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Hidden Microbial Diversity in Mangrove Depths: New Cyanobacterial Species of *Picosynechococcus* and Two New Records of *Sirenicapillaria* and *Allocoleopsis* from the Andaman Coast of Thailand

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Abstract: In Thailand, mangrove forests form a major component of the Andaman coastal ecosystems in the southern provinces. However, studies on their microbial assemblage largely revolved around groups of bacteria, fungi, and eukaryotic microalgae, while the diversity of cyanobacteria in these regions remains almost unknown. This taxonomic study applied the polyphasic approach to examine seven cyanobacterial strains collected from different mangrove environments (including soil crust, tree bark, wood, and rock surface) across Ranong, Phang-Nga, and Phuket provinces. The comprehensive analysis combining morphology, ecology, 16S rRNA phylogenetic relationships, genetic identity, ITS secondary structure, and ITS dissimilarity resulted in the first records of the genera *Picosynechococcus*, *Allocoleopsis*, and *Sirenicapillaria* in Thailand, and led to the description of a new species, *Picosynechococcus mangrovensis* sp. nov. This new species was differentiated from the type species *P. fontinalis* based on the distinct 16S rRNA gene phylogenetic position, low 16S rRNA genetic similarity, its slightly halophilic nature, and ability to form pseudo-filaments with up to 160 cells. Our research significantly expands the documented cyanobacterial diversity of Southeast Asian mangrove ecosystems, establishing a critical foundation for future ecological and biotechnological investigations in these understudied yet vital tropical habitats.

Keywords: Andaman Sea; cyanobacteria; first record; new species; polyphasic approach; tropical mangrove

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1. Introduction

Mangrove forests host diverse yet obscure communities of cyanobacteria with major ecological roles [1]. To date, at least 400 morphospecies of mangrove-associated cyanobacteria inhabiting niches such as mangrove soils, tree trunks, leaves, and pneumatophores, have been reported worldwide [1,2]. Identifying cyanobacteria is challenging due to their phenotypic plasticity and limited distinct morphological traits [3], requiring an integration of morphology, ecology, and genetic data for unambiguous delimitation [4–6]. This polyphasic approach has facilitated the discovery of new cyanobacterial taxa from Brazilian and Indian mangrove environments in the past decade [7–9].

In Thailand, mangrove forests are concentrated along the Andaman coast in the southern provinces of Ranong, Phang-Nga, Phuket, Krabi, Trang, and Satun, which constitutes 67.5% of the total mangrove coverage (277,923.2 ha) [10]. Nevertheless, research on microbial assemblages in Thailand's mangroves has been restricted to groups of bacteria [11,12], fungi [13,14], and eukaryotic microalgae [15], with limited attention to cyanobacteria. Regarding this knowledge gap, the following question needs to be addressed: What taxa of cyanobacteria grow in the Andaman mangroves of Thailand?

Several cyanobacterial genera with distinctive morphological and ecological characteristics are of relevance to coastal ecosystems. The genus *Picosynechococcus* Komárek, Johansen, and Strunecký was established for unicellular picocyanobacteria inhabiting freshwater mineral springs and slightly saline water, and characterized by solitary, sheathless, rod-like cells occasionally forming few-celled pseudo-filaments [16]. The genus has been recently reclassified within the order Chroococcales and family Geminocystaceae, distinct from Aphanothecaceae and Synechococcaceae [17]. The little-known marine genus *Sirenicapillaria* Berthold, Lefler, and Laughinghouse currently comprises three species: *S. glauca*, *S. rigida*, and *S. stauglerae*, all originally isolated from the coasts of Florida, USA [18]. These benthic mats are composed of long, erect, net-like thalli with facultative and lamellated sheaths, therefore they are often justifiably misidentified as *Microseira* McGregor and Sendall or *Lyngbya* C. Agardh ex Gomont [18]. The monospecific genus *Allocoleopsis* Moreira-Fernandes, Giraldo-Silva, D. Roush, and Garcia-Pichel was notably established to accommodate a terrestrial species, *A. franciscana* Moreira-Fernandes, Giraldo-Silva, D. Roush, and Garcia-Pichel, previously regarded as *Microcoleus steenstrupii* J.B. Petersen, that commonly colonize biological soil crusts in semi-arid to arid regions [19].

In this study, we investigated cyanobacterial diversity from mangrove environments in the provinces of Ranong, Phuket, and Phang-Nga through a comprehensive polyphasic approach, incorporating morphological, molecular, ecological, and biogeographical information to expand our understanding of cyanobacterial distribution in Thailand's Andaman mangroves. This research aims to contribute significantly to our knowledge of mangrove-associated cyanobacteria in Southeast Asia and provide insights into potentially novel taxa within this understudied ecosystem.

2. Materials and Methods

2.1. Sample Collection

The samples from mangrove environments of Ranong, Phang-Nga, and Phuket provinces were collected during low tide in February 2024 as part of a larger floristic survey of cyanobacterial diversity in southern Thailand. Samples were collected from various substrates including bark surfaces in Pa Khlok Bay, Phuket (08°02′11″ N, 98°24′54″ E), and the Andaman Coastal Research Station for Development (ACRSD), Ranong (09°22′38″ N, 98°24′11″ E), rock surfaces in Ban Bang Ben, Ranong (09°35′50″ N, 98°30′05″ E), and biological soil crusts in the intertidal zone of a mangrove forest in Ban Bang Phat, Phang-Nga (08°21′37″ N, 98°34′35″ E) (Figure 1). Collection was performed using a metal spatula and the samples were stored in 60 mL sterile polycarbonate screw-top containers and rinsed with sterilized seawater to remove any sand or sediment prior to further processing at the laboratory.

