NEW RECORDS OF CORTICOLOUS MICROALGAE AND CYANOBACTERIA FOR PHILIPPINE ALGAL FLORA FROM MT. MAKILING FOREST RESERVE

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ABSTRACT

Diversity and species composition of corticolous microalgae and cyanobacteria from Mt. Makiling Forest Reserve showed the occurrence of 15 taxa belonging to the class Cyanophyceae, Chlorophyceae, Trebouxiiophyceae, Ulvophyceae, and Klebsormidiophyceae. The collection reported in this study represents 12 orders, 13 families, 14 genera and 15 species based on morphothaxonomic characterization supported by microscopic studies. Of these taxa, the occurrence of six corticolous algae namely: Chroococcidiopsis cubana Kamárek & Hindák, Pseudanabaena galeata Böcher, Pseudanabaena catenata Lauterborn, Parachlorella kessleri (Fott & Nováková) L. Krienitz, E.H. Hegewald, Hepperle, V. Huss, T. Rohr & M. Wolf, Desmococcus olivaceus (Persoon ex Archerson) J. R. Laundon, and Apatococcus lobatus (Chodat) J.B. Petersen are reported for the first time in the Philippines. These taxonomic records are considered important information in enhancing our knowledge about the diversity and habitat distribution of this important group of primary producers found in forest reserves of the Philippines.

INTRODUCTION

Corticolous microalgae and cyanobacteria are considered dwellers of the bark of trees from the areas of different altitudes. These organisms can be observed as greenish or dark, gelatinous, red dark or brown patches, streaks or velvet masses and are exposed to air and absorb water, minerals and other nutrients directly from the atmosphere (Bhakta, et al., 2014). The environment from which these algae are found is regarded as extreme because of their minimal water resources or their extremely low or high temperature and light levels. These algae have developed distinct morphological and physiological adaptations in order to survive and proliferate under such extreme condition (Lemes-da-Silva, et al., 2010). The occurrence of small (5-15 μm), unicellular, coccoid microalgal taxa in tree bark biofilms is an example of morphology-based functional adaptation of the corticolous algae to frequent desiccation, high irradiance and temperature fluctuations (Ettl and Gätner, 2013; Lopez-Bautista et al., 2007; Štifterová and Neustupa, 2015). In addition to morphological adaptations, the constituents of the phototrophic aerial biofilms also hold diverse eco-physiological strategies, such as high intracellular osmotic values to prevent water loss to recurrent dry/rehydration cycles and photoprotection for survival in habitat with high UV levels (Štifterová and Neustupa, 2015).

The tropical rainforest is considered as one of the most diverse naturally occurring habitat on Earth that should be conserved. The wide variability of distinct and unique landscapes in this biological community can explain the high number of endemic organisms, which is also true for microorganisms, including microalgae and cyanobacteria. Several studies on taxonomy and ecology of aerophytic corticolous algae were conducted in temperate and other tropical countries with great emphasis on those occurring on forest areas (Millow, 1999; López-Bautista et al., 2006; Neustupa and Škaloud, 2008; Lemes-da-Silva et al., 2010; Zammit et al., 2011; Neustapa and Štifterová, 2010; Štifterová and Neustupa, 2015). While recent studies indicate a high taxonomic diversity of corticolous algae in the tropical regions of the world equaling or even surpassing that of temperate areas, studies of corticolous algae in these regions remain rare and Philippines is no exception. This important microflora is still very poorly known in the Philippines due to the lack of exploration and seasonal collection of samples. Hence, the present survey was carried out to assess the diversity of corticolous algae from Mt. Makiling Forest Reserve. This paper aims to add to our knowledge of the taxonomy, diversity and habitat distribution of corticolous algae present in corticolous communities found in a conserved tropical forest in Luzon, Philippines.

MATERIAL AND METHODS

Sampling of Corticolous Algae

A single preliminary collection was made to Mt. Makiling Forest Reserve (situated at 14° 08’ N, 121° 11’ E) to study the occurrence of corticolous algal flora occurring on trees on December 2017. The bark of 14 trees with a trunk diameter of more than 30 cm was sampled for surface microbial growths at a height of 120–40 cm above the soil level, evenly around the trunk perimeter. All samples were collected in sterile specimen tubes (Tarson) of 25 X 50 mm size using clean sampling bottles, forceps, polythene bags, brush, petri dish, scalpel etc. and brought to the laboratory for further analysis.

