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Morphological and Molecular Evidence of Microcystin-Producing *Microcystis aeruginosa* (Chroococcales, Cyanophyceae) from Pantabangan Lake, Nueva Ecija, Philippines

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ABSTRACT

Microcystin-producing cyanobacteria are widely known to be a significant threat to freshwater ecosystems, aquaculture, and public health. The principal aim of our study is to isolate, identify and detect *Microcystis aeruginosa* capable of producing microcystins from Pantabangan Lake, Philippines, using morphological characterisation and molecular approaches. Two strains of *M. aeruginosa* (GBJLPAN01 and GBTKPAN01) were established and their morphology and phylogeny were examined. Cells of *M. aeruginosa* were spherical, densely packed in irregular colonies and embedded within a mucilaginous sheath. Cell sizes ranged from 2 – 5 µm in diameter, and displayed a distinct blue-green colouration. Phylogenetic analyses inferred from 16S rDNA showed the clustering of our cultured strains within a well-supported *Microcystis* clade, though the genus exhibited non-monophyletic clustering. Additionally, phylogenetic analysis of *mcyE gene* sequences confirmed clustering with *M. aeruginosa* reference strains, providing molecular validation of species identification. PCR-based screening revealed the presence of *mcyE* gene in both laboratory cultures and field-collected water samples, indicating widespread potential for microcystin production in Pantabangan Lake. The presence of *mcyE* serves as a reliable molecular marker for toxigenic strains, enabling accurate assessment of microcystin biosynthetic capacity. This study provides the first verified molecular evidence of microcystin-producing *M. aeruginosa* in Pantabangan Lake, combining diagnostic morphological features with dual molecular confirmation (16S rDNA and *mcyE* phylogenies) including *mcyE* detection from both cultured isolates and field samples. Our findings highlight the need to recognise the potential risk of *Microcystis* blooms to aquatic life and water quality of the lake.

Keywords: detection, freshwater, HABs, microcystin, phylogeny, toxin

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INTRODUCTION

In aquatic environments, cyanobacterial blooms signal a significant disruption to the natural balance of planktonic communities, often reflecting underlying ecological stress or nutrient enrichment (Moreira *et al.,* 2014). They produce a wide range of secondary metabolites, including toxins that pose significant ecological and health risks. Traditionally, these toxins have been classified according to their functional effects into hepatotoxins, neurotoxins, dermatoxins, and cytotoxins (Corbel *et al.,* 2014; Du *et al.,* 2019). To date, more than 80 cyanobacterial species have been identified as toxigenic, and detection methods for cyanotoxins continue to advance in sensitivity and reliability (Nugamanova *et al.,* 2023). The toxicity associated with cyanotoxins is closely linked to both cyanobacterial proliferation and the extent of toxin biosynthesis, which are strongly influenced by abiotic factors such as light intensity, temperature, ultraviolet radiation, pH, and nutrient availability (Neilan *et al.,* 2013; Haeder *et al.,* 2014; Rastogi *et al.,* 2014; Rastogi *et al.,* 2015). Because of these dynamics, cyanotoxins represent not only a major threat to human and animal health but also an important

ecological factor that requires comprehensive understanding for effective management and mitigation. Among these, microcystins are the most frequently detected cyanotoxins in both surface and drinking water, and they are well-studied hepatotoxins due to their potential carcinogenicity (Rastogi *et al.,* 2014). Globally, microcystins are considered the most widespread cyanotoxins (Chorus & Welker, 2021), with their production primarily attributed to cyanobacteria of the genera *Microcystis*, *Anabaena*, and *Planktothrix* (Botes *et al.,* 1984; Rouhiainen *et al.,* 2000).

Microcystis is a taxonomically diverse cyanobacteria genus, with species differentiated largely based on colony morphology, cell size, mucilage structure, and the pattern of cellular aggregation. Currently, there are 57 species, 11 varieties and 7 forms of *Microcystis* being recognised (Guiry & Guiry, 2025). *Microcystis aeruginosa* (Kützing) Kützing is one of the most common and widely studied bloom-forming species and is considered among the principal producers of microcystins in freshwater environments worldwide (Carmichael, 2001; Oberholster *et al.,* 2004). In the Philippines, the occurrence of *M. aeruginosa* has been documented in several freshwater systems, including Laguna de Bay, Taal Lake, and Pasig River, as well as other inland water bodies such as, Lake Buluan and Paoay Lake (Tayaban *et al.,* 2018; Bernardo *et al.,* 2020; Tabugo *et al.,* 2023; Inog *et al.,* 2025). Notably, harmful blooms of this species have been reported in Laguna de Bay, where they often result in massive fish kills, reduced water quality, and the development of off-flavours in fish, particularly during the summer dry season when high temperature and nutrient enrichment favour cyanobacterial proliferation (Papa & Mamaril, 2011; Tayaban *et al.,* 2018). Such events highlight the urgent need for reliable detection and molecular identification of *M. aeruginosa* populations in Philippine freshwater ecosystems to better assess bloom dynamics and mitigate their ecological and socio-economic impacts.

Despite growing global awareness, many freshwater systems in developing regions, including the Philippines, remain understudied in terms of diversity of cyanobacteria and the toxins associated with the bloom-forming species. One of the less studied lakes in terms of cyanobacteria research is Pantabangan Lake. This is one of the largest artificial dams located in the province of Nueva Ecija covering a total area of 97,318 hectares, with 4,023 hectares comprising the water reservoir (Cruz *et al.,* 2006; Pulhin *et al.,* 2006; Reyes & Mendoza, 2019). The reservoir supports a multipurpose dam that provides irrigation and hydroelectric power. In addition to supplying water for domestic and industrial uses, the watershed plays a vital role in flood control, mitigating damage to agricultural lands in Central Luzon. Previous research on Pantabangan Lake has concentrated on other issues, such as fisheries productivity, hydrology, sedimentation, or its role in irrigation and hydroelectricity (Pulhin *et al.,* 2006), leaving less attention for cyanobacterial studies. Given its large role in irrigation and supply of water across Central Luzon, this pioneering study has been initiated with the main purpose of accurate species identification and molecular detection of *mcyE* to confirm the presence of toxic *Microcystis* species in Pantabangan Lake.

