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Aliinostoc bakau sp. nov. (Cyanobacteria, Nostocaceae), a New Microcystin Producer from Mangroves in Malaysia

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Abstract: A new microcystin-producing mangrove cyanobacterium, *Aliinostoc bakau* sp. nov., was isolated from a tropical mangrove in Penang, Malaysia, and characterized using combined morphological and phylogenetic approaches. Cultures were established in liquid media of different salinities (0, 7, 14, 21, 28, and 35 ppt). Optimal growth observed at both 7 and 14 ppt was consistent with the origin of the strain from an estuarine mangrove environment. Phylogenetic analysis based on the 16S rRNA gene strongly indicated that the strain is a member of the genus *Aliinostoc* and is distinct from other currently sequenced species in the genus. The sequences and secondary structure of the 16S–23S ITS region D1–D1′ and Box–B helices provided further confirmation that the new species is clearly distinct from previously described *Aliinostoc* species. Amplification of the *mcyE* gene fragment associated with the production of microcystin in *A. bakau* revealed that it is identical to that in other known microcystin-producing cyanobacteria. Analysis of the extracts obtained from this strain by HPLC-MS/MS confirmed the presence of microcystin variants (MC-LR and -YR) at concentrations of 0.60 µg/L and MC-RR at a concentration of 0.30 µg/L. This is the first record of microcystin production from *Aliinostoc* species in tropical mangrove habitats.

Keywords: cyanobacteria; cyanotoxin; *mcyE* gene; tropical; morphological; phylogenetic

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1. Introduction

Mangroves are coastal ecosystems that constitute a transitional forest between the sea and land. These ecosystems are extremely productive and provide niches for a diversity of microbes, plants, and animals, as well as breeding grounds for various benthic and pelagic species [1–3]. Cyanobacteria are key species adapted to the generally very changeable circumstances among the highly varied microbial communities observed in mangrove habitats [1,3]. They are also one of the main primary producers supporting marine fisheries that rely on mangrove ecosystems [4]. The organic material produced by these organisms at the base of the mangrove ecosystem cascades through multiple levels in the food web [5].

The majority of the mangroves in the world (50.5%) are found in Malaysia, along with Indonesia, Australia, Brazil, Mexico, and Nigeria [6]. Mangroves are a common feature in the coastal areas of Malaysia, reaching their greatest extent on the north-east coast of Sabah, where about 60% of the country's coverage occurs. In Sarawak, a further 23% is located in the deltas of the Sarawak, Rejang, and Trusan-Lawas rivers, and the

remaining 17% is found along the east and west coasts of peninsular Malaysia [7]. Most published studies of mangroves in Malaysia have focused on faunal diversity, primarily on fish diversity and larval abundance [8,9], mollusks ([10–12]), horseshoe crabs [13], mud crabs [14], and food web interactions [15]. Studies of micro-organisms have been conducted only on selected groups, for instance on the diversity and features of Actinobacteria [16,17], Proteobacteria [18], and Firmicutes [19]. The diversity of macroalgae [20–22] and diatoms [23] has also been studied.

Studies on the ecology and diversity of mangrove cyanobacteria have been conducted in several regions, including India [24–27], Brazil [28–30], Tanzania [31], and Saudi Arabia [32]; however, research on cyanobacterial diversity in Malaysia has only been conducted in freshwater ecosystem and aquaculture ponds, with identification only to genus level [33–37].

A number of cyanobacterial species and genera are widely known as producers of cyanotoxins that can pose health hazards [38–41]. Microcystins (MCs) are potent hepatotoxic cyanotoxins that have been widely studied globally [42,43]. To date, various cyanobacterial genera, including *Microcystis* Kützing ex Lemmermann, *Planktothrix* Anagnostidis and Komàrek, *Phormidium* Kützing ex Gomont, *Anabaena* Bory ex Bornet and Flahault, *Nostoc* Vaucher ex Bornet and Flahault, *Hapalosiphon* Nägeli ex É. Bornet and C. Flahault, and *Fischerella* Gomont, have been confirmed to be MC producers [44–46]. Most studies on MC production to date have focused on planktonic cyanobacteria rather than their benthic counterparts [46].

The genus *Aliinostoc* Bagchi, Dubey, & P. Singh was established with the type species *A. morphoplasticum* Bagchi, Dubey, & P. Singh [47], based on molecular assessment confirming that it was distinct from other *Nostoc*-like genera. Although morphological characteristics alone do not distinguish members of *Aliinostoc* from *Nostoc sensu stricto*, the loosely arranged filaments and motile hormogonia with gas vesicles are considered diacritical features of the genus [47,48]. The genus originally included six species, all originating from tropical regions. Three of these, *A. constrictum* Kabirnataj & al. [48], *A. soli* A. Saraf & al., and *A. tiwarii* A. Saraf, P. Singh, & al. [49] have since been moved to the new genus *Pseudoaliinostoc* N.-J. Lee, S.-D. Bang, T. Kim, J.-S. Ki, & O.-M. Lee based on molecular evaluation using 16S rRNA and the internal transcribed spacer (ITS) markers of *Pseudoaliinostoc sejongens* Lee, Bang, Kim, Ki, & Lee [50]. The genus currently includes five species, *A. morphoplasticum* [47], *A. magnakinetifex* Kabirnataj & al., *A. catenatum* Kabirnataj & al. [48], *A. alkaliphilum* Christodoulou, Economou-Amilli, M. Fiore, & Sivonen [51], and *A. vietnamicum* S. Maltseva, E. Kezlya, & Y. Maltsev [52]. All are characterized based on 16S rRNA gene sequences, as well as morphological and ecological features.

Several studies have reported toxin production by mangrove cyanobacteria. These include one record from the island of Guadeloupe [53], 18 records from two islands in Brazil [30], two records from the Red Sea coast of Saudi Arabia [54,55], and one record from the northern coast of the Persian Gulf, Iran [56]. All these records originated from subtropical mangroves. The present study aims to identify a novel cyanobacterial strain capable of producing MCs obtained from a tropical estuarine mangrove forest in Pulau Betong, Malaysia. Phylogenetic analysis shows that the new strain is included in a distinct clade with previously identified *Aliinostoc* strains. Based on morphological and molecular assessments, we establish the new species *Aliinostoc bakau* sp. nov., following the procedures of the International Code of Nomenclature for algae, fungi, and plants [57].

2. Materials and Methods

2.1. Site Description and Sampling

The microbial mat sample used in this study was obtained from a rotting tree trunk in the intertidal zone of an estuarine mangrove area in Kampung (Kg.) Pulau Betung, Penang (5°18′23.3″ N, 100°12′02.4″ E) (Figure 1), located close to a residential area and shrimp aquaculture ponds. Field samples were collected as part of a broad study of mangrove cyanobacteria, based on the visual presence of macroscopic mats, gelatinous colonies, and the crusts of cyanobacteria on both natural and artificial substrates. Each sample was stored

in a separate 60 mL sterile polycarbonate screw top container and immediately transported to the laboratory in Universiti Sains Malaysia, Penang, for further analysis.

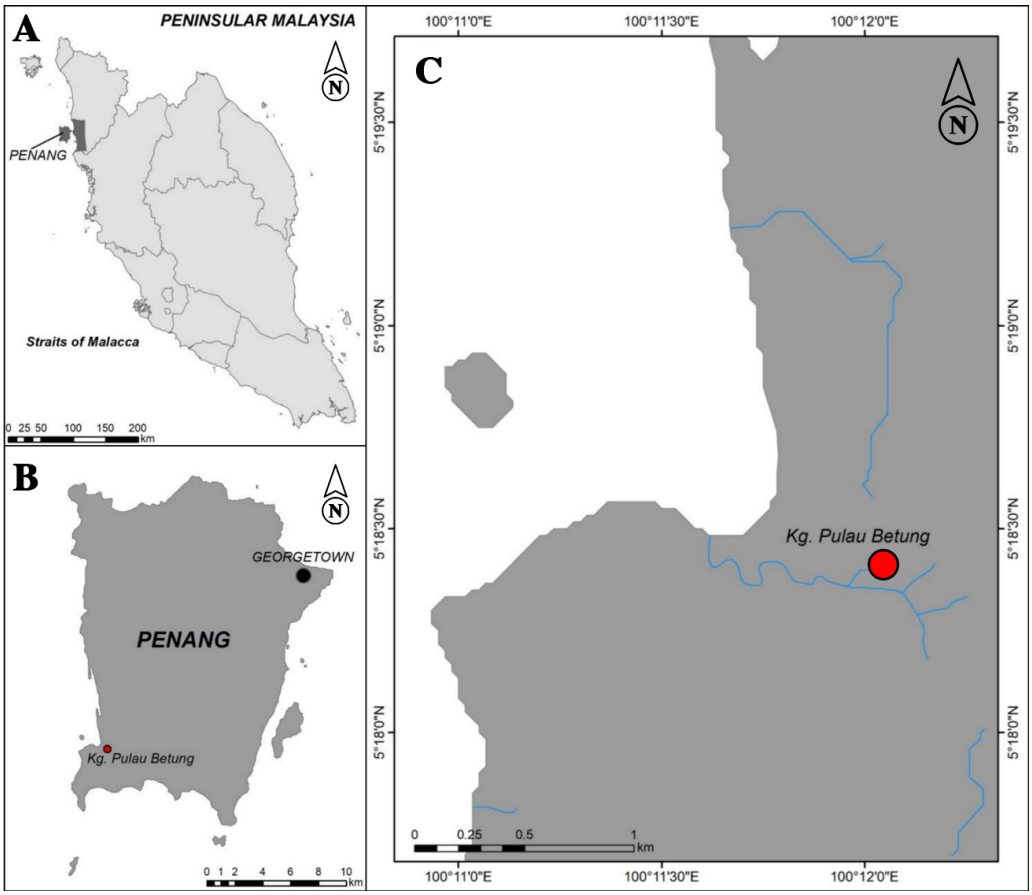

Figure 1. Location map of study site. (**A**) Peninsular Malaysia, and location of Penang (arrow). (**B**) Penang, and location of Kampung (Kg.) Pulau Betung (red circle). (**C**) Study site shown by red circle.