Micrometry, Photomicrography, and Identification

The algal biofilm was abraded off the samples with a sterile scalpel and placed into 1.5 ml Eppendorf tubes containing 0.5 ml liquid Blue-Green Medium (BG 11). Then, sterile glass beads (0.5 mm in diameter) were placed in the tube, which was mixed for 15 seconds at 1200 rpm in a vortex mixer (Štifterová and Neustupa, 2015). The homogenized samples were placed in Petri dishes within 72 h after the collection. They were cultivated on BG11 medium (Stainer et al., 1971) at a temperature of 25°C and an illumination of 40μmol m⁻² s⁻¹ provided by 18W cool fluorescent tubes (Philips TLD 18W/33) (Arguelles, et al., 2018). After growth, each filament, colony or a consortium was taken for photomicrography. Microphotographs were taken with AO Spencer microscope and Olympus CX31 binocular research microscope (Arguelles et al., 2014). The morphological features pertinent to morphothaxonomic 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 as follows: (Desikachary, 1959; Presscott, 1962; Velasquez, 1962; Komárek and Anagnostidis, 2005;
Morphotaxonomic identification was done up to the species level using all available information. In the present study, the orthographs ‘hormogonia’ and ‘heterocytes’ instead of ‘hormogones’ and ‘heterocysts’ respectively were used, as suggested by the International Association for Cyanophyte Research (IAC) (Mollenhauer et al., 1994).

RESULTS AND DISCUSSION

The study revealed 15 taxa belonging to the class Cyanophyceae, Chlorophyceae, Trebouxiophyceae, Ulvophyceae, and Klebsormidiophyceae. Morphotaxonomy of each of the isolates is presented together with a short description of the place of collection and habitat of their occurrence. Current names were used based on Guiry and Guiry (2018). All scale bars = 10 μm.

Taxonomic Enumeration

Cyanobacteria
Class Cyanophyceae
Order Chroococcales
Family Aphanothecaceae
Genus Gloeothece C. Nägeli
1. Gloeothece membranacea (Rabenhorst) Fig. 1

Basionym: Aphanocapsa membranacea Rabenhorst


Colonies consist of miniature sub-colonies enclosed together into mucilaginous envelopes, sometimes spherical or membranous; cells short cylindrical to oval with widely rounded ends, olive green to dark green in color, 6.0–7.5 μm × 3.0–5.0 μm; formation of macroscopic cell aggregation sometimes occur; mucilaginous sheath around cells are colourless, lamellate and sometimes diffluent at the margin.

Found occurring as a bluish green patch on a bark surface associated with other green microalgae. Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Order: Chroococcales
Family: Chroococcaceae
Genus Chroococcus Nägeli
1. Chroococcus minutus (Kützing) Nägeli Fig. 3

Basionym: Protococcus minutus Kützing

Cells spherical or irregularly spherical usually occurring as single or in groups of 2-4 cells, blue-green in color; colonies enclosed in an amorphous, colorless, homogenous mucilage diffusent at the margin; 5.0-7.0 μm in diameter with sheath and 3.0-4.0 μm in diameter without sheath; protoplast is slightly granulated. Found occurring as a blackish patch on a bark surface associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Order: Oscillatoriales
Family: Coleofasciculaceae
Genus: Anagnostidinema Strunecký et al.

1. Anagnostidinema amphibium (C.Agardh ex Gomont) Strunecký, Bohunická, J.R.Johansen & J.Komárek  Fig. 4

Basionym: Oscillatoria amphibia C. Agardh ex Gomont


Trichomes light blue-green in color and without a sheath, short, isopolar, flexuous, sometimes or rarely straight, 0.5-1.5 μm wide, with 15-35 cells per filament, cross walls are constricted, not attenuated and capable of gliding motility. Cells are longer than wide, cylindrical, 2.5-3.0 μm long. Apical cells are canonically rounded with a crescent-shaped characteristic.

A new record for the Philippines.

Found occurring as a brownish to blackish patch on a bark surface associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

2. Pseudanabaena catenata Lauterborn  Fig. 6


Cells usually 1.5-2.0 times longer than wide, cells are light blue-green in color, homogeneous protoplasm without aerotopes. Anterior end cells are rounded or sometimes slightly conical. Trichomes are solitary or sometimes cluster into a small tangle, straight, cylindrical and isopolar, usually with noticeable constrictions at the cross walls, 0.9-2.0 μm wide. Found occurring as a brownish to blackish patch on a bark surface associated with other filamentous cyanobacteria.

A new record for the Philippines.