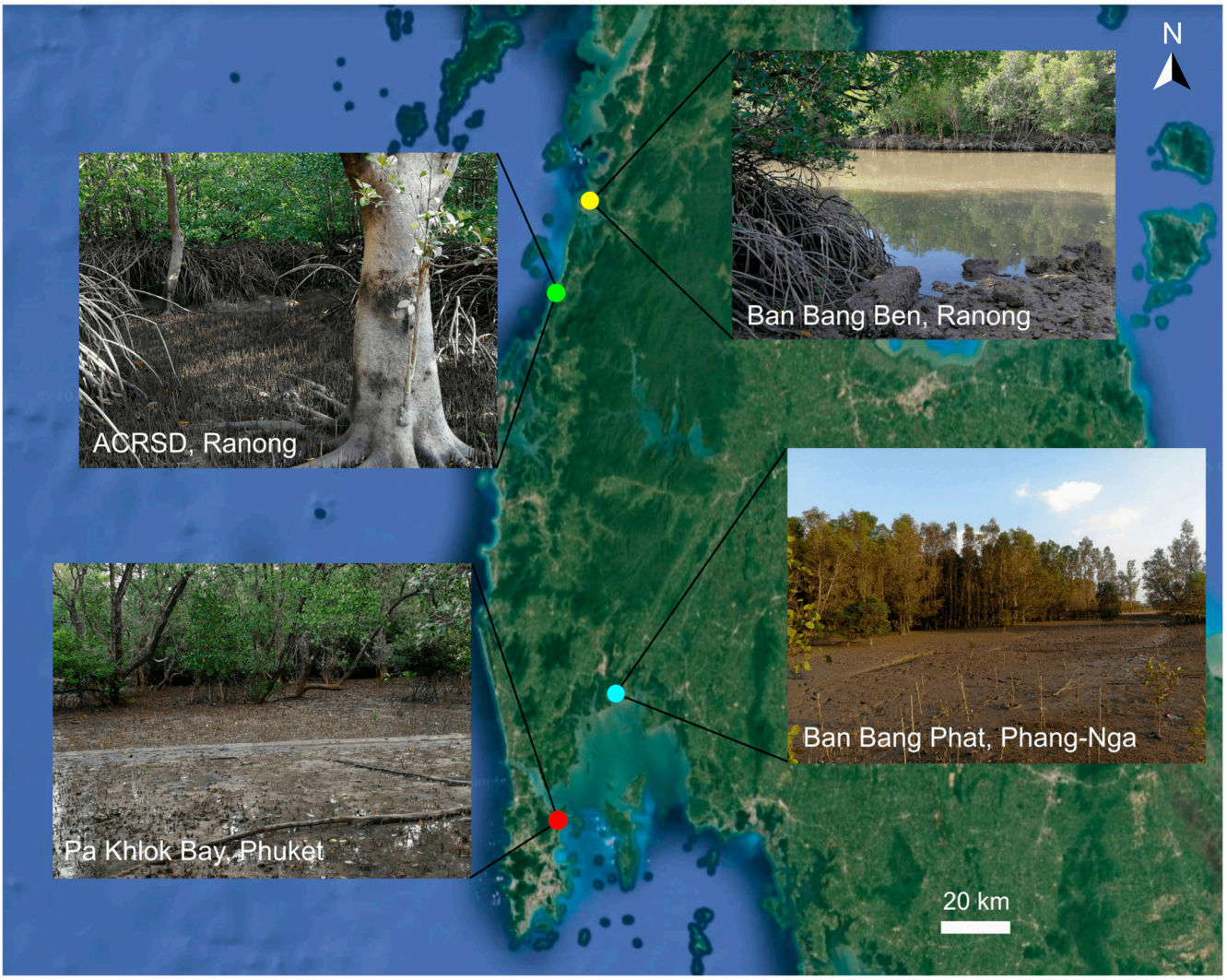


Figure 1. Map showing the sampling sites of the mangrove environments in the southern provinces of Thailand.

2.2. Isolation and Culture

Subsamples of the field specimens were enriched in f/2 liquid media [20], adjusted to a range of salinities (0, 15, 30, 45, 60, and 75 psu), and treated with 100 µg mL-1 cycloheximide to suppress any eukaryotic growth. Favorable growth was observed at a salinity of 30 psu for *Picosynechococcus mangrovensis* sp. nov. and *Sirenicapillaria glauca*, and at 15 psu for *Allocoleopsis franciscana*; therefore, cultures were maintained at these salinities thereafter. For filamentous cyanobacteria, isolation was performed by first placing the inoculum in the center of the agar medium, wherein small blocks of agar with single filaments that had migrated from the initial point of inoculation were then cut with sterile forceps and transferred to a fresh agar medium [21]. Additionally, filaments were serial-diluted and incubated in a 96-well plate containing f/2 medium for 2 weeks to achieve an unialgal status. The enriched cultures of unicellular cyanobacteria were dilution-plated and the formed single colonies were consecutively re-streaked on f/2 agar medium. Single colonies were subsequently re-streaked for culture maintenance. To achieve greater biomass for further analyses, the cells/filaments were cultured in 250 mL Erlenmeyer flasks containing 125 mL of f/2 liquid media, and incubated on an orbital shaker at 25 ± 3 °C under 12:12 h light:dark cycle with white cool fluorescent light of approximately 30 µmol m^{-2} s^{-1}.

2.3. Morphological Observation

The morphology of natural samples and cultured, isolated strains was examined utilizing an Olympus CX31 microscope (Olympus, Tokyo, Japan) equipped with a DinoEye AM4031X USB eyepiece camera (Dino-Lite, Taiwan, China) for digital image acquisition via DinoCapture 2.0 software. Comprehensive morphological characterization included documentation of trichome or filament attributes, vegetative and apical cell morphology and dimensions, presence or absence of extracellular sheath material, and reproductive mechanisms. Quantitative morphometric measurements were conducted on 50 randomly selected specimens.

2.4. Herbarium Voucher Preparation

Cells/filaments were filtered, immobilized, and attached to herbarium paper. The herbarium paper was subsequently air-dried and enclosed in a glassine envelope and packed with archival acid-free paper. The herbaria were then deposited at the Thailand Natural History Museum (THNHM), Pathum Thani, Thailand with the numbers THNHM-P-2024-0078, THNHM-P-2024-0079, THNHM-P-2024-0080, THNHM-P-2024-0081, THNHM-P-2024-0082, THNHM-P-2024-0083, and THNHM-P-2024-0084. The studied strains were preserved in 2% glutaraldehyde and stored in glass vials. Additionally, living cultures are maintained at the Applied Plankton and Aquatic Plant Research Laboratory, Center for Advanced Studies for Agricultural and Food (CASAF), Kasetsart University, Bangkok Thailand.

2.5. Molecular and Phylogenetic Analyses

DNA was extracted from unialgal cultures using the NucleoSpin Tissue kit (Macherey-Nagel, Dueren, Germany) according to manufacturer protocols. The primers 16S27F1 (5′-AGA GTT TTG ATC CTG GCT CAG-3′) [22] and 23S30R (5′-CTT CGC CTC TGT GTG CCT AGG T-3′) [23] were used for the PCR amplification of the 16S rRNA gene and 16S-23S rRNA ITS region. The 20 µL PCR reaction consisted of 1X PCR master mix of i-Taq™ iNtRON kit (Biotechnology DR, Seongnam, South Korea), 1 µL each of forward and reverse primers (10 pmol μL^{-1}), and 2 µL of DNA template. The PCR was performed using a Labcycler thermal cycler (SensoQuest GmbH, Göttingen, Germany) with the following conditions: initial denaturation at 95 °C for 3 min; 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 7 min; and a storage step at 10 °C. The PCR products were revealed using 1% agarose gel electrophoresis, later commercially purified and sequenced by U2Bio (Seoul, South Korea). Additionally, the amplicons of the 16S rRNA gene and associated ITS region were ligated into the pTG19-T cloning vector (Vivantis, Korea) and transformed into competent *Escherichia coli* DH5α cells, followed by selection of multiple positive transformants from each sample for sequencing using the primers M13F and M13R [24]. A total of one, four, and two clones of our strains *A. franciscana*, *S. glauca*, and *P. mangrovensis* were obtained, respectively.