2.2. Culture Establishment

The mat, from which the strain reported here was obtained, was dominated by *Oscillatoriales*. A small sub-sample of the field material was cultured in both full-strength BG 11 and $BG11_0$ (lacking nitrogen with 1% agarised medium) media [58]. The culture media were supplemented with 0.1 µg/L vitamin B12, artificial seawater (Instant Ocean) that was adjusted to 6 ppt salinity, and 100 µg/mL cycloheximide to eliminate eukaryotes [59]. Isolation was carried out after a 2-week incubation by removing single colonies from the original enrichment culture and transferring them onto new culture media. All cultures were incubated at 25 °C and a 12:12 h light:dark cycle under a white, fluorescent lamp ($\pm$27.03 µmol photon m^{-2} s^{-1}) for 2 weeks before microscopic examination. The present strain was only observed growing in culture and was not detected in the initial observation of the field specimen. After isolation, the strain was grown in 50 mL $BG11_0$ liquid cultures in a range of salinity conditions (0, 7, 14, 21, 28, and 35 ppt), with all other conditions as described above. Good growth was observed at a salinity of 7 ppt, and the cultures were maintained at this salinity to achieve the biomass required for further analysis.

2.3. Morphological Characterisation

Morphological examination was carried out on the strain in culture using an Olympus BX-53 (Olympus America Inc., Center Valley, PA, USA) bright field microscope at 100–2000× magnification. Detailed descriptions of morphological characteristics were

recorded, including trichome color and shape, vegetative cell length and width, shape, and dimensions of heterocyst and akinetes, apical cell shape, and the presence or absence of a sheath. Measurements were made on 30 replicates of randomly chosen specimens. Illustrations were made with the aid of a *camera lucida*. Initial morphological identification followed [49–52,60,61].

2.4. Molecular Analyses

Colonies were aseptically transferred from liquid media into 1.5 mL microcentrifuge tubes containing 1 mL $BG11_0$ (lacking nitrogen) liquid culture medium. DNA was then extracted using the Intron G-spin™ Total DNA Extraction Mini Kit for bacteria (iNtRON Biotechnology, Inc., Seongnam, Kyonggi-do, Republic of Korea), following the manufacturer's instructions. The extracted DNA was stored frozen at −20 °C. The quality of the extracted DNA was determined using a Nanodrop Quawell UV Spectrophotometer Q3000 (Thermo Fisher Scientific, Loughborough, UK). The extracted DNA was used to amplify an approximately 1300 bp product of the 16S rRNA gene using the combination of primers 2 (5′–GGG GGA TTT TCC GCA ATG GG–3′) and 3 (5′–CGC TCT ACC AAC TGA GCT A–3′) [62]. The 16S–23S ITS region was amplified using primers 1 (5′– CTC TGT GTG CCT AGG TAT CC–3′) and 5 (5′–TGT AGC TCA GGT GGT TAG–3′) [62]. Thermal cycling conditions were 94 °C for 4 min for pre-denaturation, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 15 s, extension at 72 °C for 20 s, and a final extension at 72 °C for 7 min. The genes were amplified using the polymerase chain reaction (PCR) with 25 µL of MyTaq™ Red Mix (Bioline, Little Clacton, UK), 2 µL of each 20 µM forward and reverse primer, 2 µL of approximately 5–10 ng of genomic DNA template, and 19 µL of milli-Q, giving a final volume of 50 µL. An extra reaction tube with all the chemicals except for the DNA template was used as a negative control.

PCR products were loaded onto a 1% agarose electrophoresis gel stained with 1 µL ready-to-load RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc., Seongnam, Kyonggi-do, Republic of Korea) and inserted into a gel tank with 0.5 × TBE running buffer (iNtRON Biotechnology, Inc., Seongnam, Kyonggi-do, Republic of Korea). Amplified DNA was separated by electrophoresis and visualised with the power system supplied by Major Science MP-300V (100 V, 500 mA) for 30 min. A Bioneer 100 bp DNA ladder was also loaded to determine the size of the DNA extracted. The gel was viewed using Syngene GeneFlash Bio Imaging Gel Documentation UV/VVIS. The PCR product was then purified using the MEGAquick-spinTM Total Fragment DNA Purification Kit (iNtRON Biotechnology, Inc., Seongnam, Kyonggi-do, Republic of Korea). The purified PCR product was sequenced commercially by the Bioneer Corporation (Daejeon, Republic of Korea).

2.5. Screening for Cyanotoxin Genes

The extracted DNA was screened for five cyanotoxin genes using PCR: the *mcyE* gene coding for microcystin (MCY) production in microcystin producers was amplified using the primer pair *mcyE*-F2/R4 [63]; the *ndaF* gene in nodularin (NOD) producers was amplified using the primer pair HEP-F/R [64]; the *anaC* gene for anatoxin (ATX) production was amplified using primers anxgen-F/R [65]; the *cyrJ* gene-encoding cynlindrospermopsis (CYN) was amplified with *cyn*sulf-F and *cyn*lnam-R [66]; and the gene encoding for saxitoxin (SXT) was amplified with *sxt*AF/R [67]. Extra reaction tubes with these chemicals and DNA template strains, which were previously confirmed to be from microcystin (*Microcystis aeruginosa* FSS-164), nodularin (*Iningainema pulvinus* ES0614), saxitoxin (*Dolichospermum circinale* FSS-124), anatoxin (*Anabaena* UHC0054), and cylindrospermopsin (*Raphidiopsis raciborski* FSS-127) producers, were used as positive controls. A positive result in a specific PCR would indicate that the strain contained the respective gene and, if so, the PCR product was purified and sequenced as described above. Only the *mcyE* gene was identified by the BLAST (Basic Local Alignment Search Tool) and, hence, we tested the strain for microcystin production specifically. A 20 mL sample was sent to the Cawthron Institute, New Zealand, for quantifying microcystin concentration using HPLC-MS/MS analysis [68,69].

2.6. Phylogenetic Analysis and ITS Folding

All sequences were edited and assembled using the Geneious Prime software package (Biomatters, http://www.geneious.com (accessed on 25 December 2023)). Sequences that shared more than 97% sequence identity with our strains were considered as the same operational taxonomic unit (OTU) [70]. Multiple sequence alignments were built with the MUSCLE algorithm in Geneious Prime (Biomatters Ltd., Auckland, New Zealand) and then manually checked. Phylogenetic trees were constructed using two different methods: maximum likelihood (ML) and Bayesian inference (BI). ML analyses were performed using RaxML v8 [71] in Geneious using the general time-reversible invariant-sites (GTRI) nucleotide substitution model with 1000 bootstrap replicates. BI analyses were carried out using MrBayes v3.1.2 [72] as implemented in Geneious under the GTR + I + Γ model of sequence evolution. Two independent analyses, each consisting of four simultaneous Markov chains, were run for 3,000,000 generations, and trees were sampled every 100 generations. Log likelihood and parameter values were determined with the Tracer program v1.5 [73]. The first 25% of trees were discarded as burn-in, and the remaining trees were used to compute the Bayesian posterior probability values. ML and BI trees were edited in FigTree v1.3.1 [74]. Uncorrected pairwise genetic distances (*p*-distance) were calculated in MEGA X [75].

The 16S–23S ITS region was used for the modelling of secondary structure folding. The tRNA genes were identified using tRNAscan-SE 2 [76]. The secondary structure of the D1–D1′ and Box–B helices was modelled using the Mfold WebServer with default conditions.

3. Results

The new strain was isolated from an oscillatorialean-dominated mat and showed the diagnostic traits of the genus *Aliinostoc* Bagchi, Dubey, & P. Singh; however, it has morphological characteristics that are distinct from those of previously described species in the genus. Phylogenetic analysis based on the 16S rRNA gene also placed this strain within the clade of the genus *Aliinostoc* species (see below), but as phylogenetically distinct from other *Aliinostoc* species. On this basis, we propose establishing the new species of *Aliinostoc.*

Aliinostoc bakau sp. nov. Merican, Rahim, Broady, Convey, & Muangmai (Figure 2A–G).

Description. Field specimen a greenish, slimy mat from a rotting tree branch (Figure 2A). Colonies on solid medium (Figure 2B) firmly gelatinous, dark brown; in liquid medium (Figure 2C), softly gelatinous, light brown, and amorphous. Trichomes in both agar and liquid cultures dark brown, isopolar, usually densely (Figure 2D–E), occasionally loosely, convoluted. Cells barrel-shaped to cylindrical, longer than wide, 2.0–3.5 µm wide, 3.5–7.5 µm long; constricted at cross wall; cell content granulated (Figure 2D–G). Apical cell rounded. Heterocytes both terminal and intercalary, spherical to cylindrical, 3.8–5.0 µm × 5.0–6.4 µm (Figure 2D,G). Akinetes broadly ellipsoidal, larger than vegetative cells without thickened cell walls, 4.0–6.5 µm × 5.0–7.5 µm (Figure 2E,F). Individual sheath thin, hyaline (Figure 2E,G); communal sheath, thick and brownish. Hormogonia was not observed.

