation, 17: 32, pl. I. Figure 7, 2016.

Spheroidal cells, solitary sometimes occurring as several cells that are packed together to form a group of cells, greenish in color; chloroplast is more or less covering the entire cells; cells 9 - 14 μ m in diameter.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order Trebouxiales

Family Botryococcaceae

Genus Botryococcus Kützing

2. Botryococcus braunii Kützing {Plate II(7)}

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, 499, pl. 2I, 113H; Papa, *et al.*, Philippine Journal of Systematic Biology, 2(1): 21, 2008.

Cells oval or sometimes occurring as obvoid, $2.5 - 3.5 \,\mu$ m wide, $5.5 - 10.5 \,\mu$ m long. Apical cap is present in each of the cells, placed in the edge of the mucilaginous masses. Colonies are greenish to yellowish in color, consisting of cells arranged irregularly and embedded within the edge of the crinkled mucilage.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.



Plate II (1) *Pseudanabaena minima* (G.S. An) Anagnostidis, (2) *Pseudanabaena catenata* Lauterborn, (3) *Hapalosiphon welwitschii* West & G.S.West, (4) *Chlorella vulgaris* Beyerinck [Beijerinck], (5) *Chlorella ellipsoidea* Gerneck, (6) *Chlorococcum infusionum* (Schrank) Meneghini, (7) *Botryococcus braunii* Kützing. All scale bars = 10 μm.

Order: Sphaeropleales

Family: Scenedesmaceae

Genus: Scenedesmus Meyen

1. Scenedesmus quadricauda (Turpin) Brébisson {Plate III(1)}

Basionym: Achnanthes quadricauda Turpin

Arguelles, IAMURE International Journal of Ecology and Conservation, 17:32, pl. I. Figure 8, 2016; Satpati, *et al.*, Journal of Algal Biomass Utilization. 4(1): 26, pl. 1, Figure 4; pl. 5, Figure 6, 2013; Ortega-Calvo, *et al.*, Nova Hedwigia. 57: 247, pl. 3, Figure 35, 1993; Zafaralla, Microalgae of Taal Lake, 39, pl. 9g.j, 1988; Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 119, pl. IX, Figure 8; Prescott, 1962, Algae of the Western Great Lakes Area, 280, pl. 64, Figure 2.

Cells oblong or spherical in shape, $4 - 6 \mu m \log and 2 - 3 \mu m$ wide, with visible solitary pyrenoid; cells usually occur in parallel with setae; colonies with two or four cells attached alongside each other, organized linearly in coenobia; inner cells of the coenobia are without spines and terminal cells with two spiny projections, which are usually straight but sometimes curved and with smooth cell wall.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1; Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Genus: Tetradesmus G.M. Smith

1. *Tetradesmus dimorphus* (Turpin) M.J. Wynne {Plate III(2)}

Synonym: Scenedesmus dimorphus (Turpin) Kützing

Basionym: Achnanthes dimorpha Turpin

Satpati, *et al.*, Journal of Algal Biomass Utilization. 4 (1): 30, pl. 1, Figure 5 and pl. 5, Figure 7; 2013; *Scenedesmus dimorphus* (Turpin) Kützing, Pantastico, 1977, Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity, 117, pl. IX, Figure 6; Prescott, 1962, Algae of the Western Great Lakes Area, 277, pl 63, Figure 8, 9.

Colonies usually occur in four to eight spindle-shaped cells aligned in a solitary or alternate chains; cells are uninucleated with a single pyrenoid; size is normally 10 - 13 μ m long and x 6 - 7 μ m wide; inner cells with straight and sharp apices; terminal cells sickle-shaped and are firmly curved, with sharp apices and uneven cell wall.

Found occurring as a greenish crust on submerged roots of pink lotus associated with other filamentous cyanobacteria.