Chromatograms were manually edited and assembled using Geneious Prime version 2025.0.2 (Biomatters, http://www.geneious.com, (accessed on 20 March 2025)). The 16S rRNA sequence alignment of 80 operational taxonomic units (OTUs) was constructed using the MUSCLE algorithm in Geneious Prime. The alignment incorporated publicly available sequences representing the genera *Allocoleopsis*, *Sirenicapillaria*, *Picosynechococcus*, along with sequences from closely related genera within the same respective families, all of which demonstrated high sequence identity based on BLAST+ 2.15.0 analyses (https://blast.ncbi.nlm.nih.gov/Blast.cgi, (accessed on 20 March 2025)). The phylogenetic tree was inferred by Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML analysis was performed in IQ-TREE 2 [25] with the best-fit model

selection (TIM3 + F + I + G4) implemented in ModelFinder [26]. Nodal support values were estimated using standard nonparametric bootstraps (BSs) with 1000 replicates [27]. The BI method was implemented using MrBayes v3.2 [28], with the best-fit nucleotide model (GTR + G + I) selected by Kakusan 4 [29]. Two independent Markov Chain Monte Carlo (MCMC) runs were conducted, each for 2,000,000 generations, sampling every 1000 generations. The convergence of runs was confirmed by split frequency standard deviations (<0.01). Additionally, run convergence and parameter stability were assessed using Tracer 1.6 [30]. Branch support was estimated from the posterior distribution of trees, discarding the initial 25% of samples as burn-in, based on an assessment of convergence among runs. Both ML and BI trees were visualized using FigTree v1.4.4 [31].

The analysis of the ITS region was conducted separately for operons with both $tRNA^{Ile}$ and $tRNA^{Ala}$ genes, operons with only one $tRNA^{Ile}$ gene, and operons lacking tRNA genes. The tRNA genes and the ITS domains were identified according to [32] and analyzed using Geneious Prime version 2025.0.2 (Biomatters, http://www.geneious.com, (accessed on 20 March 2025)). The secondary structures of the ITS domain were predicted using the Mfold program [33], with the draw mode set at untangle with loop fix and other parameters set to default. Additionally, the percent similarity of the 16S rRNA gene and percent dissimilarity of the 16S-23S rRNA ITS region were calculated using MEGA version 11 [34].

2.6. Salinity Tolerance and Growth

To facilitate the adaptation of cyanobacterial isolates to high salinity conditions, cultures were incubated in low light for 1 week. Subsequently, triplicates of *P. mangrovensis* were inoculated to a starting OD730 value of approximately 0.1 and cultured at salinities of 0, 15, 30, 45, 60, and 75 psu in 250 mL Erlenmeyer flasks containing 125 mL f/2 culture medium. Growth conditions were maintained as previously described. Cell growth was monitored daily for 2 weeks by measuring the optical density (OD) at 730 nm in a 1 cm light path with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). *A. franciscana* and *S. glauca* isolates were grown separately in 24 well-plates, in 2 mL culture volume, as the filaments tend to form clumps rather than a homogenous distribution in liquid medium, their growth was observed daily for 2 weeks.

3. Results

The identification of the isolated cyanobacterial strains in this study was predominantly based on the analyses of the 16S rRNA gene and ITS sequences, with morphological and ecological information used to complement our molecular findings.

3.1. 16S rRNA Gene Phylogenetic and p-Distance Analyses

Both ML and BI analyses yielded congruent topologies, with the ML tree presented in Figure 2. Genetic distances (*p*-distance) are provided in Table S1. Our phylogenetic reconstruction revealed that the isolated cyanobacteria strains were placed into three distinct monophyletic clades, representing the genera *Picosynechococcus*, *Sirenicapillaria*, and *Allocoleopsis* (Figure 2).

Our strain FFKU-PNG 10g9 (PV210898) was identified as *Allocoleopsis franciscana* based on its well-supported monophyletic clade (ML = 97%) with the type strain *A. franciscana* PCC 7113 (NR_176474). The genetic identity between our strain and the type strain reached 100%, with 99.7% sequence similarity to strain ACKU679 (OR365294) (Table S1). Furthermore, our *Sirenicapillaria* isolates (FFKU-RBBB 8, FFKU-RBBB 11, FFKU-RBBB 12, and FFKU-RBBB 15) were identified as *S. glauca* based on their placement in a strongly supported monophyletic clade (ML = 97%, BI = 0.99) with the reference strain *S. glauca*

BLCC-M125 (MZ127483). The genetic identity between our strains and the reference *S. glauca* strain was 99.6% (Table S1).