Holotype. Strain USMNA collected by Faradina M. and Nur Afiqah A. R. Strain deposited in the School of Biological Sciences Herbarium, Universiti Sains Malaysia, Malaysia, and the Faculty of Fisheries, Kasetsart University, Thailand.

Type locality. Mats from a rotting tree branch in an estuarine mangrove forest; Kampung Pulau Betung, Penang, Malaysia. (5°18′23.3″ N, 100°12′02.4″ E).

Etymology. *Aliinostoc bakau*, *Aliinostoc* (A.li.i.nos'toc.) L. adj and pronoun *alius* = other, different [47]; *bakau* (ba. ka'u) adj. *bakau* = derived from a Malay word for mangrove, referring to the origin of the strain from the mangrove environment.

GenBank accession number. 16S rRNA (MH182619)

Colonies with trichomes irregularly twisted and embedded within a common mucilage conform with the genus *Nostoc*. This genus is widespread with over 200 species recorded to date, making it difficult to distinguish morphospecies confidently using morphological evaluation [77,78]. Comparison with previously described *Nostoc* species showed a resemblance in the size of vegetative cells, heterocytes, and akinetes to *Nostoc passerinianum*

Bornet et Thuret ex Bornet et Flahault [78]; however, colony morphology, heterocyst and akinete shapes, and habitat differ from the present specimen.

The cell length of *Aliinostoc bakau* sp. nov. falls within the range of all previously recorded species of *Aliinostoc* (Table 1), but the cell width is the narrowest recorded to date. The absence of hormogonia appears to be exclusive to this species.

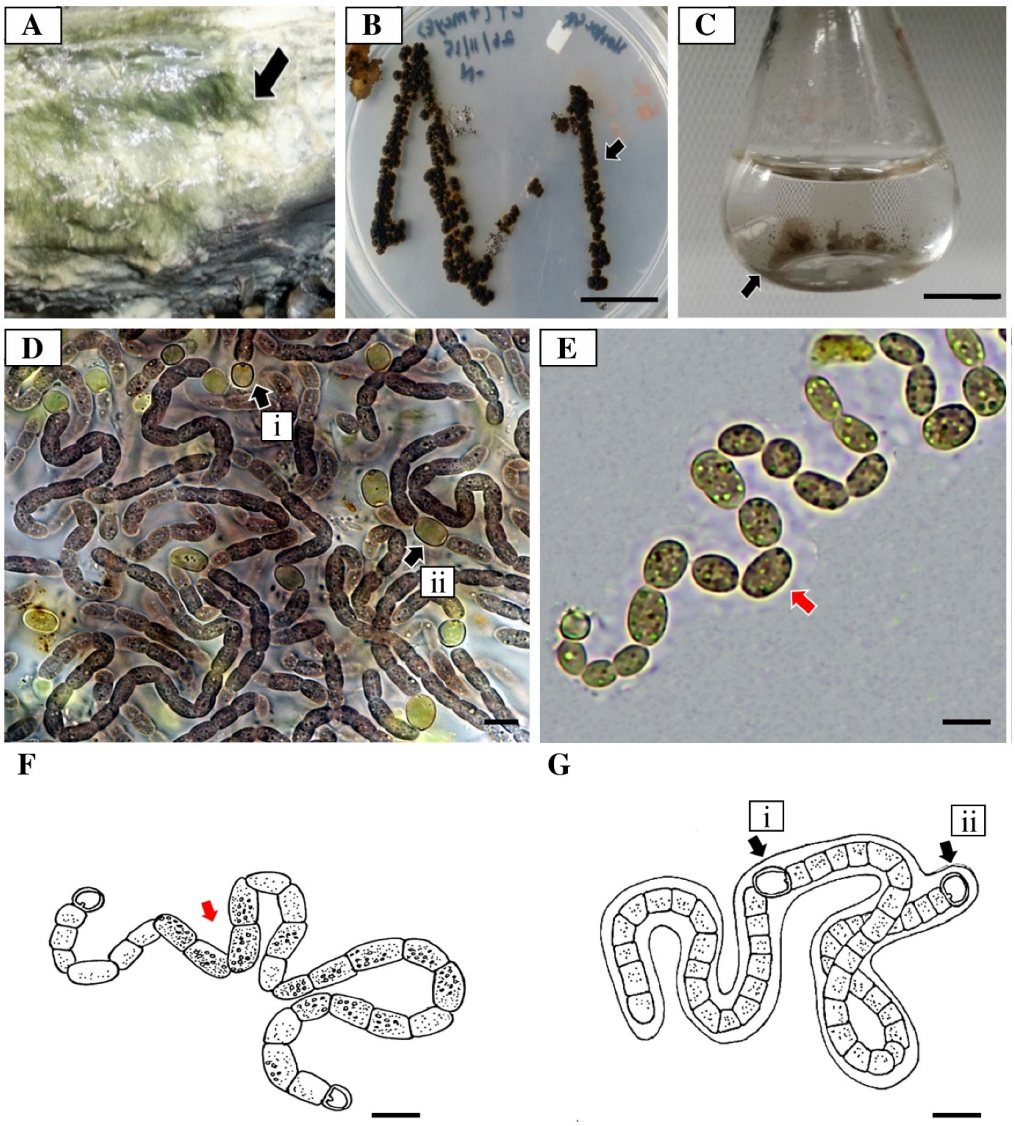

Figure 2. *Aliinostoc bakau* sp. nov. (**A**) Thin, slimy, olive-green mat on rotten tree branch (black arrow). (**B**) Firmly gelatinous, dark brown colonies on agarised medium (black arrow). (**C**) Brown, jelly-like, amorphous colony in liquid medium (black arrow). (**D**) Trichomes irregularly convoluted with barrel-shaped to cylindrical terminal heterocytes (arrow i) and intercalary heterocytes (black arrow ii) (**E**,**F**) old cells forming akinetes (red arrow). (**G**) Thin hyaline sheath surrounding the trichome, and intercalary (black arrow i) and terminal heterocyst (black arrow ii). Scale bars: 2 cm for (**B**–**C**), 10 µm for (**D**–**G**).

Table 1. Comparison of characteristics of *A. bakau* sp. nov. and five previously described species of *Aliinostoc* [47,48,51,52].

Characters	*Aliinostoc bakau* sp. nov	*Aliinostoc morphoplasticum* [47]	*Aliinostoc magnakinetifex* [48]	*Aliinostoc catenatum* [48]	*Aliinostoc alkaliphilum* [51]	*Aliinostoc vietnamicum* [52]
Macroscopic appearance in the environment	No visible colonies observed in the original collection	Spherical colonies forming yellowish-brown leathery mats	Discrete mucilaginous greenish-blue colonies	Thick mucilaginous greenish-blue mats	Does not form macroscopic mats in nature, planktic with dark brown cells	Irregular flake-like, loose mucilaginous-textured, gray or gray-blue colonies without periderm
Colony characteristics in cultures	Dark brownish-green colonies (on agar plates) and brown jelly-like amorphous colonies (in liquid medium)	No data available	No data available	No data available	Dark brown, irregular, without distinct periderm (on agar plates) and tiny clusters of filaments loosely attached to bottom (in liquid medium)	No data available
Cell length (μm)	3.8–7.5	2.6–5.2	1.4–4.3	3.2–9.5	(3.3) 3.5–6.6	4.0–5.0
Cell width (μm)	2.0–3.5	2.7–3.8	2.9–4.1	2.4–4.0	2.6–3.5	2.5–3.0 or 2.2–3.7 in diameter
Vegetative cell shape	Barrel-shaped to cylindrical, longer than wide	Barrel-shaped to spherical to oblong, isodiametric, barrel-shaped	Spherical to square and even cylindrical, isodiametric, longer than wide	Barrel-shaped to cylindrical, longer than wide	Cylindrical with rounded ends, oval or barrel-shaped	Barrel-shaped, spherical to cylindrical
Cross wall	Constricted at cross wall	Distinctly constricted at cross wall	Distinctly constricted at cross wall	Constricted at cross wall	Constricted at cross wall	Constricted at cross wall
Heterocyst shape	Spherical to cylindrical	Spherical to elliptical to ovate and oblong	Spherical to cylindrical	Spherical to cylindrical	Spherical to cylindrical to oblong or oval	Spherical to oval to cylindrical
Heterocyst length (μm)	5.0–6.4	3.7–5.7	2.5–5.5	3.0–10.2	3.9–7.0 (7.4)	6.2–8.0
Heterocyst width (μm)	3.8–5.0	3.2–4.0	2.5–5.1	3.2–5.5	(2.8) 3.1–5.0 (5.7)	4.7–6.0 or 3.0–5.7 in diameter
Sheath	Individual, thin hyaline	Individual, hyaline	Individual, hyaline	Individual, slightly coloured	Individual, thin, colourless	Individual, lightly coloured
Akinete	Present	Present	Present	Present	Present	Present
Akinete shape	Broadly ellipsoidal	Oblong	Spherical	Oval	Spherical to oval	Spherical to oval or oblong
Akinete length (μm)	5.0–7.5	5.7–6.1	6.3–10.1	3.4–11.3	No data available	6.4–8.0
Akinete width (μm)	4.0–6.5	4.5–4.7	5.2–8.4	3.3–6.4	up to 12	5.7–6.6
Hormogonia	Absent	Present with gas vesicles	Present	Present	Present with gas vesicles	Present with gas vesicles
Hormogonia motility	-	Motile	No data available	No data available	Motile	Motile
Occurrence	Mangrove on rotten tree branch	A stagnant, eutrophic-polluted pond	Garden soil	Garden soil	Alkaline brackish water	Tropical forest soil

3.1. Phylogenetic Analysis

The partial 16S rRNA gene sequences of 1316 bp were successfully amplified from *A. bakau* sp. nov. USMNA (MH182619) and trimmed to 1143 bp to provide fully bidirectional contiguous sequences. The trees constructed using ML and BI analyses were largely congruent and, therefore, only the ML tree is presented (Figure 3). Sequences of *Aliinostoc* species, including *A. bakau*, and four strains labelled as '*Nostoc*' sp. formed a well-supported clade (ML = 99%, BI = 1.00). Our new species was grouped with '*Nostoc*' sp. (MN864652) with high bootstrap support (ML = 100%, BI = 1.00) and sister to all other *Aliinostoc* species (Figure 3).