Bacillariophyta

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

1. *Nitzschia palea* (Kützing) W. Smith {Plate III(3)}

Basionym: Synedra palea Kützing

Costa, *et al.*, Hoehnea, 44(4): 574, Figure 156,157 and 158, 2017; Marra *et al.*, Biota Neotropica, 16(4): 14, Figure. 194-195, 2016; Akbulut, Turkish Journal of Botany, 27: 293, Figure 5.17, 2003.

Valves are lanceolate to linear lanceolate tapering rapidly at the poles with protracted round to capitate apices. Fibulae are distinct with central nodule with striae that are slightly visible (21 - 25 striae in 10 μ m). Valve mantle wider on keel side with a length of 22 - 57 μ m and width of 3 - 7 μ m, costae is 10 - 17 μ m.

Found occurring as a brownish crust on submerged roots of pink lotus associated with other filamentous fungi and cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1; Bayan, Station 2), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order: Naviculales

Family: Pinnulariaceae

Genus: *Pinnularia* Ehrenberg

1. *Pinnularia grunowii* Krammer {Plate III(4)}

Joh, 2012. Algal Flora of Korea: Chrysophyta: Bacillariophyceae: Pennales, Raphidineae: Naviculaceae, 3(9): 52, Figure 43,44;

Valves are linear with trinundulate margins, inflation of the middle parts of the cell is equal or slightly smaller than the other two, ends are capitate and clearly protracted from the cell. Raphe is straight and somewhat filiform. Axial area is linear and central area rectangular reaching the valve margins. Striae 8 - 14 rows in 10 μ m, radiate in middle and strongly convergent towards the ends. Valves 25 - 57 μ m in length and 6 - 10 μ m in width.

Found occurring as a brownish crust on submerged leaves of pink lotus associated with other filamentous fungi and cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order: Eunotiales

Family: Eunotiaceae

Genus: *Eunotia* Ehrenberg

1. Eunotia pectinalis (Kützing) Rabenhorst {Plate III(5)}

Basionym: Himantidium pectinale Kützing

Meeravali, et al., Journal of Algal Biomass Utilization. 8(4): 25, pl. I. Figure 5, 2017.

Valves dorsiventral, symmetrical to the transapical axis. Dorsal margin are convex while the ventral margin are concave. Apices of valves are broadly rounded, with slight constriction at the dorsal end. Raphe are distinct with small terminal nodule. Valve length is $26 - 49 \mu m$, breadth $3 - 6 \mu m$, striae is very fine 3 - 12 in $1 \mu m$.

Found occurring as a brownish crust on submerged roots and leaves of pink lotus associated with other filamentous fungi and cyanobacteria.

Order: Rhopalodiales Family: Rhophalodiaceae Genus: *Rhophalodia* Otto Müller 1. *Rhopalodia operculata* (C. Agardh) Håkanasson {Plate III(6)} Basionym: *Frustulia operculata* C. Agardh You, *et al.*, Nova Hedwigia, 89: 423, Figure 95-99, 2009; Akbulut, Turkish Journal of Botany, 7: 293, Figure 5.12, 2003

Valves are characterized by having a convex dorsal margin notched in the middle portion, and with straight ventral margin. Valve length is 16 - 48 μ m with breadth 5 - 10 μ m. Costae are distinct and frequently 2 - 4 in 10 μ m. Areolae are arranged in rows, 29 - 40 in 10 μ m, occurring usually 2 - 3 rows between the costae.

Found occurring as a brownish crust on submerged leaves of pink lotus associated with other filamentous fungi and cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mayondon, Station 1), E.DLR. Arguelles *s.n.* Photograph prepared from the mounted specimen.

Order: Stephanodiscales

Family: Stephanodiscaceae

Genus: Cyclotella (Kützing) Brébisson

1. Cyclotella meneghiniana Kützing {Plate III(7)}

Costa, *et al.*, Hoehnea, 44(4): 566, Figure 8-9, 2017; Leira, *et al.*, Anales del Jardín Botánico de Madrid, 74 (2): 7, Figure 2e, 2017; Marra *et al.*, Biota Neotropica, 16(4): 8, Figure 2, 2016; Akbulut, Turkish Journal of Botany, 27: 297, Figure 7.12-14, 2003.