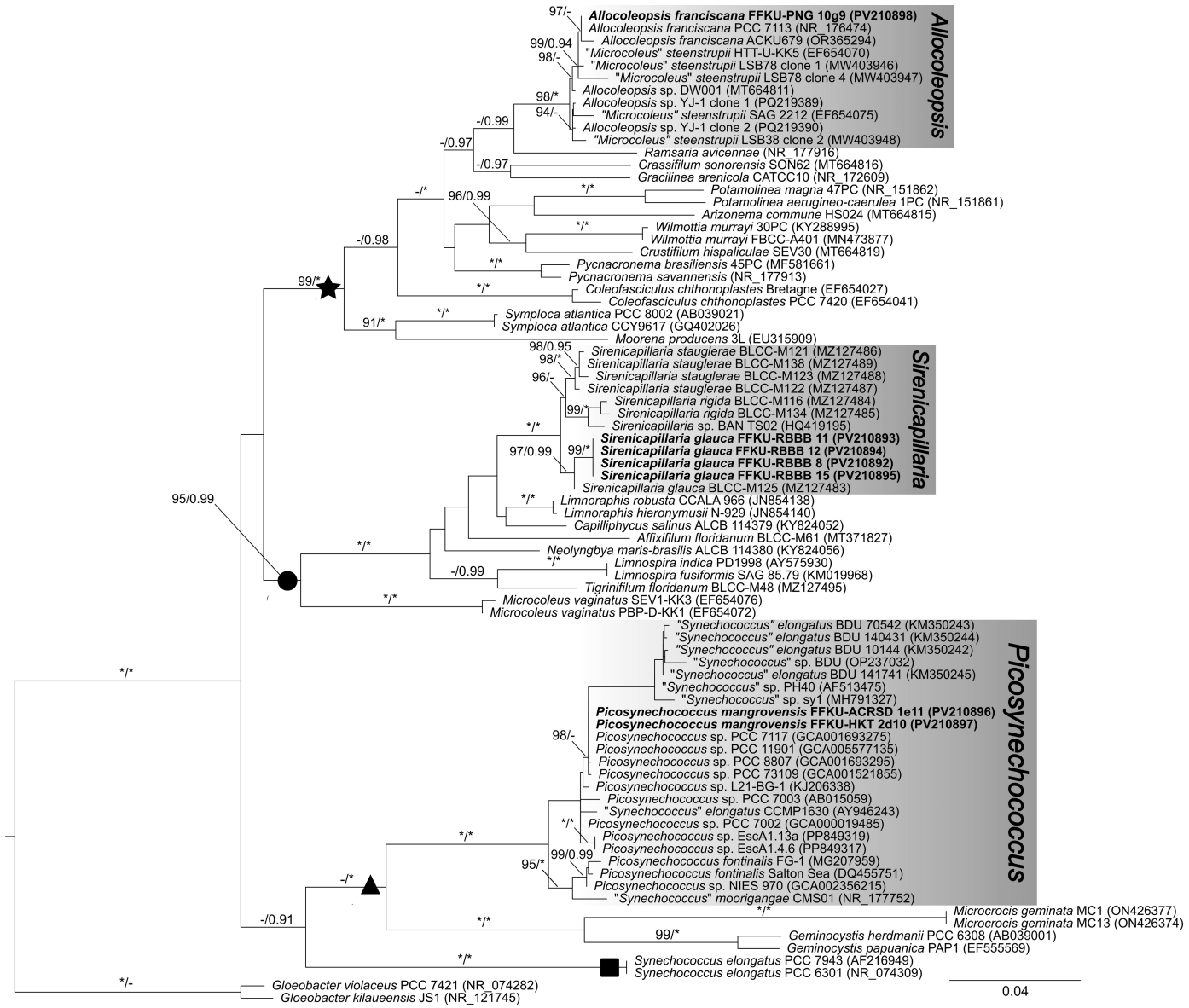


Figure 2. Maximum likelihood tree based on the 16S rRNA gene sequence dataset of 1517 base pairs (bp), including gaps. ML bootstrap support values (≥90%) (left) and BI posterior probabilities (≥90%) (right) are shown on the branches. Asterisks (*) indicate 100% bootstrap support and/or 1.00 posterior probabilities. ML bootstrap values below 90, BI posterior probabilities below 0.90, or topology without support are labelled with "-". Strains isolated from this study are bolded. Taxonomic groups are indicated by symbols, with each symbol marking the branch where the respective taxonomic group is delimited: star for Coleofasciculales (including Coleofasciculaceae and Wilmottiaceae), circle for Microcoleaceae, triangle for Geminocystaceae, and square for Synechococcaceae.

In contrast to the clear species-level identifications of our *A. franciscana* and *S. glauca* isolates, our *Picosynechococcus* strains FFKU-ACRSD 1e11 (PV210896) and FFKU-HKT 2d10 (PV210897) represented a taxonomically distinct lineage. These strains formed a moderately supported clade with several unnamed *Picosynechococcus* strains (e.g., PCC 7117 from low salinity brine pond, PCC 11901 from brackish water, PCC 73109 from seawater), exhibiting variable genetic identities ranging from 98.5% to 100% within this group (Table S1). More importantly, our *Picosynechococcus* isolates were phylogenetically distant from the type

strain *P. fontinalis* FG-1 (MG207959), with this separation receiving strong statistical support (ML = 100%, BI = 1.00) and significantly lower percent similarities (97.3–98.1%, Table S1) below the genetic identity threshold of ≤98.7% [35], suggesting that our *Picosynechococcus* isolates likely represent a novel species within the genus.

3.2. 16S–23S rRNA ITS Secondary Structure and p-Distance Analyses

Only one operon type containing both tRNA genes was identified in the *Picosynechococcus* strains analyzed. The ITS region of *Picosynechococcus* spp. (FFKU-ACRSD 1e11, FFKU-HKT 2d10, NIES 970, PCC 7002, PCC 7117, PCC 7003, PCC 73109, PCC 8807, PCC 11901, BDU 10144, BDU 70542, BDU 140431, BDU 141741) exhibited an identical D1-D1′ helical structure (Figure 3A). In contrast, the Box-B secondary structures varied across strains, particularly in the terminal loop and the bilateral bulge preceding it (Figure 4A–J). The Box-B folding pattern was nearly identical across strains, with minor differences in the nucleotide sequences of their terminal loops: AAAAAAA (FFKU-ACRSD 1e11 and FFKU-HKT 2d10) (Figure 4A), AAAAAAAA (PCC 73109, PCC 8807) (Figure 4F), and AAGAAAAA (BDU 141741) (Figure 4I). ITS sequences were not available for the type species, *P. fontinalis*. Our strains showed 0.9–2.0% dissimilarity in ITS sequences compared to certain strains (BDU 141741, BDU 10144, BDU 70542, BDU 140431) and 9.2–14.5% dissimilarity compared to others (NIES 970, PCC 73109, PCC 11901, PCC 7002, PCC 7003, PCC 8807) (Table 1).