Genetic distances within the *Aliinostoc* clade were 0–4.4%, and *A. bakau* and other *Aliinostoc* species were at least 2.5% divergent. The sequences of *A. bakau* obtained here were identical to that of '*Nostoc*' sp. (MN864652) from a mangrove in Iran and, therefore, the latter strain should be re-examined with the consideration of re-naming it *A. bakau.*

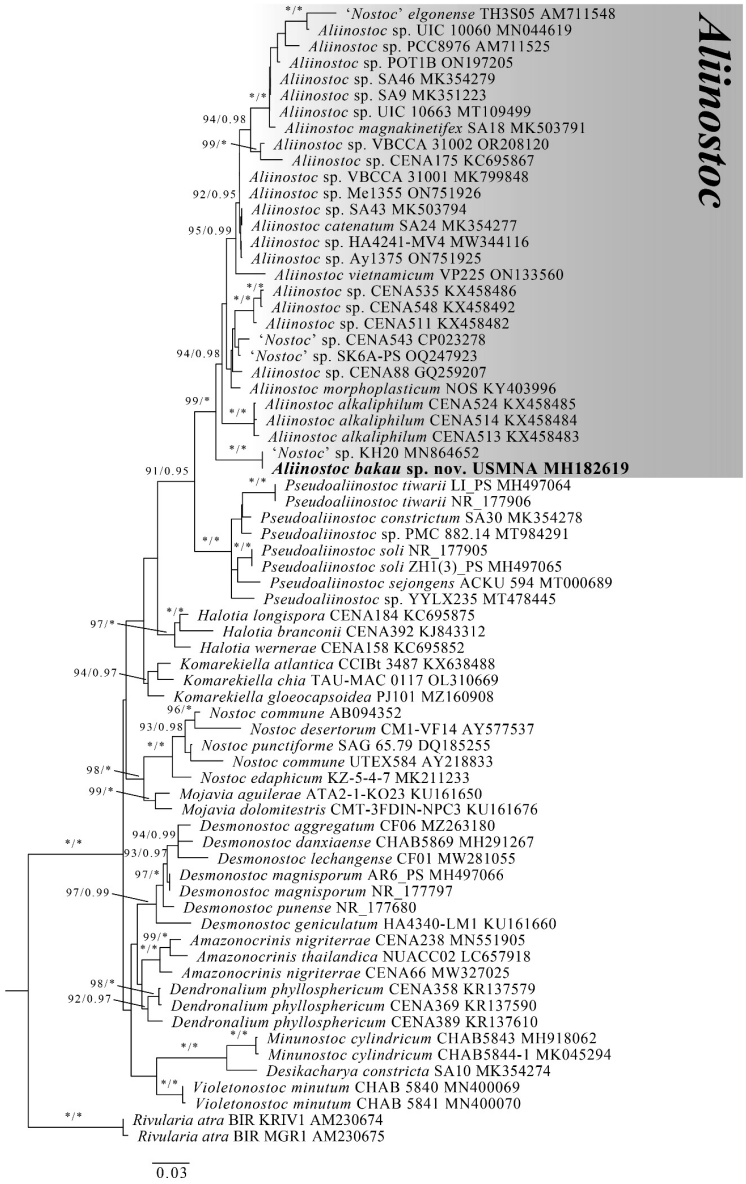

Figure 3. ML tree based on the partial 16S rRNA gene sequence showing the phylogenetic position of *Aliinostoc bakau* sp. nov. (MH182619). ML bootstrap values (left) and Bayesian posterior probabilities (right) are indicated at the nodes. Bootstrap values of >90% for ML and >0.90 for BI are presented, and full support is indicated by asterisk (*).

3.2. ITS Secondary Structure

The ITS region sequence of *A. bakau* was 641 bp long, containing the sequences coding for two tRNA molecules ($tRNA^{Ala}$ and $tRNA^{Ile}$). The D1–D1′ and Box–B helices, a semi-conserved region of the 16S–23S ITS region, were analyzed. The putative secondary structures of *A. bakau*, together with other *Aliinostoc* species, are presented in Figures 4 and 5.

The length of the D1–D1′ helix of *A. bakau* was 99 bp, whereas the length of other five *Allinostoc* species, including *A. catenatum* SA24 (MK503792), *A. magnakinetifec* SA18 (MK354276), *A. morphoplasticum* NOS (KY403996), *A. alkaliphilum* CENA513 (OK042917), and *A. vietnamicum* VP225 (ON133559), were relatively shorter, ranging from 54 to 93 bp. The folded D1–D1′ structure of *A. bakau* differs markedly from other *Aliinostoc* species, both in terms of the sequence and folding pattern. Particularly, the 4 bp long basal stem (GACC-GGUC) of *A. bakau* differs significantly from the common 6 bp long basal stem (GACCUA-UAGGUC) in other *Aliinostoc* species (Figure 4). Additionally, the D1–D1′ helix

structure of *A. bakau* was distinct from all other taxa for having the longest and a larger basal bulge with a small protrusion (Figure 4).

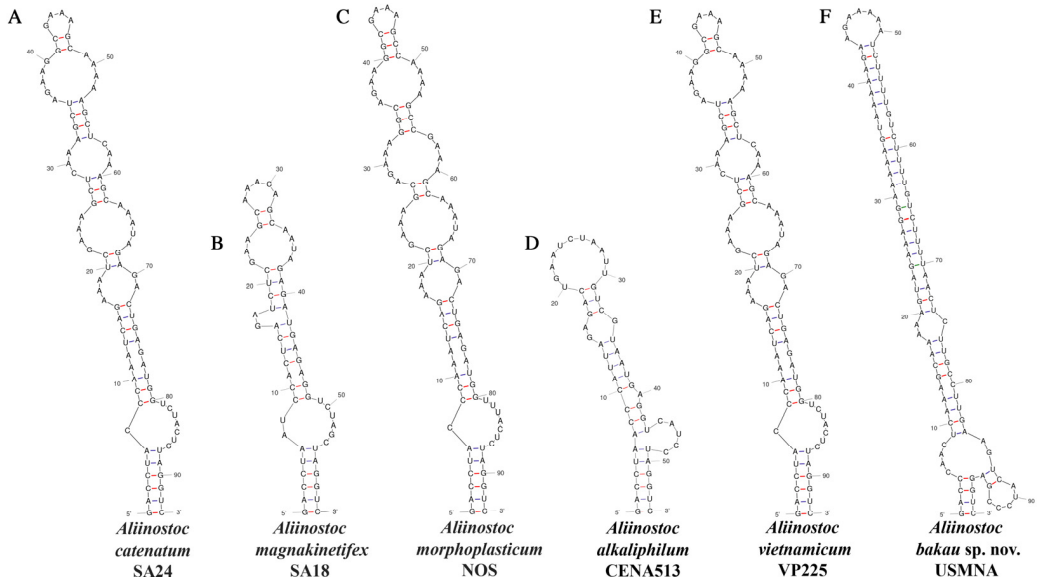

Figure 4. Secondary structure of D1–D1′ helix (16S–23S ITS) of *Aliinostoc* species, including (**A**) *A. catenatum* SA24 (MK503792), (**B**) *A. magnakinetifex* SA18 (MK503791), (**C**) *A. morphoplasticum* NOS (KY403996), (**D**) *A. alkaliphilum* CENA513 (OK042917), (**E**) *A. vietnamicum* VP225 (ON133559), and (**F**) *A. bakau* sp. nov. USMNA (PP061848).

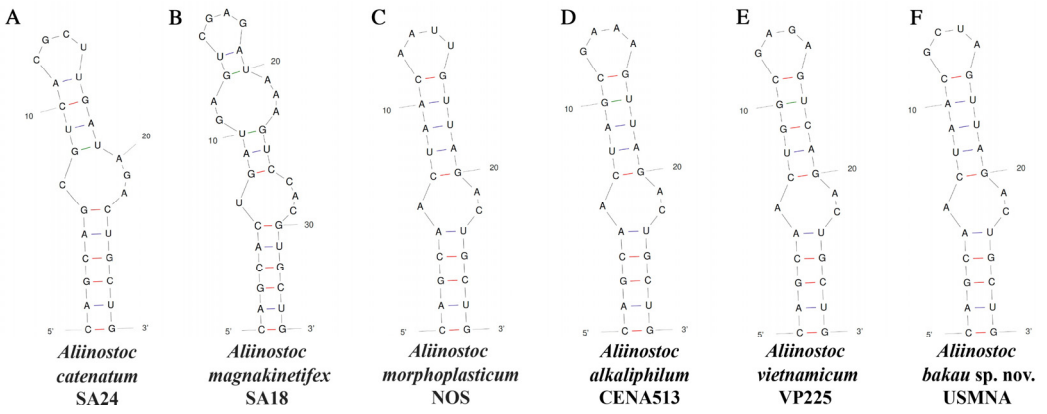

Figure 5. Secondary structure of Box–B helix (16S – 23S ITS) of *Aliinostoc* species, including (**A**) *A. catenatum* SA24 (MK503792), (**B**) *A. magnakinetifex* SA18 (MK503791), (**C**) *A. morphoplasticum* NOS (KY403996), (**D**) *A. alkaliphilum* CENA513 (OK042917), (**E**) *A. vietnamicum* VP225 (ON133559), and (**F**) *A. bakau* sp. nov. USMNA (PP061848).