Valves are small and radially symmetrical (disc-shaped) with a narrow mantle; central area is flat and smooth covering 1/3 of the valve surface with marginal chambered striae. Diameter 8 - 16 μ m, striae 6 - 9 in 10 μ m.

Found occurring as a brownish crust on submerged roots associated with other filamentous fungi and cyanobacteria.



Plate III (1) Scenedesmus quadricauda (Turpin) Brébisson, (2) Tetradesmus dimorphus (Turpin) M.J. Wynne, (3) Nitzschia palea (Kützing) W. Smith, (4) Pinnularia grunowii Krammer, (5) Eunotia pectinalis (Kützing) Rabenhorst, (6) Rhopalodia operculata (C. Agardh) Håkanasson, (7) Cyclotella meneghiniana Kützing. All scale bars = 10 μm.

Diverse collections of microalgae and cyanobacteria associated with Nymphaea pubescens, collected in Laguna de Bay, Philippines, were observed. A total of 21 species were recorded and described in detail, including their habitats and places of collection. The taxa represent 13 orders, 16 families, 18 genera, and 21 species, based on the recent combined taxonomical approach [12-17]. This study recorded for the first time in the Philippines the occurrence of Chroococcus schizodermaticus West, a cyanobacteria first reported in submerged roots of Nymphaea pubescens found in Laguna de Bay, Philippines. It was observed that the algal mats in submerged leaves and roots of pink lotus are composed mainly of unicellular and filamentous eukarvotic algae and filamentous cvanobacteria. The algal groups observed on pink lotus have been recorded to be similar in other taxonomic studies done for macrophytes in aquatic ecosystems found in other countries [2,4,40,46]. Algal epiphytes associated with Nymphaea lotus in two different freshwater bodies in southern Ghana documented a total of twenty-eight genera of algal epiphytes, wherein three genera (Scenedesmus, Chlorella, and Chlorococcum) were observed to be similar to the findings of the current study [47]. Also, taxonomic study on the composition of epiphytic diatoms on Charophycean macrophytes found in a dinaric karstic ecosystem reported the occurrence of similar algal taxa such as Cyclotella meneghiniana, Eunotia pectinalis, Rhopalodia sp., Pinnularia sp., and Nitzschia sp. [48]. Submerged macrophytes are important habitats and resources for many aquatic organisms. The interaction and dependence of many trophic levels in these plants signifies the importance of studying the diversity and ecological interactions of these organisms in such ecosystems. Environmental stress originating from human activity effects can be determined by the presence of particular algal taxa in the system. In this study, several algal taxa were identified, which can be used as biological indicators for the aquatic ecosystem health assessment of Laguna de Bay. Some of the algal taxa identified, such as Nitzschia palea, Cyclotella meneghiniana, Oscillatoria tenuis, Pseudanabaena catenata, Chroococcus minutus, Scenedesmus quadricauda, Chlorella vulgaris, and Phormidium autumnale, are indicative of eutrophic systems, but a larger part of the taxa were examples of a cosmopolitan community [13,46,49]. This systematic account shows the need to further study epiphytic microalgae and cyanobacteria in different macrophytes, since little work has been completed on the quantification and identification of these epiphytes relative to macrophyte meadows in aquatic environments.

Conclusions

The present study reported a collection of epiphytic algae observed in pink lotus found in Laguna de Bay (Philippines). Taxonomic analyses of such important epiphytes are crucial in understanding the ecology of epiphytons in several macrophytes found in different aquatic environments. The taxonomic records contributed by this study are considered important information in increasing knowledge about the diversity and habitat distribution of microalgae and cyanobacteria in the Philippines.

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