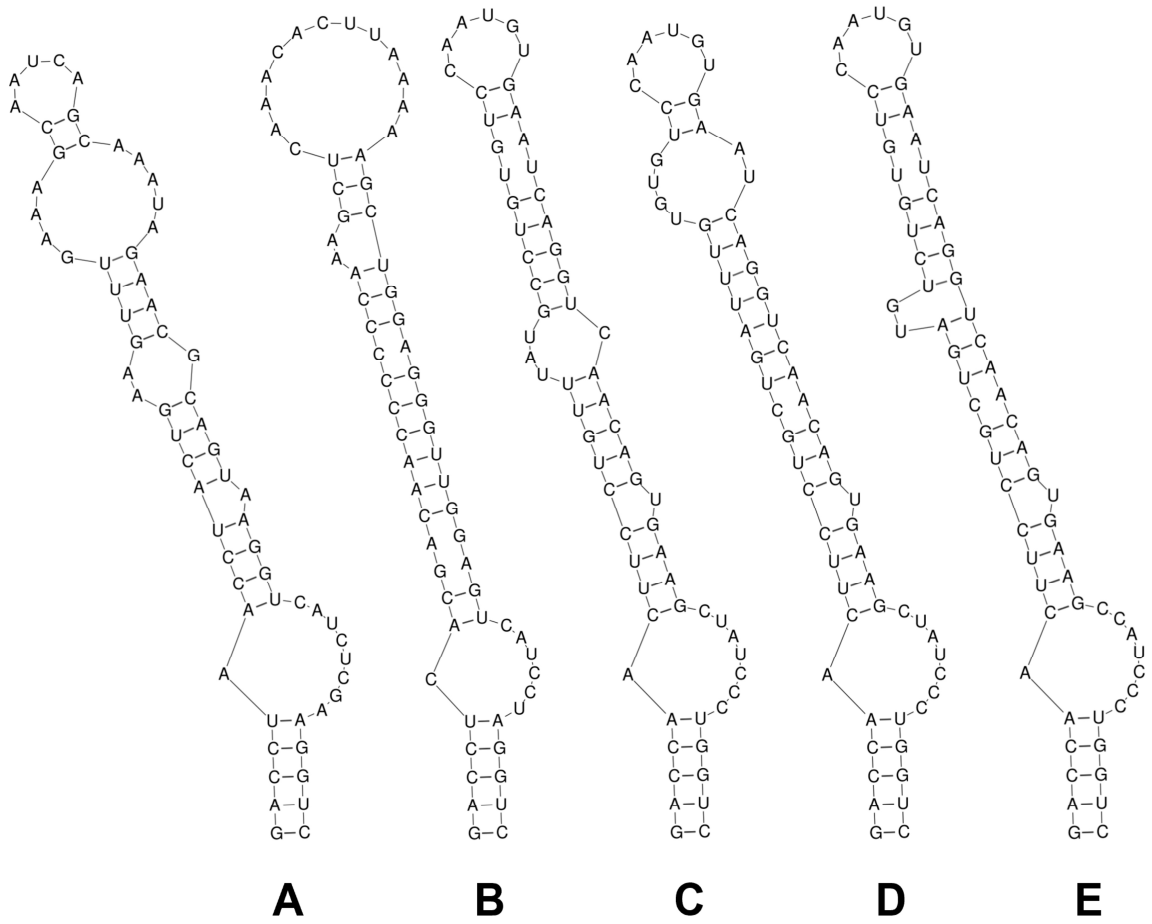


Figure 3. Secondary structures of the D1-D1′ helices of the 16S–23S rRNA ITS region. (**A**) *Picosynechococcus mangrovensis* FFKU-ACRSD 1e11 and FFKU-HKT 2d10. (**B**) *Sirenicapillaria glauca* BLCC-M125 clone B, FFKU-RBBB 8, FFKU-RBBB 11, FFKU-RBBB 12, FFKU-RBBB 15. (**C**) *Allocoleopsis franciscana* FFKU-PNG 10g9 and PCC7113. (**D**) *Allocoleopsis* sp. YJ-1 clone 1, clone 2, and LSB38 clone 2. (**E**) *Allocoleopsis* sp. LSB78 clone 1 and clone 4.

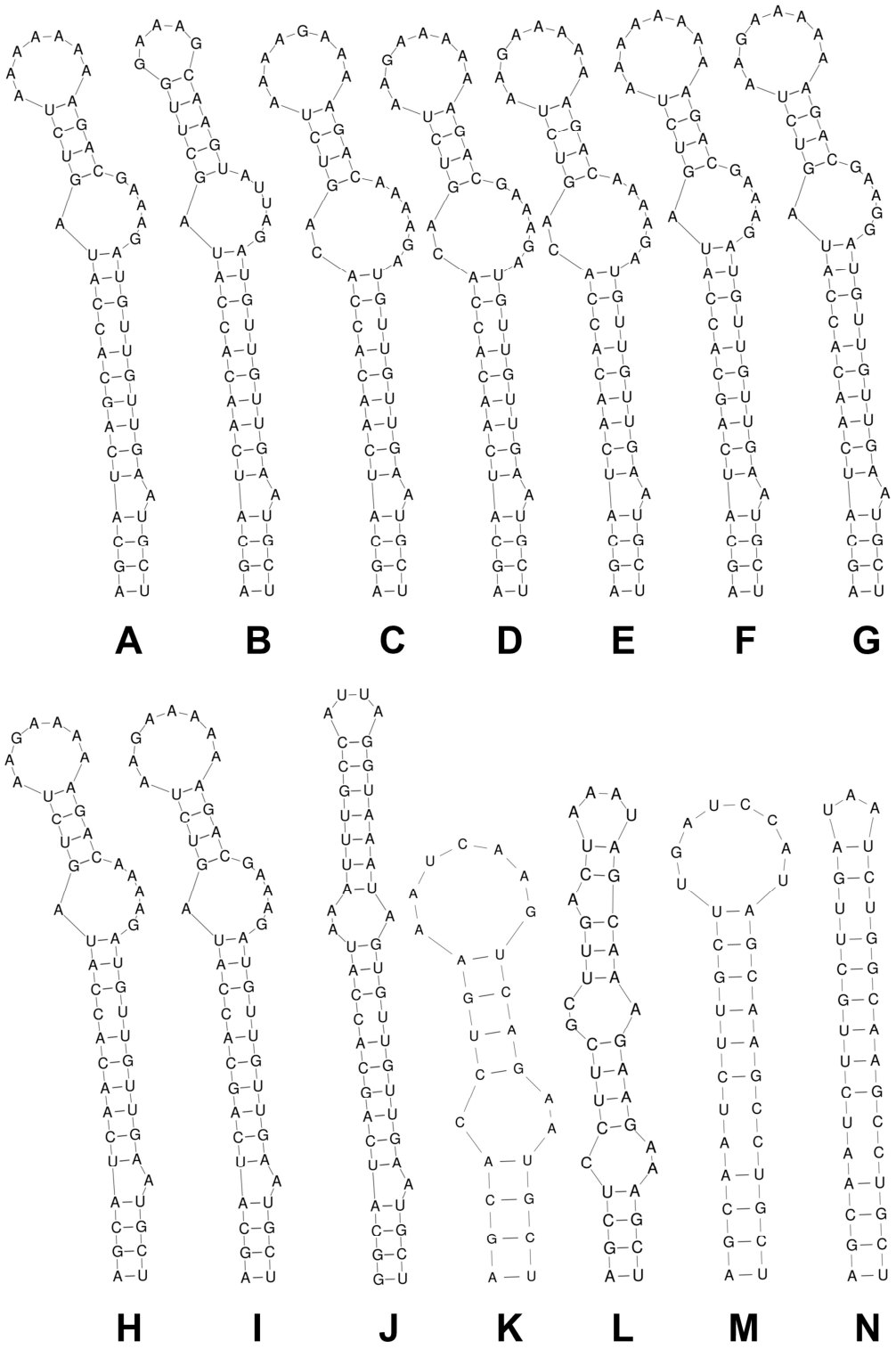


Figure 4. Secondary structures of the Box-B helices in the 16S–23S rRNA ITS region. (**A**) *Picosynechococcus mangrovensis* FFKU-ACRSD 1e11 and FFKU-HKT 2d10. (**B**) *Picosynechococcus* sp. NIES 970. (**C**) *Picosynechococcus* sp. PCC 7002. (**D**) *Picosynechococcus* sp. PCC 7117. (**E**) *Picosynechococcus* sp. BDU 140431. (**F**) *Picosynechococcus* sp. PCC 73109 and PCC 8807. (**G**) *Picosynechococcus* sp. BDU 10144. (**H**) *Picosynechococcus* sp. BDU 70542 and PCC 11901. (**I**) *Picosynechococcus* sp. BDU 141741. (**J**) *Picosynechococcus* sp. PCC 7003. (**K**) *Sirenicapillaria glauca* BLCC-M125 clone B, FFKU-RBBB 8, FFKU-RBBB 11, FFKU-RBBB 12, FFKU-RBBB 15. (**L**) *Allocoleopsis franciscana* FFKU-PNG 10g9 and PCC7113. (**M**) *Allocoleopsis* sp. YJ-1 clone 1, clone 2, and LSB38 clone 2. (**N**) *Allocoleopsis* sp. LSB78 clone 1 and clone 4.

Table 1. Percent dissimilarity based on 16S–23S rRNA ITS region of *Picosynechococcus mangrovensis* sp. nov. and its allied strains. Strains of this study are bolded.