For Box–B sequences, the length of *A. bakau* was 27 bp, which was comparable to that of *A. alkaliphilum*, *A. morphoplasticum*, and *A. vietnamicum*. The remaining species, *A. catenatum* and *A. magnakinetifex*, had a longer length, up to 35 bp. The Box–B structures of *A. bakau* were clearly distinct from *A. catenatum* and *A. magnakinetifex* by the length of the basal stem and sequences of internal and terminal loop (Figure 5). On the other hand, the pattern in the Box–B secondary structure of *A. bakau* was identical to *A. alkaliphilum*, *A. morphoplasticum*, and *A. vietnamicum* by having a 5 bp long basal stem (CAGCA–UGCUG), a 3-residue asymmetrical internal loop, and a 4-residue terminal loop (Figure 5). The differences among these four species were mainly based on the nucleotide sequence at position 10 (*A. bakau* vs. *A. alkaliphilum* and *A. vietnamicum*), and 18 (*A. bakau* vs. *A. vietnamicum*),

and terminal loop (AAUU for *A. morphoplaticum*, GAAA for *A. alkaliphilum*, GAGA for *A. vietnamicum*, and GCUA for *A. bakau*) (Figure 5).

The comparison of secondary structure provides additional information in confirming the identity of the new species in comparison with previous records.

3.3. Microcystin Production from A. bakau

A partial *mcyE* gene sequence (733 bp) associated with the microcystin biosynthetic pathway was successfully amplified from the strain. Phylogenetic analysis of this sequence with comparable sequences from other microcystin-producing cyanobacteria revealed that the sequence of *A. bakau* sp. Nov. USMNA (MT982365) was similar to those obtained from '*Nostoc*' sp., *Anabaena* sp., *N. linckia*, and *Anabaena lemmermannii*, and formed a clade with other *mcyE*-producing strains, with 0.99/100 bootstrap values for ML and BI analyses (Figure 6).

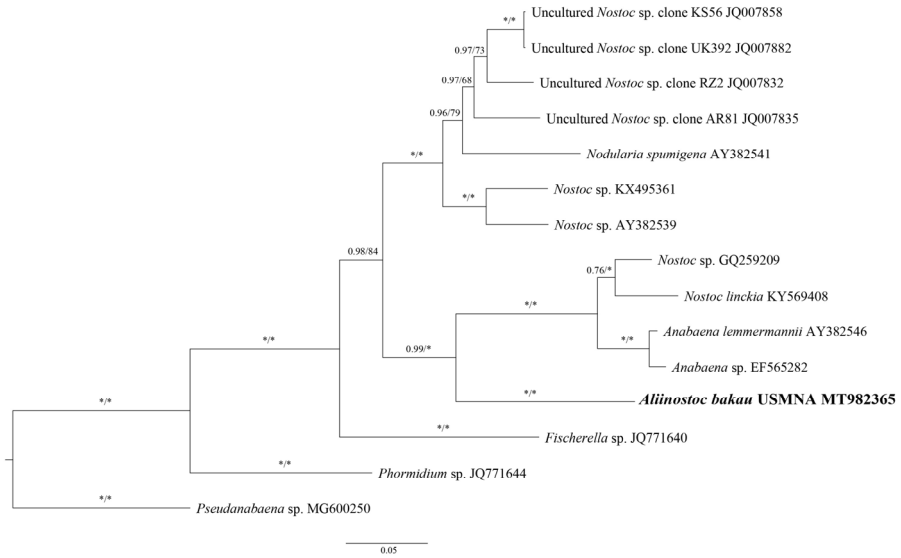

Figure 6. Phylogenetic tree based on the partial *mcyE* gene sequence showing the phylogenetic position of *Aliinostoc bakau* USMNA (MT982365). ML bootstrap values (left) and Bayesian posterior probabilities (right) are indicated at the nodes. Asterisk (*) indicates full support (100%, 1.0) in both analyses.

Analysis of the extract from strain USMNA by HPLC-MS/MS confirmed the presence of microcystin variants MC-LR and MC-YR at concentrations of 0.60 µg/L and MC-RR at a concentration of 0.30 µg/L.

4. Discussion

This study establishes a new microcystin-producing species of cyanobacteria, *Aliinostoc bakau* sp. nov., isolated from a tropical estuarine mangrove environment in Malaysia. The genus *Aliinostoc* is morphologically indistinguishable from *Nostoc* except for the loosely arranged filaments and formation of motile hormogonia with gas vesicles [47]. The new species differs from the other five currently recognised species of *Aliinostoc* in the absence of hormogonia (Table 1). Although the formation of hormogonia with gas vesicles has been proposed to be diagnostic for the genus [47], the current study suggests otherwise.

The capacity to produce motile hormogonia has been reported to be inconsistently present or entirely lacking in *Nostoc*-like strains [58,79]. Environmental conditions, including exposure to various light wavelengths, have been shown to either induce differentiation into hormogonia or suppress such development [80–82]. Furthermore, under specific conditions, hormogones may be retained within the colony, undergoing developmental processes without subsequent release, resulting in an increased number of trichomes within the colony [83]. While the formation of hormogonia has been previously described in both

A. magnakinetifex and *A. catenatum*, the motility of this structure has not been explicitly mentioned [48].

The genus was erected based on the type species, *A. morphoplasticum*, which originated from a stagnant, eutrophic, polluted pond. The specimen was collected amongst benthic rocks and other submerged substrates [47]. Gas vesicles are common in planktonic cyanobacteria and they provide buoyancy [84]; however, non-planktonic species have been reported to form them only under certain conditions [85]. In *A. alkaliphilum*, *A. morphoplasticum*, and *A. vietnamicum*, the formation of hormogonia with gas vesicles observed in culture suggests that these species have a planktonic phase in their life cycle. The gas vesicles may play an important role in facilitating hormogonia dispersal by increasing their time in suspension in the water column before sedimentation [86]. The hormogonia of *A. magnakinetifex* and *A. catenatum,* both collected from garden soil, lacked gas vesicles. The absence of hormogonia in *A. bakau*, and the absence of gas vesicles together with unknown motility in the hormogonia of *A. magnakinetifex* and *A. catenatum*, indicate that the possession of motile hormogonia with gas vesicles is not diagnostic for the genus. In contrast to akinetes, hormogonia represent a transient life stage lasting for only 1–2 days [87]. Hence, we propose the shape and dimensions of both heterocytes and akinetes as diagnostic morphological features for the identification of the genus.

Genetically, *A. bakau* is well separated from the previously described species of *Aliinostoc.* Based on available GenBank 16S rRNA sequences, the current strain is identical to '*Nostoc*' sp. (MN864652; deposited in December 2019), which was isolated from a mangrove environment in Iran. Although '*Nostoc*' sp. (MN864652) lacks morphological characterization to enable a reliable comparison with our strain, the genetic evidence presented here strongly supports the correct identity for that strain to be *A. bakau*, highlighting the need for caution in the use of species names assigned in such publicly accessible databases. Similarly, based on the analyses conducted here, we suggest that three further strains, '*Nostoc*' *elgonese* TH3S05 (AM 711548), '*Nostoc*' sp. CENA543 (CP023278), and '*Nostoc*' sp. SK6A-PS (OQ247923) should also be reclassified into the genus *Aliinostoc*. The names applied in GenBank for these previously deposited strains were assigned before the genus *Aliinostoc* was established [47].

The modelling of the secondary structure of the ITS region indicated that the structure of the D1–D1' and Box–B regions was distinct in both sequence and folding patterns from other *Aliinostoc* species. Previous studies have shown that the analysis of 16S gene phylogeny, coupled with the secondary structure of the ITS region, provides a better tool for the separation of cyanobacteria species [49–52,62,77,88,89].

The amplification of a fragment of the *mcyE* gene involved in MC biosynthesis from the *A. bakau* strain USMNA confirmed that the species has a genetic determinant vital for MC production, consistent with the result of HPLC-MS/MS analysis. This is the first member of the genus *Aliinostoc* shown to be capable of toxin production and only the fifth species of toxin-producing cyanobacteria identified from mangrove ecosystems worldwide. MC production has been detected in benthic mats in Red Sea mangrove swamps, with the presence of variants MC-YR, MC-LR, and MC-RR at higher concentrations of 4.89–9.74 μg/L [54] than recorded in the current study (0.0–0.60 μg/L). World Health Organization provisional guidelines [90] for microcystin consumption stipulate a maximum concentration of 1 μg/L.

The role of toxin-producing cyanobacteria in mangrove ecosystems is currently unknown. Previous studies have reported that known major producers of cyanotoxins are planktonic and able to form toxic blooms [91]; however, in mangrove habitats, blooms of benthic cyanobacteria are common [30,54–56]. Where they are present in algal mats, cyanotoxin producers may pose ecological risks to grazers if the toxins are produced at sufficiently high concentrations. Invertebrates that feed on toxic cyanobacteria can serve as vectors transferring cyanotoxins to higher trophic levels and even into the human food chain, for instance by the consumption of contaminated seafood [56]. Studies conducted in tropical and temperate lakes have reported MC accumulations ranging from 0.5 to 1917 and 4.5–215.2 μg/kg wet mass in muscle tissue and whole fish, respectively [92]. These

levels are generally higher than health guideline values (24 µg/kg of whole organism) for MC levels in seafood and, therefore, pose a significant risk for human consumption [93].