MATERIALS AND METHODS

Sample Collection and Processing

Water sampling was conducted in Pantabangan Lake, Nueva Ecija, Philippines, in January 2025 and December 2025. Samples were collected from three distinct stations within the lake (Sites 1 – 3; Figure 1), representing varying field conditions, including a surface bloom area and non-bloom sites. Site 1 (15.82893°N, 121.16953°E) exhibited a dense surface accumulation of *Microcystis* (Figure 1B), whereas Site 2 (15.84419°N, 121.13888°E) showed no visible bloom (Figure 1C), and Site 3 (15.82670°N, 121.14982°E) represented open-water conditions without bloom (Figure1D). Sampling was performed using a 20 µm plankton net deployed vertically. At each station, 500 mL of water was collected in duplicate. Water temperature ranged from 25.7 to 26.3 °C, while pH values ranged from 6.25 to 6.68 during the sampling period. All samples were transported to the Algal Diversity and Bioresources

Laboratory, Central Luzon State University, for further processing and analysis. Transportation time was approximately 45 minutes, and samples were maintained at ambient temperature during transit. Cells of *Microcystis* were isolated using a capillary pipette under an inverted microscope (Olympus CKX41, Japan). Established cultures (GBJLPAN01, GBTKPAN01) were cultivated using a sterilized distilled water enriched with commercially available BG11 media (Qingdao Hope Bio-Technology Co., China) and/or MA media (Ichimura, 1979). Cultures were maintained in a fabricated culture cabinet with a temperature of 24 ± 2°C and 12:12h light:dark photoperiod. Environmental water samples collected from the lake were processed immediately after on-site sampling, with 100 mL of each sample filtered upon arrival at the laboratory using a 0.22 µm polycarbonate membrane filter (MF-Millipore, Merck, Ireland). The filter papers were then placed in sterile microtubes and stored at -20 °C for DNA extraction.

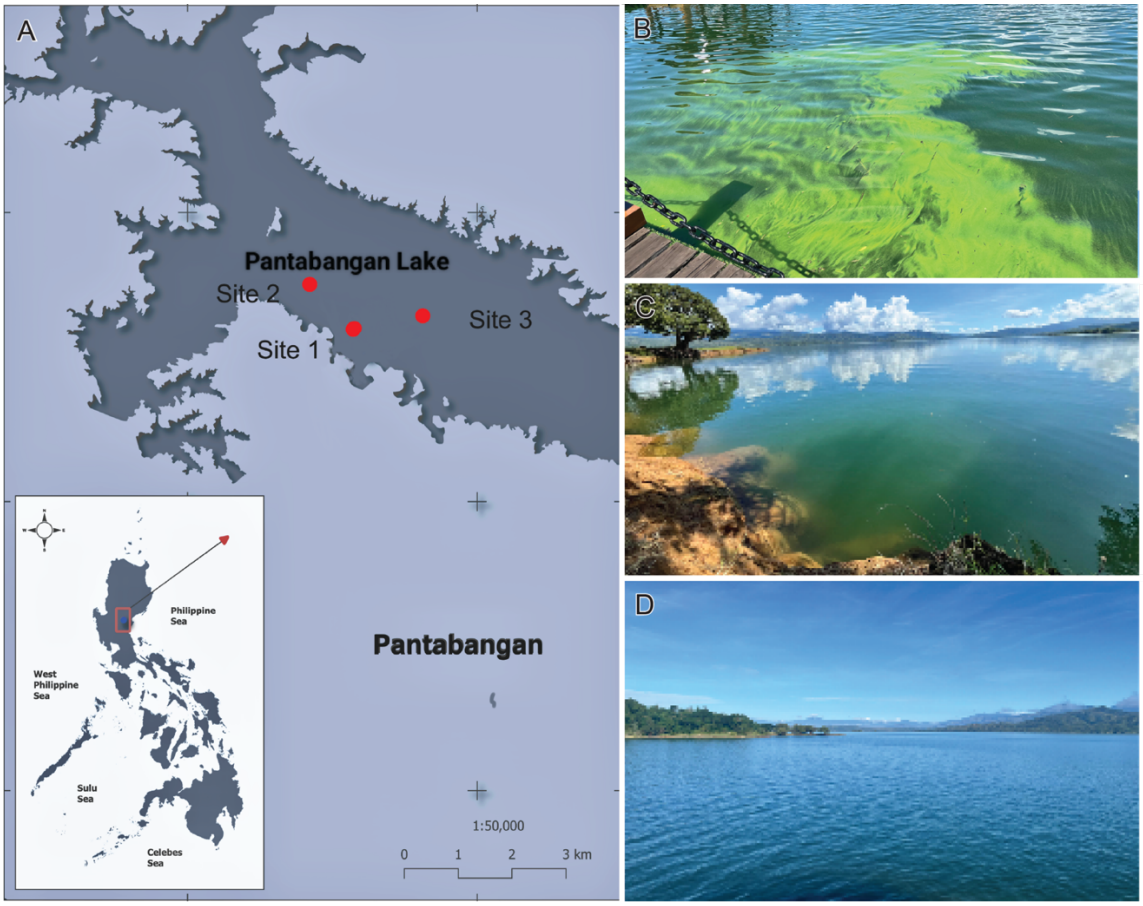

Figure 1. Sampling sites and field conditions in Pantabangan Lake, Philippines. (A) Map showing the location of Pantabangan Lake within the Philippines and the three sampling stations (Sites 1 – 3) indicated by red dots. (B) Surface bloom observed at Site 1, characterised by dense *Microcystis* accumulation. (C) Site 2 showing no visible bloom during sampling. (D) Site 3, showing open-water view of Pantabangan Lake

Morphological Characterisation

The morphological features of the cultured and field-collected *Microcystis* colonies were observed using a light microscope (Olympus BX60, Japan) equipped with differential interference contrast and fluorescence devices. Colony and cellular traits were characterised based on standard taxonomic references with slight modifications (Valerio *et al.,* 2009; Conklin *et al.,* 2020). Main features examined included colony shape, size, and mucilage

characteristics, as well as individual cell morphology such as cell shape, colour, granulation, and presence of aerotopes (gas vesicles). Vegetative cell diameter was measured from at least 30 cells per sample to capture intraspecific variability. In an attempt to distinguish morphospecies of *Microcystis*, colony compactness, mucilage appearance (homogeneous or diffuse), and cell arrangement within colonies were also taken into account (Watanabe *et al.,* 1980; Komárek & Anagnostidis, 1998).