Found occurring as a brownish to blackish patch on a bark surface associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Order Oscillatoriales
Family Oscillatoriaceae

Genus Phormidium Kützing ex Gomont

1. Phormidium chalybeum (Mertens ex Gomont) Anagnostidis and Komárek

Fig. 7

Basionym: Oscillatoria chalybea Mertens ex Gomont


Trichomes blue-green in color, long and straight, 9.0-11.00 μm wide, constricted at cross walls, straight or slightly attenuated at the ends, capable of gliding motility. Cells are usually shorter than wide, (4.1) 5.0-9.0 μm long. Apical cells without a sheath, conically to broadly rounded and without a calyptra, protoplasm is finely granulated.

Found occurring as a brownish to bluish-green patch on a bark surface associated with other green microalgae.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), 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

Fig. 8

Martinez-Goss, et al., The Philippine Scientist, 51: 78, pl. III, fig. 5, 2014; Komárek et al., 2003, Filamentous Cyanobacteria In: Freshwater Algae of North America. Ecology and Classification, 177, fig. 34e; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 52; Velasquez, Philippine Journal of Science, 91(3): 342, pl. 8. Fig. 105, 1962; Desikachary, 1959, Cyanophyta, 387, pl. 68, Fig. 3.

Cells are blue-green in color, barrel-shaped or nearly spherical; trichomes are constricted at the crosswalls, 3.0-4.5 μm broad, not attenuated at the ends; cells 5.0 μm long, mostly shorter than broad; heterocytes nearly spherical, about 6.0 μm broad; filaments are entangled and aggregate at maturity in a mass, macroscopically or microscopically.

Found occurring as a brownish to blackish gelatinous patch on a bark surface associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Chlorophyta

Class: Chlorophyceae

Order: Chlamydomonodales

Family: Chlorococcaceae

Genus: Chlorococcum Meneghini

1. Chlorococcum infusionum (Schrank) Meneghini

Fig. 10

Arguelles, Tropical Life Sciences Research, 30(1): 7, pl. 1. Fig.7, 2019; Arguelles, IAMURE International Journal of Ecology and Conservation, 17:30, pl. I. fig. 5, 2016; Saha, et al., Indian Journal of Microbiology, 47: 219, fig. 29, 2007; Martinez, 1984, A Checklist of Blue-Green Algae of the Philippines, 39; Desikachary, 1959, Cyanophyta, 588, pl. 137, fig. 5.
Cells are spherical, greenish in color, usually occurring as solitary but sometimes several cells are packed together to form a mass of compact cells; parietal chloroplasts are present in the cell with a single pyrenoid nearly covering the entire cell; cells 7.0-9.0 μm in diameter; zoospores are cylindrical or oval in shape, 2.0-4.5 um wide and 5.0-11.5 um long, characterized by having a papilla with an eye spot and contractile vacuoles. Found occurring as a greenish patch on a bark surface associated with other filamentous cyanobacteria.

A new record for the Philippines.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Order Prasiolales
Family Prasiolaceae
Genus Desmococcus F. Brand
1. Desmococcus olivaceus (Persoon ex Archerson) J. R. Laundon

Cells are in sarcinoid aggregates (2-4 celled colonies), 4.0-8.0 μm in diameter; cells occur as spherical, hemispherical or irregular, 2.0-5.0 μm diameter; chloroplasts are observed to be parietal; pyrenoid is indistinguishable; aplanosporangia are spherical with an ornamented wall. Found occurring as a greenish patch on a bark surface associated with other filamentous cyanobacteria.

A new record for the Philippines.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Genus Apatococcus F. Brand
1. Apatococcus lobatus (Chodat) J.B. Petersen Fig. 13

Basionym: Lepraria olivacea Persoon ex Archerson


Cells are in sarcinoid aggregates (2-4 celled colonies), 4.0-8.0 μm in diameter; cells occur as spherical, hemispherical or irregular, 2.0-5.0 μm diameter; chloroplasts are observed to be parietal; pyrenoid is indistinguishable; aplanosporangia are spherical with an ornamented wall. Found occurring as a greenish patch on a bark surface associated with other filamentous cyanobacteria.

A new record for the Philippines.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.
Cells solitary or clumped together forming 2-, 3-, or 4-celled packets, frequently clustered together; cells globose to somewhat compressed, 7.0–13.0 μm across, walls regularly thickening with age; large chloroplasts either lobed or plate-shaped. Found occurring as a greenish patch on a bark surface associated with other filamentous cyanobacteria and green microalgae.