In previous investigations of sub-tropical mangroves, the prevalence of toxin-producing cyanobacteria was primarily attributed to non-heterocytous species [30,54–56]. A study from Brazil reported the highest number of microcystin (MC) producers, with 18 out of 55 identified species originating from two sampling localities [54]. The current study unveils a novel finding in the identification of a heterocytous cyanobacteria with toxin-producing abilities identified from a total of 33 species recorded from a tropical mangrove ecosystem [94]. *A. bakau*'s ability to differentiate specialized cells allows exceptional adaptation to environmental stressors, including osmotic stress, desiccation, increasing temperatures, limited nutrient availability, and prolonged darkness [95,96]. The ability to form heterocyst allows *A. bakau* to survive in a nitrogen deprived environment, while akinetes increase resilience to fluctuating environmental conditions [95]. The formation of specialised cells highlights the potential long-term impact of *A. bakau*'s survival mechanisms in a changing environment.

Escalating human population pressures in South-east Asia, including in Malaysia, have led to the extensive destruction of mangrove forest habitats, primarily attributable to anthropogenic activities including land reclamation, aquaculture expansion, oil palm expansion, the development of human settlements, and industrial expansion [97]. These human-induced alterations pose a substantial threat to the microbial communities inhabiting tropical mangrove environments. While the response of toxin-producing cyanobacteria to environmental stimuli remains unknown within mangrove ecosystems, an earlier study on phytoplankton diversity and community composition in a Malaysian mangrove revealed the dominance of a single species in a disturbed habitat [98]. Further research is needed to understand both the survival strategies of *A. bakau* and the physical stimuli that induce toxin production in order to fully assess potential future threats. An environmental monitoring programme should be established, particularly when animals or plants intended for human consumption are harvested from this habitat.

The general lack of microbiological studies, and specifically of cyanobacteria, in mangrove habitats, is exemplified by our identification of the first strain capable of MC production in Malaysia, a country with some of the most important remaining expanses of mangrove forest globally. Further studies across other mangroves in these regions are required to facilitate identification of other toxin-producing cyanobacteria and to clarify their functional roles in the mangrove environment.

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References

1. Alongi, D.M. A simple mass balance framework for estimating limits to sustainable mangrove production: Some examples from managed forests in Southeast Asia. Cyanobacteria in Extreme Environments. *Int. J. Eco. Environ. Sci.* **2005**, *11*, 209–224.
2. Sundararaman, M.; Boopathi, T.; Gopinath, S. Status of mangrove ecosystem: Exploring the potential role of cyanobacteria in restoration and afforestation. *Algae Int. J. Ecol. Environ. Sci.* **2007**, *31*, 147–155.
3. Rigonato, J.; Kent, A.D.; Alvarenga, D.O.; Andreote, F.D.; Beirigo, R.M.; Vidal-Torrado, P.; Fiore, M.F. Drivers of cyanobacterial diversity and community composition in mangrove soils in south-east Brazil. *Environ. Microbiol.* **2013**, *15*, 1103–1114. [CrossRef]
4. Essien, J.P.; Antai, S.P.; Benson, N.U. Microalgae biodiversity and biomass status in Qua Iboe Estuary mangrove swamp, Nigeria. *Aquat. Ecol.* **2008**, *42*, 71–81. [CrossRef]
5. Dadheech, P.K.; Glöckner, G.; Casper, P.; Kotut, K.; Mazzoni, C.J.; Mbedi, S.; Krienitz, L. Cyanobacterial diversity in the hot spring, pelagic and benthic habitats of a tropical soda lake. *FEMS Microbiol. Ecol.* **2013**, *85*, 389–401. [CrossRef]
6. Alvarenga, D.O.; Rigonato, J.; Branco, L.H.Z.; Fiore, M.F. Cyanobacteria in mangrove ecosystems. *Biodivers. Conserv.* **2015**, *24*, 799–817. [CrossRef]
7. Bianchi, M.C.G.; Chopin, F.; Farme, T.; Franz, N.; Fuentevilla, C.; Garibaldi, L.; Laurenti, A.L.G. *The State of World Fisheries and Aquaculture*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2014.
8. Abu Hena, M.K.; Idris, M.H.; Rajaee, A.H.; Siddique, M.A.M. Length-weight relationships of three fish species from a tropical mangrove estuary of Sarawak, Malaysia. *J. Appl. Ichthyol.* **2017**, *33*, 858–860. [CrossRef]
9. Azmir, I.A.; Esa, Y.; Amin, S.M.N.; Md Yasin, I.S.; Md Yusof, F.Z. Identification of larval fish in mangrove areas of Peninsular Malaysia using morphology and DNA barcoding methods. *J. Appl. Ichthyol.* **2017**, *33*, 998–1006. [CrossRef]
10. Singh, H.R.; Baharin, N.K. Gastropod community structure from varying levels of mangrove disturbance in Selangor, Malaysia. *Malays. For.* **2016**, *79*, 54–63.
11. Ismail, M.H.; Chong, V.C.; Ramli, R.; Sasekumar, A. Rediscovery of *Ellobium scheepmakeri* (Petit De La Saussaye, 1850) (Mollusca: Gastropoda: Ellobiidae), a rare ellobiid in Bukit Belimbing mangrove forest, Peninsular Malaysia. *Molluscan Res.* **2017**, *37*, 222–226. [CrossRef]
12. Vaezzadeh, V.; Zakaria, M.P.; Bong, C.W. Aliphatic hydrocarbons and triterpene biomarkers in mangrove oyster (*Crassostrea belcheri*) from the west coast of Peninsular Malaysia. *Mar. Pollut. Bull.* **2017**, *124*, 33–42. [CrossRef]
13. Noor Jawahir, A.R.; Samsur, M.; Shabdin, M.L.; Khairul Adha, A.R. Distribution of two species of Asian horseshoe crabs at west coast of Sarawak's Waters, East Malaysia. Egypt. *J. Aquat. Res.* **2017**, *43*, 135–140. [CrossRef]
14. Sharif, N.; Kahar, N.; Rodrigues, K.; Ransangan, J.; Kian, A. 2016. Genetic diversity of mud crabs, *Scylla tranquebarica* in Sabah, Malaysia based on cytochrome C oxidase (COI) gene sequence. *Songklanakarin J. Sci. Technol.* **2016**, *38*, 365–372.
15. Le, Q.D.; Haron, N.A.; Tanaka, K.; Ishida, A.; Sano, Y.; Dung, L.V.; Shirai, K. Quantitative contribution of primary food sources for a mangrove food web in Setiu lagoon from East coast of Peninsular Malaysia, stable isotopic (δ13C and δ15N) approach. *Reg. Stud. Mar. Sci.* **2017**, *9*, 174–179. [CrossRef]
16. Azman, A.S.; Zainal, N.; Mutalib, N.S.A.; Yin, W.F.; Chan, K.G.; Lee, L.H. *Monashia flava* gen. nov., sp. nov. An actinobacterium of the family Intrasporangiaceae. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 554–561. [CrossRef]
17. Law, J.W.F.; Ser, H.L.; Duangjai, A.; Saokaew, S.; Bukhari, S.I.; Khan, T.M.; Ab Mutalib, N.S.; Chan, K.G.; Goh, B.H.; Lee, L.H. *Streptomyces colonosanans* sp. nov., a novel actinobacterium isolated from Malaysia mangrove soil exhibiting antioxidative activity and cytotoxic potential against human colon cancer cell lines. *Front. Microbiol.* **2017**, *8*, 877. [CrossRef]
18. Moh, T.H.; Lau, N.S.; Furusawa, G.; Amirul, A.A.A. Complete genome sequence of *Microbulbifer* sp. CCB-MM1, a halophile isolated from Matang Mangrove Forest, Malaysia. *Stand. Genom. Sci.* **2017**, *12*, 36. [CrossRef]
19. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. *Environ. Pollut.* **2017**, *231*, 1552–1559. [CrossRef]
20. Aikanathan, S.; Sasekumar, A. The community structure of macroalgae in a low shore mangrove forest in Selangor, Malaysia. *Hydrobiologia* **1994**, *285*, 131–137. [CrossRef]
21. Billah, M.M.; Kamal, A.H.M.; Idris, M.H.B.; Ismail, J.B. Seasonal variation in the occurrence and abundance of mangrove macroalgae in a Malaysian Estuary. *Cryptogam. Algol.* **2016**, *37*, 109–120. [CrossRef]
22. Md Isa, H.; Mustafa Kamal, A.H.; Idris, M.H.; Rosli, Z.; Ismail, J. Biomass and habitat characteristics of epiphytic macroalgae in the Sibuti Mangroves, Sarawak, Malaysia. *Trop. Life Sci. Res.* **2017**, *28*, 1–21. [CrossRef]
23. Tan, S.N.; Teng, S.T.; Lim, H.C.; Kotaki, Y.; Bates, S.S.; Leaw, C.P.; Lim, P.T. Diatom *Nitzschia navis-varingica* (Bacillariophyceae) and its domoic acid production from the mangrove environments of Malaysia. *Harmful Algae* **2016**, *60*, 139–149. [CrossRef]
24. Mani, P. Natural phytoplankton communities in Pichavaram mangroves. *Ind. J. Mar. Sci.* **1992**, *21*, 278–280.
25. Nagasathya, A.; Thajuddin, N. Cyanobacterial diversity in the hypersaline environment of the saltpans of south eastern coast of India. *Asian J. Plant Sci.* **2008**, *7*, 473–478. [CrossRef]
26. Silambarasan, G.; Ramanathan, T.; Kathiresan, K. Diversity of marine cyanobacteria from three mangrove environment in Tamil Nadu Coast, south east coast of India. *Curr. Res. J. Biol. Sci.* **2012**, *4*, 235–238.
27. Sakthivel, K.; Kathiresan, K. Cyanobacterial diversity from mangrove sediment of south east coast of India. *Asian J. Biodivers.* **2013**, *4*, 190–203. [CrossRef]
28. Nogueira, N.M.C.; Ferreira-Correia, M.M. Cyanophyceae/Cyanobacteria in red mangrove forest at mosquitos and coqueiros estuaries, São Luís, state of Maranhão, Brazil. *Braz. J. Biol.* **2001**, *61*, 347–356. [CrossRef]