DNA Amplification and Sequencing

DNA from both cultured and environmental samples was extracted using the Cetyltrimethylammonium bromide (CTAB) method, as described by Jensen *et al.* (2023). During the extraction process, aseptic techniques were carefully followed to prevent contamination. Prior to PCR amplification, the extracted DNA was diluted with nuclease-free water to achieve optimal working concentrations and to minimise potential interference from buffer components such as EDTA and Tris, which can inhibit enzymatic reactions.

For the PCR amplification of 16S rDNA and *mcyE* sequence, TaKaRa Ex Taq (Takara, Shiga, Japan) was used in a total reaction volume of 10 µL, consisting of 6.15 µL nuclease-free water, 1 µL PCR buffer, 0.8 µL dNTP mix, 0.5 µL of primers, 0.05 µL Ex Taq polymerase, and 1 µL genomic DNA template. Primers used for 16S rDNA were CYA106F (5′ CGG ACG GGT GAG TAA CGC GTG A 3′) and CYA781R (5′ GAC TAC TGG GGT ATC TAA TCC CAT T 3′), and for *mcyE* were mcyE-F2 (5′ GAA ATT TGT GTA GAA GGT GC 3′) and mcyE-R8 (5′ CAA TGG GAG CAT AAC GAG 3′) (Nübel *et al.,* 1997). The thermal cycling conditions for 16S rDNA were: an initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 45 s, and extension at 72 °C for 2 min; with a final extension at 72 °C for 10 min. Those for *mcyE* were: an initial denaturation at 95 °C for 3 min; followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 30 s, and extension at 72 °C for 2 min; with a final extension at 72 °C for 10 min. The PCR products were checked by electrophoresis using Mupid-One (Takara, Japan) with a 2 % agarose gel containing 1 µL of SYBR Safe DNA Gel Stain (Thermo Fisher, USA). Unpurified PCR products were sent to Apical Scientific (Selangor, Malaysia) for sequencing of 16S rDNA. The validated sequence data generated using the forward primer and reverse primer were edited and aligned using a pairwise alignment tool, and consensus sequences were conducted using the BioEdit 7.2 (Hall, 1999) application.

Phylogenetic Analysis

The sequences obtained were first checked against the GenBank database using BLAST to confirm their identities. For phylogenetic analysis, a dataset of 55 taxa representing 16S rDNA sequences was assembled (Table S1). The tree is rooted using bacteria sequences obtained from the freshwater i.e., *Shigella sonnei*, *Escherichia coli*, and *Escherichia fergusonii* (Olowe *et al.,* 2017). Multiple sequence alignment was done with MAFFT v7 (Katoh & Standley, 2013) and the output was carefully examined and adjusted manually using BioEdit v7.2.5 (Hall, 1999) to improve alignment quality. The best substitution model was selected with jModelTest2 v2.1.6 (Darriba *et al.,* 2012) based on the Akaike information criterion (AIC). Phylogenetic trees were then constructed using both maximum likelihood (ML) and Bayesian inference methods. ML analysis was performed in PhyML (Guindon *et al.,* 2010) with 1,000 bootstrap replicates to test the reliability of the clades. Bayesian inference was carried out with MrBayes v3.2.7a (Ronquist *et al.,* 2012). The resulting trees were compared and evaluated using both bootstrap support and posterior probabilities.

RESULTS

Morphology

Colonies of *Microcystis aeruginosa* (GBJLPAN01 and GBTKPAN01) were irregular-shaped with distinct holes in cultures (Figure 2A). Cells in field water samples showed

a darker pigmentation (Figure 2B). Each colony was surrounded within a transparent and slightly overlapping mucilage layer (Figure 2C). Cells appeared tightly packed or loosely dispersed in colonies (Figure 2C). Cells measured 2 – 5 µm in diameter, spherical to sub-spherical in shape (Figure 3D & 3E). Cells contained distinct aerotopes (gas vesicles), which appeared granular (Figure 3D). Autofluorescence of chlorophyll was visible throughout the cell (Figure 3E). No heterocysts, akinetes, or specialized terminal cells were observed, consistent with the genus *Microcystis*.

Scanning electron microscopy (SEM) revealed the detailed surface morphology and colonial organisation of the examined *Microcystis* isolate (Figure 3A – E). In low-magnification views, cells were observed to be numerous, small, and predominantly spherical, occurring either as solitary units or loosely associated aggregates (Figure 3A). Individual cells exhibited a smooth to slightly textured surface and were relatively uniform in size, although minor size variation was evident across the field. At higher magnification, cells appeared coccoid to subspherical, with well-defined boundaries and no visible external appendages (Figure 3B). The cell surface was generally smooth, lacking ornamentation such as spines or ridges. Cells were observed forming incipient colonies, where individuals were loosely embedded in an amorphous matrix, suggesting the presence of extracellular mucilage (Figure 3C). The arrangement of cells within these aggregates was irregular, without a defined geometric pattern. More compact colonial structures were evident in Figure 3D, where cells were densely clustered, forming cohesive aggregates. Cells within these appeared closely packed, with reduced intercellular indicating progressive colony formation. At the highest magnification (Figure 3E), individual cells displayed a distinctly smooth, rounded morphology, with subtle surface undulations. No clear pores, spines, or specialized surface structures were observed.