	Taxa	1	2	3	4	5	6	7	8	9	10	11	12
1	***P. mangrovensis* FFKU-ACRSD 1e11**												
2	***P. mangrovensis* FFKU-HKT 2d10**	0											
3	"*Synechococcus*" *elongatus* BDU 141741	0.9	0.9										
4	"*Synechococcus*" *elongatus* BDU 10144	0.9	0.9	1.1									
5	"*Synechococcus*" *elongatus* BDU 70542	1.1	1.1	1.3	0.9								
6	"*Synechococcus*" *elongatus* BDU 140431	1.3	1.3	1.7	1.5	0.9							
7	*Picosynechococcus* sp. NIES 970	14.5	14.5	5.2	5.4	4.9	5.4						
8	*Picosynechococcus* sp. PCC 73109	9.4	9.4	0.9	0.9	1.1	1.3	5.1					
9	*Picosynechococcus* sp. PCC 11901	10.4	10.4	1.5	1.1	0.2	1.1	4.9	1.2				
10	*Picosynechococcus* sp. PCC 7117	2.0	2.0	1.5	1.5	1.3	1.5	0.6	1.6	1.5			
11	*Picosynechococcus* sp. PCC 7002	10.8	10.8	1.7	1.7	1.1	1.3	5.5	1.3	1.0	1.3		
12	*Picosynechococcus* sp. PCC 7003	10.9	10.9	5.4	5.4	5.4	5.7	5.7	5.1	5.1	6.1	5.3	
13	*Picosynechococcus* sp. PCC 8807	9.2	9.2	0.9	0.9	1.1	1.3	5.6	0.2	1.1	1.6	1.3	5.1

For *S. glauca* strains, two operon types were observed: one containing both tRNA genes (FFKU-RBBB 11, FFKU-RBBB 12), and another lacking tRNAs (FFKU-RBBB 8, FFKU-RBBB 15, BLCC-M125). Interestingly, the D1-D1′ and Box-B secondary structures were identical for both operon types across all *S. glauca* strains, while the structures for both domains remained distinct from each other (Figures 3B and 4K). ITS sequence analysis revealed 0–1.1% dissimilarity in tRNA-lacking operons of *S. glauca*, while the dissimilarity between operon types was 27.0–34.0% (Table 2). An ITS dissimilarity of >3% has been widely used as strong evidence for species separation in cyanobacterial taxonomy [36–45]. Overall, *S. glauca* was 8.6–19.1% dissimilar from *S. rigida* and *S. stauglerae* sequences lacking tRNAs, and 5.9–9.6% dissimilar from those with both tRNAs, thereby satisfying the threshold requirement (Table 2).

Table 2. Percent dissimilarity based on 16S–23S rRNA ITS region of *Sirenicapillaria* species. Sequences with both tRNAs are labelled with double asterisks (**) and those lacking tRNAs are not labelled. Strains of this study are bolded.

Taxa	1	2	3	4	5	6	7	8
***Sirenicapillaria glauca* FFKU-RBBB 15**								
***S. glauca* FFKU-RBBB 8**	0							
***S. glauca* FFKU-RBBB 12** **	29.9	34.0						
***S. glauca* FFKU-RBBB 11** **	29.9	34.0	0					
S. glauca BLCC-M125 clone B	1.1	1.1	27.0	27.0				
S. rigida BLCC-M116 clone A	8.6	8.6	27.4	27.4	8.8			
S. rigida BLCC-M116 clone B **	34.7	39.0	5.9	5.9	28.4	24.4		
S. stauglerae BLCC-M121 clone C	19.1	19.1	38.4	38.4	18.9	18.9	39.2	
S. stauglerae BLCC-M121 clone A **	44.8	49.6	9.6	9.6	37.9	38.6	9.7	23.9

Similarly, two distinct operon types were observed in *Allocoleopsis* sequences: one containing a single $tRNA^{Ile}$ gene (FFKU-PNG 10g9 and the type strain PCC 7113) and another lacking tRNAs (YJ-1, LSB38, LSB78) (Table 3). The D1-D1′ secondary structure was identical in strains FFKU-PNG 10g9 and PCC 7113 (Figure 3C) but differed from strains YJ-1, LSB38 (Figure 3D), and LSB78 (Figure 3E), particularly in the region between the first bilateral bulge and the terminal loop. The Box-B domain varied across operon types and among sequences lacking tRNAs, both in folding pattern (Figure 4L–N) and length (37 bp for FFKU-PNG 10g9 and PCC 7113; 32 bp for YJ-1 and LSB38; 33 bp for LSB78). The

ITS dissimilarity between operon types reached 14.0–17.9% (Table 3). FFKU-PNG 10g9 and PCC 7113 were 0.4% dissimilar from each other, while ITS data were not available for ACKU679 (Table 3). LSB78 clones were 5.7–7.8% dissimilar from clones of YJ-1 and LSB38, while the dissimilarity among the latter sequences ranged from 0.2% to 3.0% (Table 3).

Table 3. Percent dissimilarity based on 16S-23S rRNA ITS region of *Allocoleopsis* strains. Sequences with a single tRNA are labelled with an asterisk (*) and those lacking tRNAs are not labelled. Strains of this study are bolded.

	Taxa	1	2	3	4	5	6
1	***Allocoleopsis franciscana* FFKU-PNG 10g9 ***						
2	*A. franciscana* PCC 7113 *	0.4					
3	*Allocoleopsis* sp. YJ-1 clone 1	14.0	14.1				
4	*Allocoleopsis* sp. YJ-1 clone 2	14.3	14.4	0.2			
5	"*Microcoleus*" *steenstrupii* LSB38 clone 2	14.2	14.4	2.7	3.0		
6	"*Microcoleus*" *steenstrupii* LSB78 clone 4	17.3	17.5	7.2	7.5	5.7	
7	"*Microcoleus*" *steenstrupii* LSB78 clone 1	17.6	17.9	7.5	7.8	5.8	0.2

3.3. Taxonomic Description

Genetic analyses and comprehensive morphological examinations have led to the establishment of a new taxon within the genus *Picosynechococcus*, while simultaneously confirming the presence of *Allocoleopsis franciscana* and *Sirenicapillaria glauca* in the Andaman coastal waters of Thailand. The taxonomic characterization of each species is as follows:

3.3.1. *Picosynechococcus mangrovensis* sp. nov. Lim and Muangmai (Figure 5A–H)

Description: Cells bright blue-green, with homogenous content, solitary (Figure 5C), or connected in pseudo-filamentous formations of up to 160 cells (Figure 5D–H), oval to cylindrical, 1.7–4.0 µm long, 1.3–2.5 µm wide, with gliding motility. Cells divide by binary fission in one plane perpendicular to the longitudinal axis. Slightly halophilic, grows well in salinities ranging from 0 to 45 psu, with optimal growth at 30 psu.

Diagnosis: Morphologically similar to *P. fontinalis* and other *Picosynechococcus* strains, overlapping in cell length and cell width. Distinguished by its ability to form very long pseudo-filaments of up to 160 cells, slightly halophilic nature, its phylogenetic position and lower similarity in 16S rRNA gene sequence to other *Picosynechococcus* species (Figure 2).

Habitat: This species grew on bark or wood surfaces (Figure 5A).

Etymology: The species epithet refers to its occurrence in mangrove environments.

Type locality: Isolated from the bark surface of a stump within the intertidal zone of a mangrove environment in Pa Khlok Bay, Phuket, Thailand (08°02′11″ N, 98°24′54″ E).

Holotype: Portion of a culture of the reference strain FFKU-HKT 2d10, preserved in a metabolically inactive form, placed on herbarium paper and deposited at the Thailand Natural History Museum (THNHM), Pathum Thani, Thailand with the number THNHM-P-2024-0084.