A new record for the Philippines.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

Class: Ulvophyceae
Order: Trentepohliales
Family: Trentepohliaceae
Genus: Trentepohlia Martius

1. Trentepohlia monilia De Wildeman Fig. 14

Filaments are slightly constricted at the crosswalls; occurring naturally as long filaments with high tendency to separate and shatter apart into smaller fragments; cells are cylindrical to oval in shape with rounded ends, 10.0 μm long and 4.0 μm wide; cell wall moderately thickened; chloroplasts are parietal and band-shaped containing one pyrenoid covering 1/2–2/3 of the cell inner surface. Found occurring as a greenish mat on a bark surface associated with other filamentous cyanobacteria.

Specimen: LUZON, Laguna, Los Baños (Mt. Makiling Forest Reserve), E.DLR. Arguelles s.n. Photograph prepared from the mounted specimen.

In spite of the fact that the total area studied is relatively small in comparison with the country area and also considering that just one ecosystem was studied, the present survey showed a diverse collection of corticolous microalgae and cyanobacteria in the sampling area. A total of 15 taxa belonging to the class Cyanophyceae, Chlorophyceae, Trebouxiophyceae, Ulvophyceae, and Klebsormidio phyceae were described in detail including a short description of their habitat and place of collection. This study reported the occurrence of six corticolous algae namely: Chroococcidiopsis cubana Komárek & Hindák, Pseudanabaena galeata Böcher, Pseudanabaena catenata Lauterborn, Parachlorella kessleri (Fott & Nováková) L. Krienitz, E.H. Hegewald, Hepperle, V. Huss, T. Rohr & M. Wolf, Desmococcus olivaceus (Persoon ex Archerson) J. R. Laundon, and Apatococcus lobatus (Chodat) J.B. Petersen for the first time in the Philippines. The corticolous algal flora observed in this survey is not very much distinct from those of other regions, several species and genera found during the survey have been also described in prior studies on corticolous algae in different world regions. Bhakta, et al., (2014) reported 19 corticolous microalgae and cyanobacteria Similipal Biosphere reserve, Mayurbhanj, Odisha, India and two of them (Desmococcus olivaceus and Nostoc commune) were also observed in this study. Desmococcus olivaceus and the genus Klebsormidium...
were observed in aerial algal biofilms collected from the tropical forest remnants in the northwest region of São Paulo State, Brazil by Lemes-Da-Silva et al., (2010), besides another seven species of green microalgae. In collaboration with the preliminary survey done by Arguelles, (2016) on the Philippine subaerial epiphytic algal community, three species (Chlorococcum infusionum, Hapalosiphon welshitchi, and Chroococcus minutus) are shared.

The results show that representative taxa coming from green microalgae and cyanobacteria mainly represented the algal communities observed in the studied environment. Different adaptation strategies against dehydration in aerial habitats such as the presence of sporopollenin-like compounds in the cell wall and a special carbohydrate and alcohol pattern contributed to the successful growth and proliferation of corticolous microalgae and cyanobacteria in such extreme environments (Lemes-Da-Silva, et al., 2010). On the other hand, ecophysiological protective mechanisms to avoid excessive irradiances such as the production of carotenoids and thallus formation are also considered important mechanisms being used by corticolous algae in the aero-terrestrial environment (Lemes-Da-Silva, et al., 2010). Due to the limited number of studies on corticolous algae in the tropical regions and especially in the Philippine archipelago, these records can be considered an important contribution to the knowledge about the diversity and geographical distribution of microalgae and cyanobacteria in the country. However, morphology-based taxonomy of species has been considered sometimes problematic to species identification. Therefore, it is suggested that detailed studies focusing on molecular data should be conducted to give a more reliable and accurate taxonomic delimitation. Acknowledgments: The author express gratitude for the support of the National Institute of Molecular Biology and Biotechnology (BIOTECH) who provided the equipment as well as other chemicals needed for the completion of the study. The help of Mr. Marc Villaruel for the preparation of the algal photomicrographs is acknowledged with gratitude.

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ARGUELLES, E.D.L.R. 2016. Morphotaxonomic account of epiphytic microalgae and cyanobacteria in bark surfaces of trees found at Mt. Makiling Forest Reserve. Taxonomic account of this group of microorganism builds up important information on algal diversity as part of the living resources of the Philippines. The results of this study will add to the pool of data important in understanding the ecology and habitat distribution of microalgae and cyanobacteria in the Philippines.

CONCLUSION

The present study reported a compilation of some noteworthy corticolous microalgae and cyanobacteria in bark surfaces of trees found at Mt. Makiling Forest Reserve. Taxonomic account of this group of microorganism builds up important information on algal diversity as part of the living resources of the Philippines. The results of this study will add to the pool of data important in understanding the ecology and habitat distribution of microalgae and cyanobacteria in the Philippines.

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