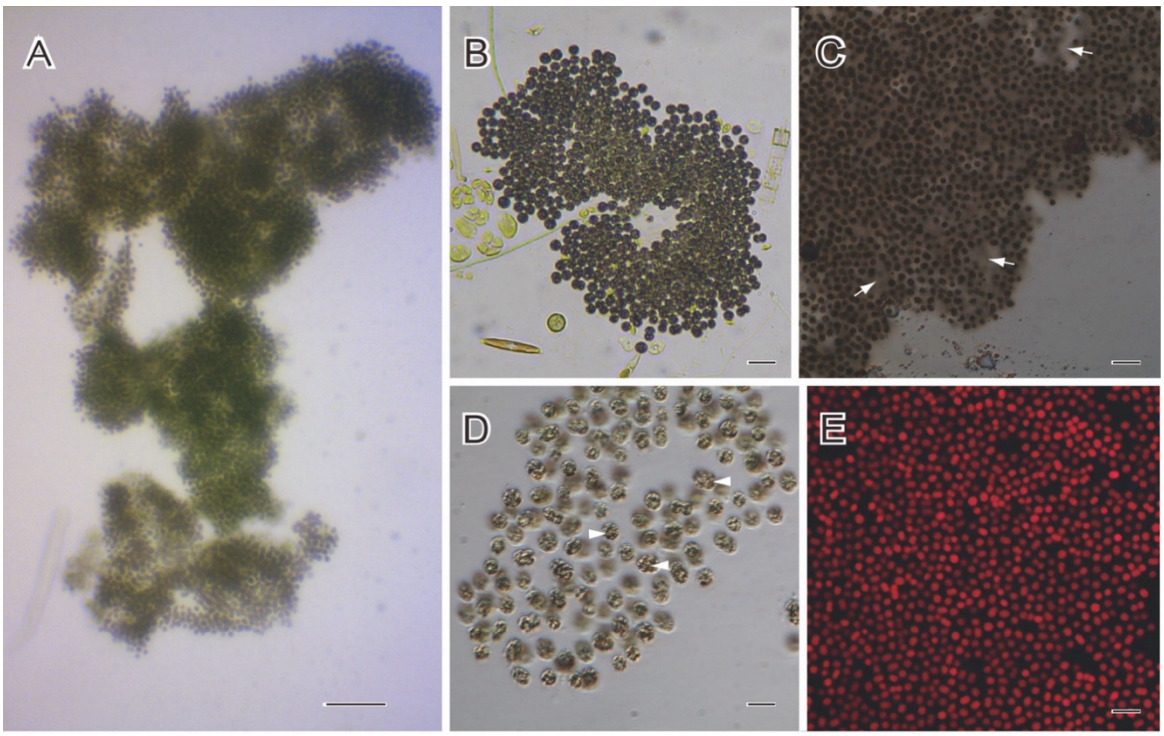

Figure 2. Light microscopy of *M. aeruginosa*. (A) Colonies of *M. aeruginosa* in culture. (B) Colonies of *M. aeruginosa* from the wild. (C) Cells covered in mucilage (white arrows) (D) Cells of *M. aeruginosa* showing aerotopes (white arrowheads). (E) Autofluorescence of *M. aeruginosa* under UV excitation. Scale bar equals 10 µm

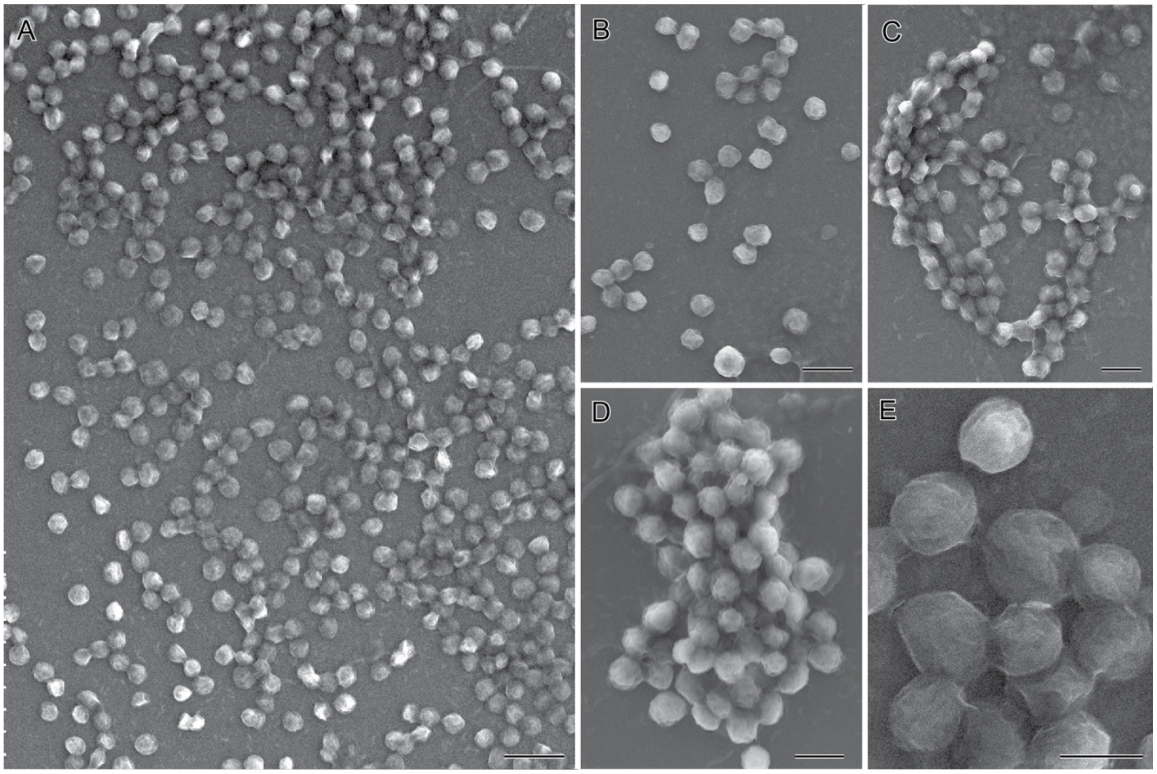

Figure 3. Scanning Electron microscopy of *M. aeruginosa* (GBTKPAN01) (A, B) Colonies composed of numerous coccoid cells forming irregular, loosely to densely aggregated clusters. (C) Intermediate magnification showing cell arrangement within colonies. (D – E) High magnification views of individual cells, which are spherical to subspherical with smooth to slightly textured surfaces and clearly defined boundaries. No heterocysts or akinetes were observed. Scale bars: (A) = 30 µm; (B – C) = 10 µm; (D, E) = 5 µm