Materials examined: FFKU-HKT 2d10, FFKU-ACRSD 1e11.

GenBank accession number: PV210896, PV210897 (16S rRNA), PV210904, PV210905 (ITS).

Note: The strains FFKU-HKT 2d10 from Phuket and FFKU-ACRSD 1e11 from Ranong were not observed in environmental samples but bloomed in enrichment cultures and were subsequently isolated through serial dilution. The strains were isolated from an algal consortium of *Lyngbya*-like and *Persinema* sp. mats, respectively. A detailed morphological comparison of our *P. mangrovensis* and other closely related species was presented in Table 4.

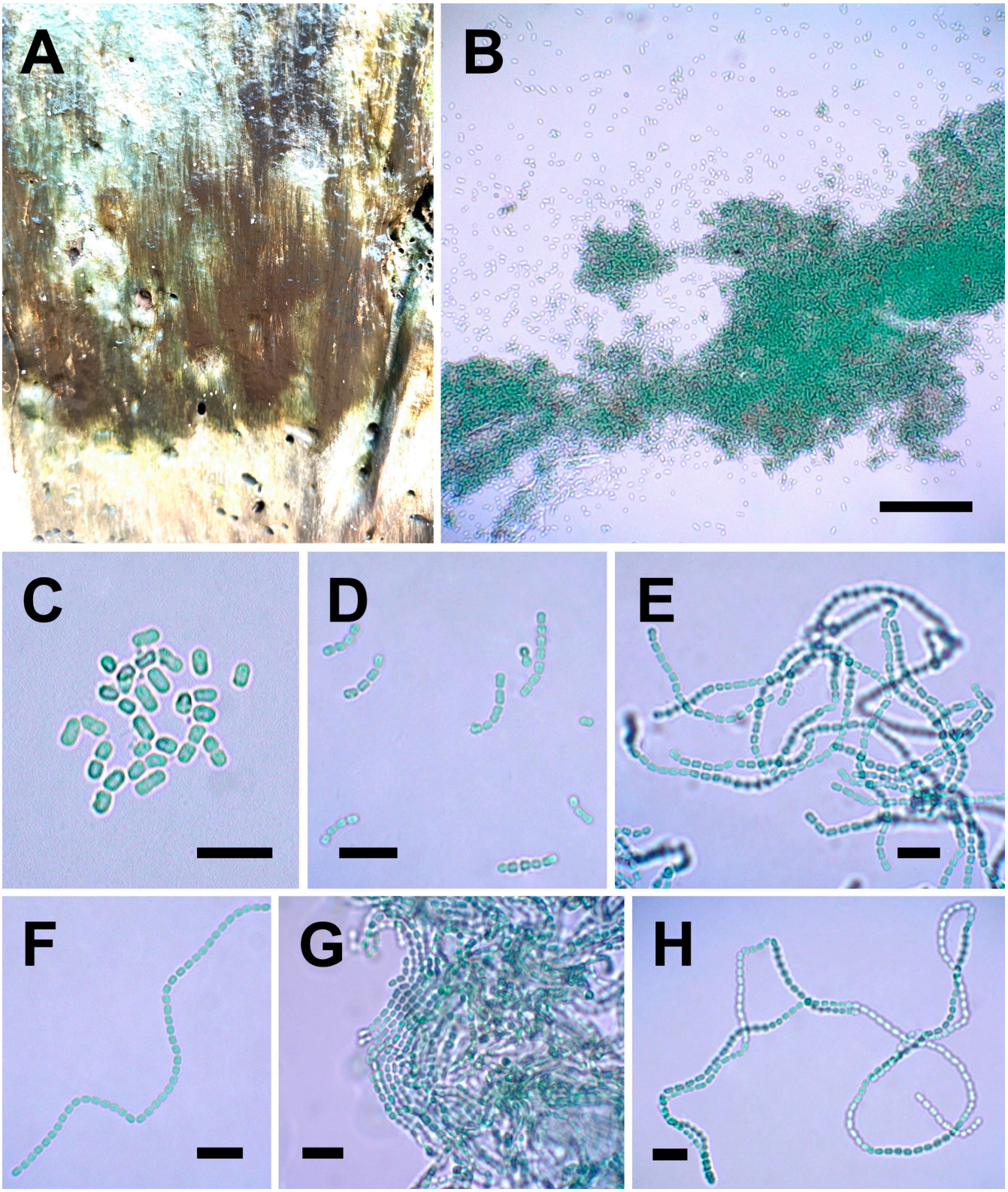


Figure 5. Morphology of *Picosynechococcus mangrovensis* strains (**A**,**C**–**E**) FFKU-HKT 2d10 and (**B**,**F**–**H**) FFKU-ACRSD 1e11. (**A**) Mat consortium of *Picosynechococcus* and *Lyngbya*-like cyanobacteria in nature. (**B**) Solitary and clustered cells in enrichment culture. (**C**,**D**) Cells solitary or in short pseudo-filaments. (**E**–**H**) Cells forming very long pseudo-filaments. Scale bar: (**B**) 70 µm, (**C**–**H**) 10 µm.

Table 4. Morphological comparison of *Picosynechococcus mangrovensis* sp. nov. and closely related taxa.

Taxon	*P. mangrovensis*	*P. fontinalis*	*"Synechococcus" moorigangae*	*Picosynechococcus* sp.
Strains	FFKU-ACRSD 1e11, FFKU-HKT 2d10	FG-1	CMS01	PCC 11901
Cell morphology	solitary, oval to cylindrical, connected in short to very long filamentous formations up to 160 cells	solitary, slightly oval	solitary, oval to cylindrical, form short chain filaments of 4 to 16 cells	solitary, elongated, form short filaments of 2 to 6 cells regardless of growth phase
Cell length (μm)	1.7–4.0	1.2–3.0	1.2–3.0	1.5–3.5
Cell width (μm)	1.3–2.5	0.8–2.0	0.8–2.0	1–1.5
Habitat/locality	on tree bark, Ranong, Thailand; on stump, Phuket, Thailand	planktic, periphytic, or metaphytic in freshwater pool, Bulgaria	Sundurban mangrove, India	Planktonic, Johor Strait, Singapore
Reference	This study	[16]	[46]	[47]

3.3.2. *Allocoleopsis franciscana* Moreira-Fernandes, Giraldo-Silva, D. Roush, and Garcia-Pichel (Figure 6A–G)

Description: Thallus-forming mat on biological soil crust in nature (Figure 6A), fasciculated (Figure 6B), or erect (Figure 6C) in culture. Solitary filaments, or forming rope-like bundles (Figure 6D). Trichomes dark olive green, heteropolar, slightly constricted at cross walls, with thin, hyaline sheath that open at the ends. Vegetative cells longer than wide to isodiametric (Figure 6E), 3.4–8.7 μm long and 3.4–5.0 μm wide. Apical cells rounded, elongated, conical or bent (Figure 6F). Reproduction by hormogonia formation with or without the help of necridia (Figure 6G).

Collection locality: Isolated from biological soil crusts in the intertidal zone of a mangrove forest in Ban Bang Phat, Phang-Nga (08°21′37″ N, 98°34′35″ E).