29. Rigonato, J.; Alvarenga, D.O.; Andreote, F.D.; Dias, A.C.F.; Melo, I.S.; Kent, A.; Fiore, M.F. Cyanobacterial diversity in the phyllosphere of a mangrove forest. *FEMS Microbiol. Ecol.* **2012**, *80*, 312–322. [CrossRef]
30. Silva, C.S.P.; Genuario, D.B.; Vaz, M.G.M.V.; Fiore, M.F. Phylogeny of culturable cyanobacteria from Brazilian mangroves. *Syst. Appl. Microbiol.* **2014**, *37*, 100–112. [CrossRef]
31. Kyaruzi, J.J.; Kyewalyanga, M.S.; Muruke, M.H.S. Cyanobacteria composition and impact of seasonality on their in-situ nitrogen fixation rate in a mangrove ecosystem adjacent to Zanzibar town. *West Indian Ocean. J. Mar. Sci.* **2003**, *2*, 35–44. [CrossRef]
32. Hussain, M.I.; Khoja, T.M. Intertidal and subtidal blue-green algal mats of open and mangrove areas in the Farasan Archipelago (Saudi Arabia), Red Sea. *Bot. Mar.* **1993**, *36*, 377–388. [CrossRef]
33. Harith, M.N.; Hassan, R. Preliminary study on cyanobacteria composition and selected water quality parameters from freshwater fish (Tor tambroides) ponds in Serian, Sarawak. In Proceedings of the Natural Resources and Environmental Management and Environmental Safety and Health (NREM & ESH), Kuching, Sarawak, Malaysia, 27–29 November 2007.
34. Harith, M.N.; Hassan, R. Diversity and similarity among cyanobacteria assemblages from selected aquatic ecosystems in Sarawak using β—indices. *Borneo J. Res Sci. Technol.* **2011**, *1*, 28–37. [CrossRef]
35. Sinang, S.C.; Poh, K.B.; Shamsudin, S.; Sinden, A. Preliminary assessment of cyanobacteria diversity and toxic potential in ten freshwater lakes in Selangor, Malaysia. *Bull. Environ. Contam. Toxicol.* **2015**, *95*, 542–547. [CrossRef]
36. Sinden, A.; Sinang, S.C. Presence and abundance of cyanobacteria in selected aquaculture ponds in Perak, Malaysia and the relationships with selected physicochemical parameters of water. *J. Teknol.* **2015**, *76*, 187–194. [CrossRef]
37. Azizan, A.A.; Radzi, R.; Omar, W.M.W.; Convey, P.; Merican, F.M.M.S. First records of morphological diversity and ecology of periphytic cyanobacteria from Tukun River, Penang Forest Reserve, Malaysia. *Trop. Life Sci.* **2020**, *31*, 85–105. [CrossRef]
38. Codd, G.A.; Morrison, L.F.; Metcalf, J.S. Cyanobacterial toxins: Risk management for health protection. *Toxicol. Appl. Pharmacol.* **2005**, *203*, 264–272. [CrossRef]
39. Falconer, I.R.; Humpage, A.R. Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. *Int. J. Environ. Res. Public Health* **2005**, *2*, 43–50. [CrossRef]
40. Steffensen, D.; Burch, M.; Nicholson, B.; Drikas, M.; Baker, P. Management of toxic blue-green algae (cyanobacteria) in Australia. *Environ. Toxicol.* **1999**, *14*, 183–195. [CrossRef]
41. Stewart, I.; Seawright, A.A.; Shaw, G.R. Cyanobacterial poisoning in livestock, wild mammals, and birds—An overview. In *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*; Hudnell, H.K., Ed.; Springer: New York, NY, USA, 2008; pp. 613–637.
42. Quiblier, C.; Wood, S.A.; Echenique-Subiabre, I.; Heath, M.; Villeneuve, A. A review of current knowledge on toxic benthic freshwater cyanobacteria–ecology, toxin production and risk management. *Water Res.* **2013**, *47*, 5464–5479.
43. Ikehara, T.; Kuniyoshi, K.; Yamaguchi, H.; Tanabe, Y.; Sano, T.; Yoshimoto, M.; Oshiro, N.; Nakashima, S.; Yasumoto-Hirose, M. First report of *Microcystis* strains producing MC-FR and-WR Toxins in Japan. *Toxins* **2019**, *11*, 521. [CrossRef]
44. Sivonen, K.; Börner, T. Bioactive compounds produced by cyanobacteria. In *The Cyanobacteria: Molecular Biology, Genomic and Evaluation*; Herrero, A., Flores, E., Eds.; Caister Academic Press: Hethersett, UK, 2008; pp. 159–197.
45. Fiore, M.F.; Genuário, D.B.; da Silva, C.S.P.; Shishido, T.K.; Moraes, L.A.B.; Neto, R.C.; Silva-Stenico, M.E. Microcystin production by a freshwater spring cyanobacterium of the genus *Fischerella*. *Toxicon* **2009**, *53*, 754–761. [CrossRef]
46. Catherine, A.; Bernard, C.; Spoof, L.; Bruno, M. Microcystins and nodularins. In *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; Meriluoto, J., Spoof, L., Codd, G.A., Eds.; John Wiley & Sons: Chichester, UK, 2017; pp. 109–126.
47. Bagchi, S.N.; Dubey, N.; Singh, P. Phylogenetically distant clade of *Nostoc*-like taxa with the description of *Aliinostoc* gen. nov. and *Aliinostoc morphoplasticum* sp. nov. *Int. J. Syst. Evol. Microb.* **2017**, *67*, 3329–3338. [CrossRef] [PubMed]
48. Kabirnataj, S.; Nematzadeh, G.A.; Talebi, A.F.; Saraf, A.; Suradkar, A.; Tabatabaei, M.; Singh, P. Description of novel species of *Aliinostoc*, Desikacharya and *Desmonostoc* using a polyphasic approach. *Int. J. Syst. Evol. Microb.* **2020**, *70*, 3413–3426. [CrossRef] [PubMed]
49. Saraf, A.; Dawda, H.G.; Suradkar, A.; Behere, I.; Kotulkar, M.; Shaikh, Z.M.; Kumat, A.; Batule, P.; Mishra, D.; Singh, P. Description of two new species of *Aliinostoc* and one new species of *Desmonostoc* from India based on the polyphasic approach and reclassification of *Nostoc punensis* to *Desmonostoc punense* comb. nov. *FEMS Microbiol. Lett.* **2018**, *365*, fny272. [CrossRef]
50. Lee, N.J.; Bang, S.D.; Kim, T.; Ki, J.S.; Lee, O.M. *Pseudoaliinostoc sejongens* gen. & sp. nov. (Nostocales, Cyanobacteria) from floodplain soil of the Geum River in Korea based on polyphasic approach. *Phytotaxa* **2021**, *479*, 55–70.
51. Christodoulou, M.; Jokela, J.; Wahlsten, M.; Saari, L.; Economou-Amilli, A.; Fiori, M.d.L.; Sivonen, K. Description of *Aliinostoc alkaliphilum* sp. nov. (Nostocales, Cyanobacteria), a new bioactive metabolite-producing strain from Salina Verde (Pantanal, Brazil) and taxonomic distribution of bioactive metabolites in Nostoc and Nostoc-like genera. *Water* **2022**, *14*, 2470.
52. Maltseva, S.; Kezlya, E.; Krivova, Z.; Gusev, E.; Kulikovskiy, M.; Maltsev, Y. Phylogeny and fatty acid profiles of *Aliinostoc vietnamicum* sp. nov. (Cyanobacteria) from the soils of Vietnam. *J. Phycol.* **2022**, *58*, 789–803. [CrossRef]
53. Guidi-Rontani, C.; Jean, M.R.; Gonzalez-Rizzo, S.; Bolte-Kluge, S.; Gros, O. Description of new filamentous toxic Cyanobacteria (Oscillatoriales) colonizing the sulfidic periphyton mat in marine mangroves. *FEMS Microbiol. Lett.* **2014**, *359*, 173–181. [CrossRef]
54. Mohamed, Z.A.; Al-Shehri, A.M. Biodiversity and toxin production of cyanobacteria in mangrove swamps in the Red Sea off the southern coast of Saudi Arabia. *Bot. Mar.* **2015**, *58*, 23–34. [CrossRef]
55. Mohamed, Z.A.; Hashem, M.; Alamri, S.; Mostafa, Y. Cyanotoxins and their environmental health risk in marine and freshwaters of Saudi Arabia. *Arab. J. Geosci.* **2020**, *13*, 285. [CrossRef]