Molecular Identification and Phylogeny

BLASTn analysis of the 16S rDNA sequences generated from strains GBJLPAN01 and GBTKPAN01 provided initial taxonomic identification as *Microcystis aeruginosa*, showing high sequence similarity (E-value 0.0) to other *M. aeruginosa* sequences. For phylogenetic analysis, downloaded sequences included representative genera from the family Microcystaceae were aligned and trimmed resulting matrix of 55 operational taxonomic units (OTUs) comprising 686 base pairs, of which 426 sites (62%) were conserved. The phylogenetic tree generated by maximum likelihood (ML) analysis is shown in Figure 4. Posterior probability values derived from Bayesian inference (BI) analysis were also shown. Tree topologies obtained from both analyses showed similar positions of all cyanobacteria clades but differed in clade support for some genera. The phylogenetic analyses based on 16S rDNA sequences showed maximum support (BI posterior probability/ML bootstrap support, 1.0/100%) of the inclusion GBJLPAN01 and GBTKPAN01 to other the clade of *Microcystis* however, relatively moderate bootstrap values and short branch lengths within the clade indicate low genetic divergence and limited resolution among species. Subclades composed of taxa such as *M. flos-aquae*, *M. ichthyoblabe*, *M. viridis*, and *M. robusta* showed moderate support suggesting partial phylogenetic structuring but unresolved interspecific relationships, consistent with the known genetic homogeneity of the genus. The isolates obtained in this study (GBJLPAN01 and GBTKPAN01) clustered tightly with reference *M. aeruginosa* sequences. The genus *Microcystis* clearly separated from other members of the family Microcystaceae taxa, including *Synechococcus*, *Gloeothece*, and *Geminocystis*, as well as from the distant outgroup.

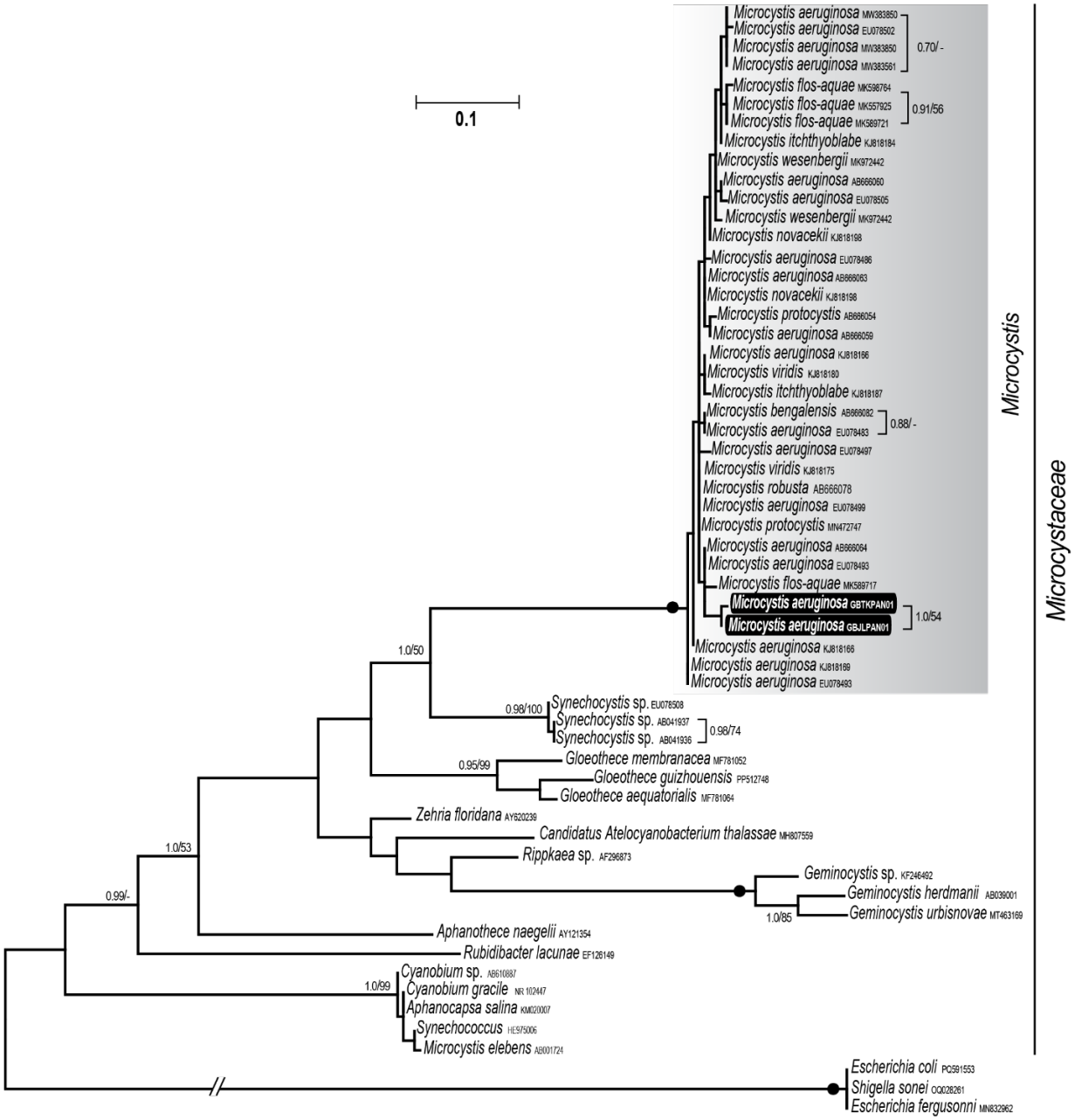

Figure 4. ML phylogeny of cyanobacteria inferred from 16S rDNA (686 bp) based on the GTR + G + I model. (PP, ≥ 0.7) (BS, ≥ 50%) values of ML were indicated black dot indicate maximum support for both BI and ML (PP/BS = 1.00/100) DNA sequence analyzed in this study are highlighted in black box

Detection of *mcyE*

The gel electrophoresis image showed the amplification of *mcyE* sequence in both cultured and environmental samples from Pantabangan Lake (Figure 5A & 5B). Clear amplicons of approximately 274 bp were observed in all environmental DNA samples (SP1, SP1D, SP2, SP2D, SP3, SP3D) as well as in the cultured isolate *M. aeruginosa* (GBJLPAN01, GBTKPAN01). The presence of these bands confirms the amplification of the target *mcyE* sequences. In contrast, no amplification was detected in the negative controls (NCW – nuclease - free water, and NCGA – green

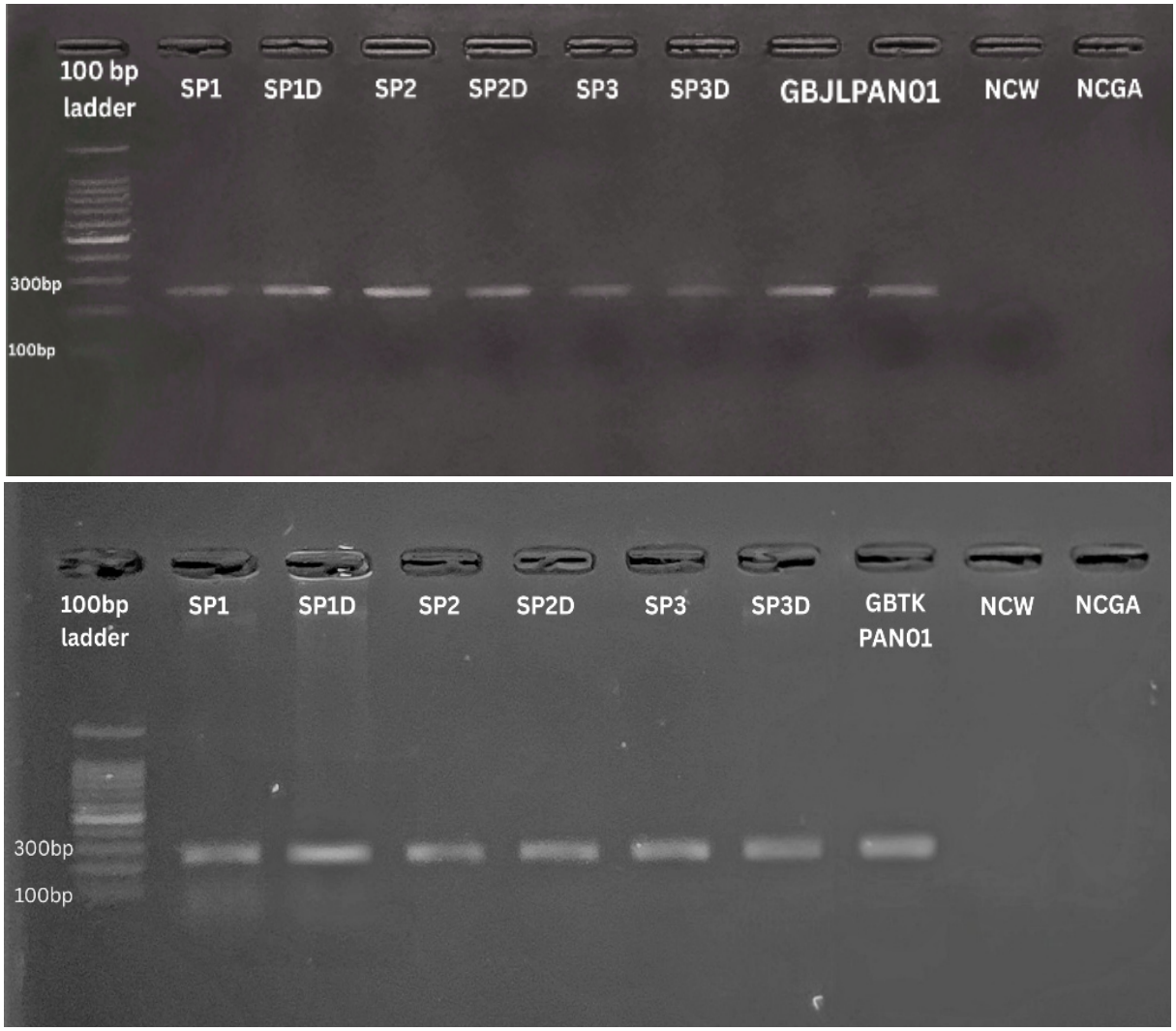

Figure 5. Gel images of amplified mcyE sequences from (A) January 2025 and (B) December 2025 collections. Environmental samples (SP1, SP2, SP3), diluted environmental samples (SP1D, SP2D, SP3D), *Microcystis aeruginosa* cultures (GBJLPAN01 in January, GBTKPAN01 in December), negative control water (NCW), and negative control green algae (NCGA)

algae DNA), demonstrating that the observed signals are specific to the microcystin-producing *Microcystis* and not due to contamination or non-specific amplification.

Sequencing of the amplified *mcyE gene* generated additional sequences. Phylogenetic analysis of these sequences revealed that the Pantabangan isolate clustered within well-supported clade of *M. aeruginosa* with maximum support (1.0/100%) (Figure 6). The isolate grouped closely with other *M. aeruginosa* reference sequences, along with related taxa such as *Microcystis flos-aquae* and *Microcystis viridis*. Several uncultured cyanobacterial sequences were also found within this clade. In addition, other cyanobacterial genera, including other filamentous cyanobacteria, *Planktothrix*, *Anabaena*, and *Phormidium*, formed distinct and separate lineages the same with other distant taxa *Cylindrospermopsis*, *Oscillatoria*, *Leptolyngbya* which served as deeper branching lineages.