56. Zaheri, A.; Bahador, N.; Yousefzadi, M.; Arman, M. Molecular identification and toxicity effects of cyanobacteria species isolated from the Khoor-e-Khooran mangrove forest, Persian Gulf. *Iran. J. Fish. Sci.* **2021**, *20*, 572–589.
57. Turland, N.J.; Wiersema, J.H.; Barrie, F.R.; Greuter, W.; Hawksworth, D.L.; Herendeen, P.S.; Knapp, S.; Kusber, W.-H.; Li, D.-Z.; Marhold, K.; et al. (Eds.) *International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code): Adopted by the Nineteenth International Botanical Congress, Shenzhen, China, July 2017*; Koeltz Botanical Books: Glashütten, Germany, 2018.
58. Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M.; Stanier, R.Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **1979**, *111*, 1–61. [CrossRef]
59. Bolch, C.J.; Blackburn, S.I. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kütz. *J. Appl. Phycol.* **1996**, *8*, 5–13. [CrossRef]
60. Guiry, M.D.; Guiry, G.M. AlgaeBase. Available online: https://www.algaebase.org (accessed on 5 November 2023).
61. Komárek, J. Modern classification of cyanobacteria. In *Cyanobacteria: An Economic Perspective*; Sharma, N.K., Rai, A.K., Stal, L.J., Eds.; Wiley: Chichester, UK, 2014; pp. 21–39.
62. Boyer, S.L.; Flechtner, V.R.; Johansen, J.R. Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Mol. Biol. Evol.* **2001**, *18*, 1057–1069. [CrossRef]
63. Rantala, A.; Rajaniemi-Wacklin, P.; Lyra, C.; Lepistö, L.; Rintala, J.; Mankiewicz-Boczek, J.; Sivonen, K. Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (*mcyE*) PCR and associations with environmental factors. *Appl. Environ. Microbiol.* **2006**, *72*, 6101–6110. [CrossRef]
64. Jungblut, A.D.; Neilan, B.A. Molecular identification and evolution of the cyclic peptide hepatotoxins, microcystin and nodularin, synthetase genes in three orders of cyanobacteria. *Arch. Microbiol.* **2006**, *185*, 107–114. [CrossRef]
65. Rantala-Ylinen, A.; Känä, S.; Wang, H.; Rouhiainen, L.; Wahlsten, M.; Rizzi, E.; Berg, K.; Gugger, M.; Sivonen, K. Anatoxin-a synthetase gene cluster of the cyanobacterium *Anabaena* sp. strain 37 and molecular methods to detect potential producers. *Appl. Environ. Microbiol.* **2011**, *77*, 7271–7278. [CrossRef]
66. Mihali, T.K.; Kellmann, R.; Muenchhoff, J.; Barrow, K.D.; Neilan, B.A. Characterization of the gene cluster responsible for cylindrospermopsin biosynthesis. *Appl. Environ. Microbiol.* **2008**, *74*, 716–722. [CrossRef]
67. Al-Tebrineh, J.; Gehringer, M.M.; Akcaalan, R.; Neilan, B.A. A new quantitative PCR assay for the detection of hepatotoxigenic cyanobacteria. *Toxicon* **2011**, *57*, 546–554. [CrossRef]
68. Lawrence, J.F.; Niedzwiadek, B.; Menard, C.; Lau, B.P.; Lewis, D.; Kuper-Goodman, T.; Carbone, S.; Holmes, C. Comparison of liquid chromatography/mass spectrometry, ELISA, and phosphatase assay for the determination of microcystins in blue-green algae products. *J. AOAC Int.* **2001**, *84*, 1035–1044. [CrossRef]
69. Spoof, L.; Vesterkvist, P.; Lindholm, T.; Meriluoto, J. Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed- phase liquid chromatography–electrospray ionisation mass spectrometry. *J. Chromatogr. A* **2003**, *1020*, 105–119. [CrossRef] [PubMed]
70. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **2011**, *28*, 2731–2739. [CrossRef] [PubMed]
71. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef]
72. Ronquist, F.; Huelsenbeck, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **2003**, *19*, 1572–1574. [CrossRef] [PubMed]
73. Rambaut, A.; Drummond, A. *Tracer MCMC Trace Analysis Tool*; Univeristy of Oxford: Oxford, UK, 2003. Available online: https://tree.bio.ed.ac.uk/software/tracer/ (accessed on 2 October 2023).
74. Rambaut, A. FigTree v1.3.1.; Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, 2010. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 2 October 2023).
75. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
76. Lowe, T.M.; Chan, P.P. tRNAscan-SE On-line: Integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res.* **2016**, *44*, W54–W57. [CrossRef] [PubMed]
77. Řeháková, K.; Johansen, J.R.; Casamatta, D.A.; Xuesong, L.; Vincent, J. Morphological and molecular characterization of selected desert soil cyanobacteria: Three species new to science including *Mojavia pulchra* gen. et sp. nov. *Phycologia* **2007**, *46*, 481–502. [CrossRef]
78. Komárek, J. *Cyanoprokaryota 3. Teil/Part 3: Heterocytous Genera*; Spektrum Akademischer Verlag: Berlin, Germany, 2013.
79. Rajaniemi, P.; Hrouzek, P.; Kaštovska, K.; Willame, R.; Rantala, A.; Hoffmann, L.; Komárek, J.; Sivonen, K. Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 11–26. [CrossRef]
80. Zhong, Z.P.; Wu, Y.H.; Xu, J.; Feng, L.J.; Shi, D.J. Differentiation of hormogonia and photosynthetic characterization of *Nostoc flagelliforme*. *Acta Bot. Sin.* **2000**, *42*, 570–575.
81. Damerval, T.; Guglielmi, G.; Houmard, J.; de Marsac, N.T. Hormogonium differentiation in the cyanobacterium *Calothrix*: A photoregulated developmental process. *Plant Cell* **1991**, *3*, 191–201. [CrossRef]

82. Campbell, D.; Houmard, J.; de Marsac, N.T. Electron transport regulates cellular differentiation in the filamentous cyanobacterium *Calothrix*. *Plant Cell* **1993**, *5*, 451–463. [CrossRef] [PubMed]
83. Nicoletti, M. The nutraceutical potential of cyanobacteria. In *The Pharmacological Potential of Cyanobacteria*; Lopes, G., Silva, M., Vasconcelos, V., Eds.; Academic Press: Cambridge, MA, USA, 2022; pp. 287–330.
84. Walsby, A.E. Gas vesicles. *Annu. Rev. Plant Physiol.* **1975**, *26*, 427–439. [CrossRef]
85. Armstrong, R.E.; Hayes, P.K.; Walsby, A.E. Gas vacuole formation in hormogonia of *Nostoc muscorum*. *Microbiology* **1983**, *129*, 263–270. [CrossRef]
86. Pfeifer, F. Distribution, formation and regulation of gas vesicles. *Nat. Rev. Microbiol.* **2012**, *10*, 705–715. [CrossRef] [PubMed]
87. Pawlowski, K.; Bergman, B. Plant Symbioses with Frankia and Cyanobacteria. In *Biology of the Nitrogen Cycle*; Bothe, H., Ferguson, S.J., Newton, W.E., Eds.; Elsevier: Amsterdam, The Netherlands, 2007; pp. 165–178.
88. Bohunická, M.; Pietrasiak, N.; Johansen, J.R.; Gómez, E.B.; Hauer, T.; Gaysina, L.A.; Lukešová, A. *Roholtiella*, gen. nov. (Nostocales, Cyanobacteria)—A tapering and branching cyanobacteria of the family Nostocaceae. *Phytotaxa* **2015**, *197*, 84–103. [CrossRef]
89. Cai, F.; Li, X.; Geng, R.; Peng, X.; Li, R. Phylogenetically distant clade of Nostoc-like taxa with the description of *Minunostoc* gen. nov. and *Minunostoc cylindricum* sp. nov. *Fottea* **2019**, *19*, 13–24. [CrossRef]
90. World Health Organization. *Guidelines for Drinking Water Quality Policies and Procedures Used in Updating the WHO Guidelines for Drinking Water Quality*; WHO Document Production Services: Geneva, Switzerland, 2009.
91. Chorus, I.; Bartram, J. *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*; CRC Press: London, UK, 1999.
92. Poste, A.E.; Hecky, R.E.; Guildford, S.J. Evaluating microcystin exposure risk through fish consumption. *Environ. Sci. Technol* **2011**, *45*, 5806–5811. [CrossRef]
93. Mulvenna, V.; Dale, K.; Priestly, B.; Mueller, U.; Humpage, A.; Shaw, G.; Allinson, G.; Falconer, I. Health risk assessment for cyanobacterial toxins in seafood. *Int. J. Environ. Res. Public Health* **2012**, *9*, 807–820. [CrossRef]
94. Rahim, N.A.A.; Merican, F.M.M.S.; Radzi, R.; Omar, W.M.W.; Nor, S.A.M.; Broady, P.; Convey, P. Unveiling the diversity of periphytic cyanobacteria (Cyanophyceae) from tropical mangroves in Penang, Malaysia. *Trop. Life Sci. Res.* **2023**, *34*, 57–94.
95. Kaplan-Levy, R.N.; Hadas, O.; Summers, M.L.; Rücker, J.; Sukenik, A. Akinetes: Dormant Cells of Cyanobacteria. In *Dormancy and Resistance in Harsh Environments*; Lubzens, E., Cerda, J., Clark, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 5–27.
96. Garg, R.; Maldener, I. The formation of spore-like akinetes: A survival strategy of filamentous cyanobacteria. *Microb. Physiol.* **2021**, *31*, 296–305. [CrossRef]
97. Gerona-Daga, B.M.E.; Salmo, S.G. A systematic review of mangrove restoration studies in Southeast Asia: Challenges and opportunities for the United Nation's Decade on Ecosystem Restoration. *Front. Mar. Sci.* **2022**, *9*, 987737. [CrossRef]
98. Hilaluddin, F.; Yusoff, F.M.; Natrah, F.M.I.; Lim, P.T. Disturbance of mangrove forests causes alterations in estuarine phytoplankton community structure in Malaysian Matang mangrove forests. *Mar. Environ. Res.* **2020**, *158*, 104935. [CrossRef] [PubMed]