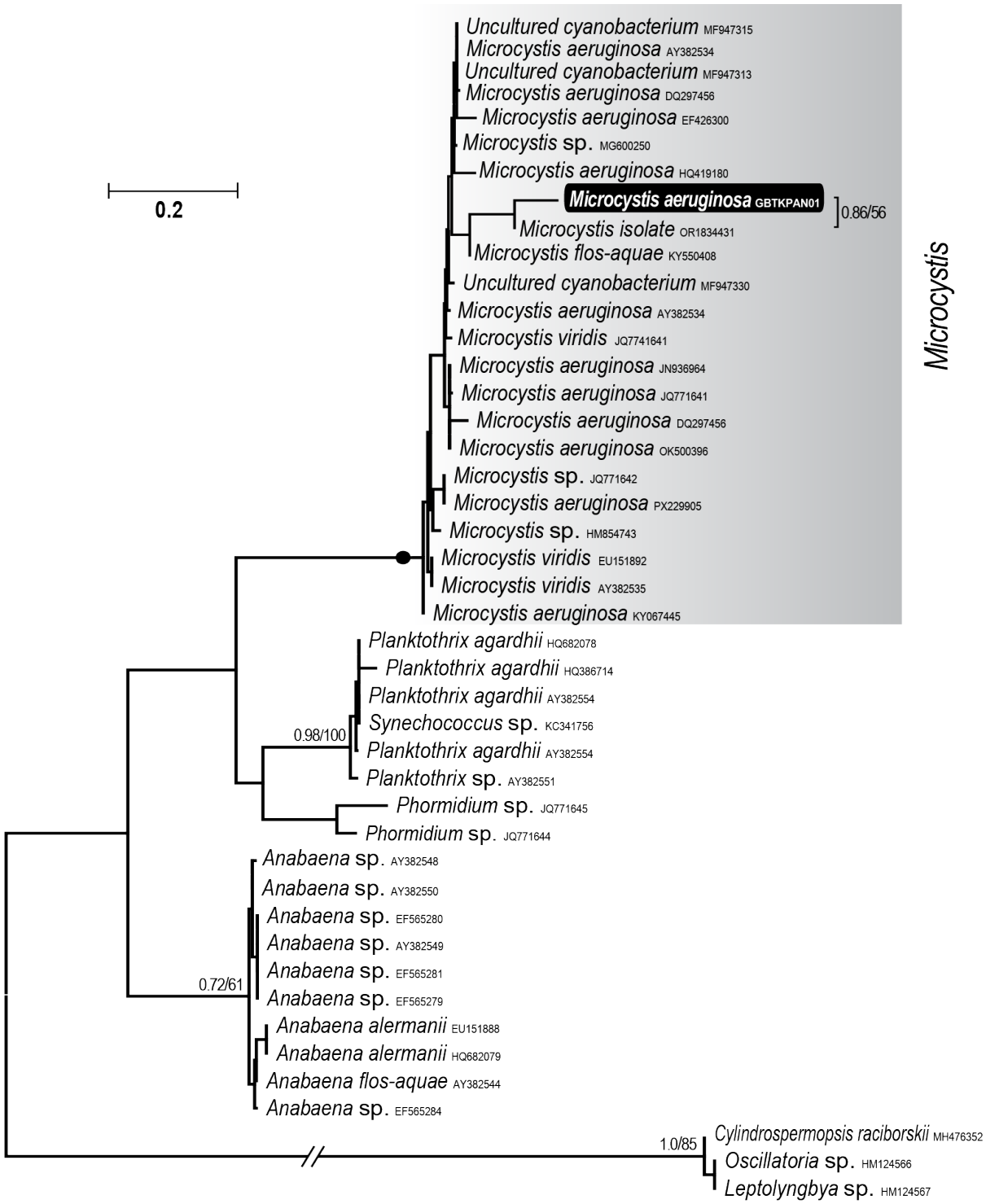

Figure 6. ML phylogeny of cyanobacteria inferred from *mcyE* gene (247 bp) based on the GTR + G + I model. (PP, ≥ 0.7) (BS, ≥ 50%) values of ML were indicated black dot indicate maximum support for both BI and ML (PP/BS = 1.00/100) DNA sequence analysed in this study are highlighted in black box

DISCUSSION

Taxonomic Identification

The features we observed in both fixed field material and cultured isolates match the diagnostic traits of *Microcystis aeruginosa*. The irregular-shaped colonies with distinct holes observed in our cultures represent a main diagnostic feature distinguishing *M. aeruginosa* from other *Microcystis* species. The characteristic colonial morphology contrasts markedly with *M. wesenbergii*, which typically forms large, regularly spherical colonies (often >50 μm) with cells arranged predominantly in a single peripheral layer around a largely cell-free center (Komárek, J., & Komárková, J. 2002). The presence of holes and irregular colony boundaries we documented is inconsistent with *M. flos-aquae*, which characteristically forms smaller, more compact spherical colonies with tightly aggregated cells and more uniform structural integrity (Otsuka *et al*. 2001; Komárek & Komárková, 2002). Under SEM observations, our cells revealed both as solitary units and in loosely associated aggregates, progressing to more compact colonial structures with densely clustered, cohesive aggregates. This progression from loose to tight cell packing within the same population is characteristic of *M. aeruginosa* and differs from the more consistently organized arrangements seen in *M. smithii*, where cells are arranged in distinct groups or clusters, or *M. botrys*, which shows more structured, grape-like clustering patterns (Komárek & Komárková, 2002). The transparent, slightly overlapping mucilage layers surrounding each colony demonstrate the characteristic extracellular matrix production of *M. aeruginosa*. Our SEM analysis confirmed cells were loosely embedded in an amorphous matrix, indicating substantial mucilage production. This mucilage architecture differs from *M. viridis*, which typically exhibits more defined, regular colony boundaries with less prominent mucilage, and from *M. panniformis*, which often shows more structured mucilage organization (Otsuka *et al.* 2001; Komárek & Komárková, 2002). The irregular arrangement of cells within colonies, without defined geometric patterns is diagnostic for *M. aeruginosa*. This contrasts with the more organized cellular arrangements characteristic of other species, such as the peripheral cell distribution in *M. wesenbergii* or the clustered organisation in *M. smithii* (Otsuka *et al.* 2001; Komárek & Komárková, 2002).

Phylogenetic reconstruction based on 16S rDNA identified nine *Microcystis* species forming a non-monophyletic group, with *M. aeruginosa* (including our Pantabangan Lake isolates) being the most abundantly represented, followed by *M. bengalensis*, *M. protocystis*, *M. wesenbergii*, *M. novacekii*, *M. viridis*, *M. ichthyoblabe*, *M. flos-aquae* and *M. robusta*. Many taxonomists argue that these morphospecies are not truly distinct and may in fact represent a single, highly variable species, *M. aeruginosa* (Otsuka *et al.,* 1999; Komárek *et al.,* 2002). On the other hand, some researchers contend that the taxa are indeed separate species, but the commonly used molecular markers such as 16S rRNA and ITS regions lack sufficient resolution to reliably distinguish them (Tanabe *et al.,* 2007; Humbert *et al.,* 2013). Notably, more recent studies using alternative markers such as the cpcBA-IGS sequences (phycocyanin intergenic spacer and flanking regions) have shown that certain morphospecies, for example *M. wesenbergii*, can form a phylogenetically distinct clade, supporting the view that at least some species boundaries within *Microcystis* are real (Tan *et al.,* 2010). In this study, we identified the Pantabangan Lake strain as *M. aeruginosa* based on its characteristic colony morphology, following the diagnostic features described in standard cyanobacterial taxonomy (Komárek & Anagnostidis, 1998), and its clustering within a clade containing the majority of *M. aeruginosa* sequences. Although the 16S rRNA gene is widely used for cyanobacterial identification, our findings are consistent with earlier studies showing that it does not provide sufficient resolution to clearly define the monophyly or intraspecific diversity of *M. aeruginosa*. The high level of sequence conservation in this gene often results in poor separation among closely related *Microcystis* taxa, leading to ambiguous phylogenetic clustering and limited species-level discrimination. Consequently, analyses based solely on the 16S rRNA gene may underestimate genetic diversity and mask ecologically

meaningful differences among strains in natural populations. To address these limitations, future work should incorporate additional molecular markers such as cpcBA-IGS sequences with higher discriminatory power to improve taxonomic resolution. Accordingly, we treat the 16S rDNA phylogeny as complementary evidence and prioritize diagnostic morphological traits for species delimitation, thereby identifying the Pantabangan isolates and bloom species as *M. aeruginosa.*

Molecular analysis of the *mcyE gene* was conducted to provide independent verification of species identification. Although the *mcyE gene* is not exclusive to *Microcystis* and has been reported in other microcystin-producing cyanobacterial genera such as *Planktothrix* and *Anabaena* (e.g., Rantala *et al*., 2003; Vaitomaa *et al*., 2003), phylogenetic reconstruction demonstrated that our obtained sequences clustered within the *Microcystis* mcyE clade with maximum statistical support and were clearly distinct from other cyanobacterial lineages. Within the *Microcystis* clade, our isolates clustered predominantly with *M. aeruginosa* reference strains, although the phylogenetic tree also included strains identified as *M. viridis* and *M. flos-aquae* within the same well-supported clade. This phylogenetic pattern reflects the well-documented challenges in *Microcystis* taxonomy, where morphological species designations do not always correspond to distinct molecular lineages (*Otsuka et al*., 2001; Willame *et al*., 2006). The clustering of multiple morphospecies within a single molecular clade suggests either these represent ecotypes or morphological variants of a single evolutionary lineage, or the *mcyE gene* region lacks sufficient resolution to distinguish between closely related *Microcystis* species.

Detection of *mcyE* sequence

Our PCR based analyses targeting *mcyE* sequence demonstrated the presence of potential microcystin production of our cultured *M. aeruginosa* isolate and environmental samples from Pantabangan Lake. This represents, to the best our knowledge, the first report of applying molecular detection of *mcyE* to both cultured and field samples in this reservoir, filling a gap in Philippine studies where most efforts have focused on chemical-based detection of microcystin in other freshwater systems (Cuvin-Aralar *et al.,* 2002; Baldia *et al.,* 2003). While HPLC and LC-MS based analyses remain the most accurate and comprehensive method for toxin confirmation and quantification, and ELISA-based rapid kits provide cost-effective field screening (Szlag *et al.,* 2015; Kumar *et al.,* 2020), both approaches only measure toxins present at the time of sampling and may underestimate risk due to rapid degradation. In contrast, molecular assays detect the genetic potential for toxin production and provide an early warning of bloom toxicity (Duan *et al.,* 2022). We acknowledge that detection of the toxin biosynthesis gene does not confirm that microcystins are actively synthesized or released into the environment at harmful concentration. Nonetheless, combining molecular, chemical, and kit-based methods provides a more comprehensive framework for assessing bloom risks in freshwater systems.

The successful amplification of the *mcyE* gene across all environmental and cultured samples indicates the widespread presence of microcystin-producing cyanobacteria in Pantabangan Lake. The absence of amplification in negative controls confirms that the detected bands were specific to the target gene. The phylogenetic clustering of the Pantabangan isolate with known *M. aeruginosa* strains suggests that the dominant toxin-producing species in the lake is closely related to previously characterized toxic lineages.

Records and Distribution of *M. aeruginosa* in the Philippines

The documented occurrences of *M. aeruginosa* in the Philippines span six decades, beginning with reports in Laguna de Bay as early as 1964, where blooms were linked to water quality deterioration and fisheries risk (Notario, 1964). This was later followed by Cuvin-Aralar *et al*. (2002), who identified *M. aeruginosa* in Laguna de Bay. Baldia *et al*. (2003) also documented *M. aeruginosa* in Laguna de Bay, highlighting microcystin production during algal bloom

events, which emphasized the species' ecological and public health relevance in the lake. While most published research on *M. aeruginosa* in the Philippines has centered on Laguna de Bay and primarily emphasised toxin detection, morphological characterizations remain limited. More recently, employment of molecular techniques in accurately identifying cyanobacteria in the Philippines was reported. Tayaban *et al*. (2018) examined aquaculture ponds in Central Luzon using 16S rDNA sequencing confirmed the presence of *M. aeruginosa*. Another study applied high-throughput sequencing to Lake Buluan in Mindanao, where *M. aeruginosa* was detected alongside a diverse assemblage of phytoplankton, highlighting the utility of metabarcoding for bloom monitoring (Tabugo *et al.,* 2023). Our present study expands the report of occurrence of *M. aeruginosa* in Pantabangan Lake Nueva Ecija.

CONCLUSION

This study provides the first molecular evidence of microcystin-producing *Microcystis aeruginosa* in Pantabangan Lake, Nueva Ecija. Morphological characterisation confirmed the typical colony structure, mucilage embedding, and pigmentation of *M. aeruginosa*, while phylogenetic analysis based on 16S rRNA sequences placed the isolates within a well-supported *Microcystis* clade. The successful detection of the *mcyE* sequences in both cultured strains and environmental water samples highlights the potential for widespread toxin production within the lake. Given that Pantabangan Lake is a critical source of irrigation, hydroelectric power, and domestic water supply in Central Luzon, the presence of toxin-producing cyanobacteria presents ecological, economic, and public health concerns. These findings emphasise the importance of continuous monitoring of cyanobacterial blooms using both morphological and molecular tools to ensure early detection and effective risk assessment. Moreover, this pioneering work contributes valuable baseline data on cyanobacteria in Pantabangan Lake, supporting future efforts in bloom management, water quality protection, and the sustainable use of this vital freshwater resource.

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