Herbarium deposition: Portion of a culture of the strain FFKU-PNG 10g9 was preserved in a metabolically inactive form, placed on herbarium paper and deposited at the Thailand Natural History Museum (THNHM), Pathum Thani, Thailand with the number THNHM-P-2024-0082.

GenBank accession number: PV210898 (16S rRNA), PV210899 (ITS).

Note: A detailed morphological comparison of our *Allocoleopsis franciscana* and other strains was presented in Table 5.

Table 5. Morphological comparison of *Allocoleopsis* strains.

Strains	FFKU-PNG 10g9	PCC 7113	ACKU 679	YJ-1	LSB78	LSB38
Filament morphology in culture	erect or fasciculate, solitary or forming rope-like bundles	solitary, forming rope-like bundles	fasciculate	solitary, forming rope-like bundles	forming rope-like bundles	
Cell length (μm)	3.4–8.7	6–7.5	4.1–6.8	4–7	3.4–6.7	4–9.4
Cell width (μm)	3.4–5.0	5–6	4.6–5.4	5–7	5.3–7.7	4.4–8.4
Trichome	heteropolar, cross-walls slightly constricted	heteropolar, cross-walls slightly constricted	heteropolar, cross-walls slightly constricted	heteropolar, cross-walls slightly constricted	-	-
Apical cell	rounded, elongated, conical or bent	rounded, slightly conical	rounded to conical	rounded to conical	-	-
Reproduction	hormogonia, necridia	-	hormogonia, necridia	hormogonia	-	-
Habitat/locality	mangrove soil, Phang-Nga, Thailand	orchid greenhouse soil and biocrust, USA	freshwater gravel, South Korea	moist soil nearby an IKEA building, Wuhan, China	desert soil crust, Taleb Larbi, Algeria	
References	This study	[19]	[48]	[49]	[50]	

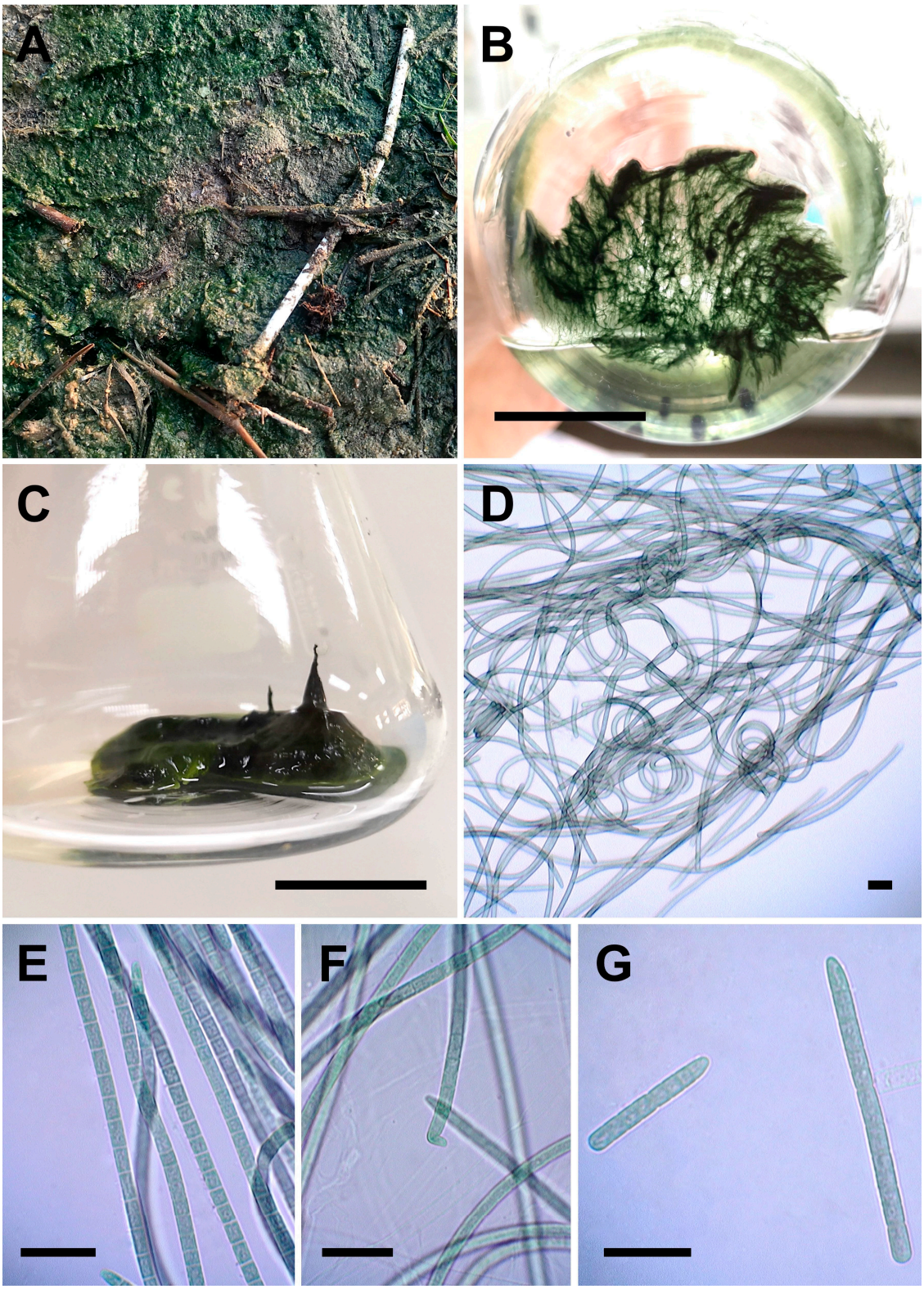


Figure 6. Morphology of *Allocoleopsis franciscana* FFKU-PNG 10g9. (**A**) Thallus on biological soil crust. (**B**) Fasciculated filaments in culture. (**C**) Thallus tightly aggregated and erect in culture. (**D**) Flexuous filaments. (**E**) Trichomes with distinct cross walls and conical apices. (**F**) Bent apical cell. (**G**) Hormogonia formation. Scale bar: (**B**,**C**) 4 cm, (**D**–**G**) 20 μm.

3.3.3. *Sirenicapillaria glauca* Berthold, Leftler, and Laughinghouse (Figure 7A–G)

Description: In liquid culture, thallus dark green, forming free-floating globose colony with hair-like filaments radiating from a common center (Figure 7A). Filaments straight, 10.9–18.0 µm in diameter. Sheath colorless, thin, firm, open at the ends. Trichomes pale to dark green, cylindrical, mostly not tapered towards the ends, rarely forming knot-like arrangements (Figure 7B) or having two trichomes occupying the same sheath. Vegetative cells discoid, shorter than wide, with cross walls slightly constricted and heavily granulated, 0.9–2.2 µm long, 8.9–11.8 (12.8) µm wide. Apical cell rounded, sometimes slightly narrowed, no calyptra. Reproduction by straight fragmentation with or without the help of necridic cells, diagonal fragmentation (Figure 7C), false branching (Figure 7D–F), hormogonia (Figure 7G).

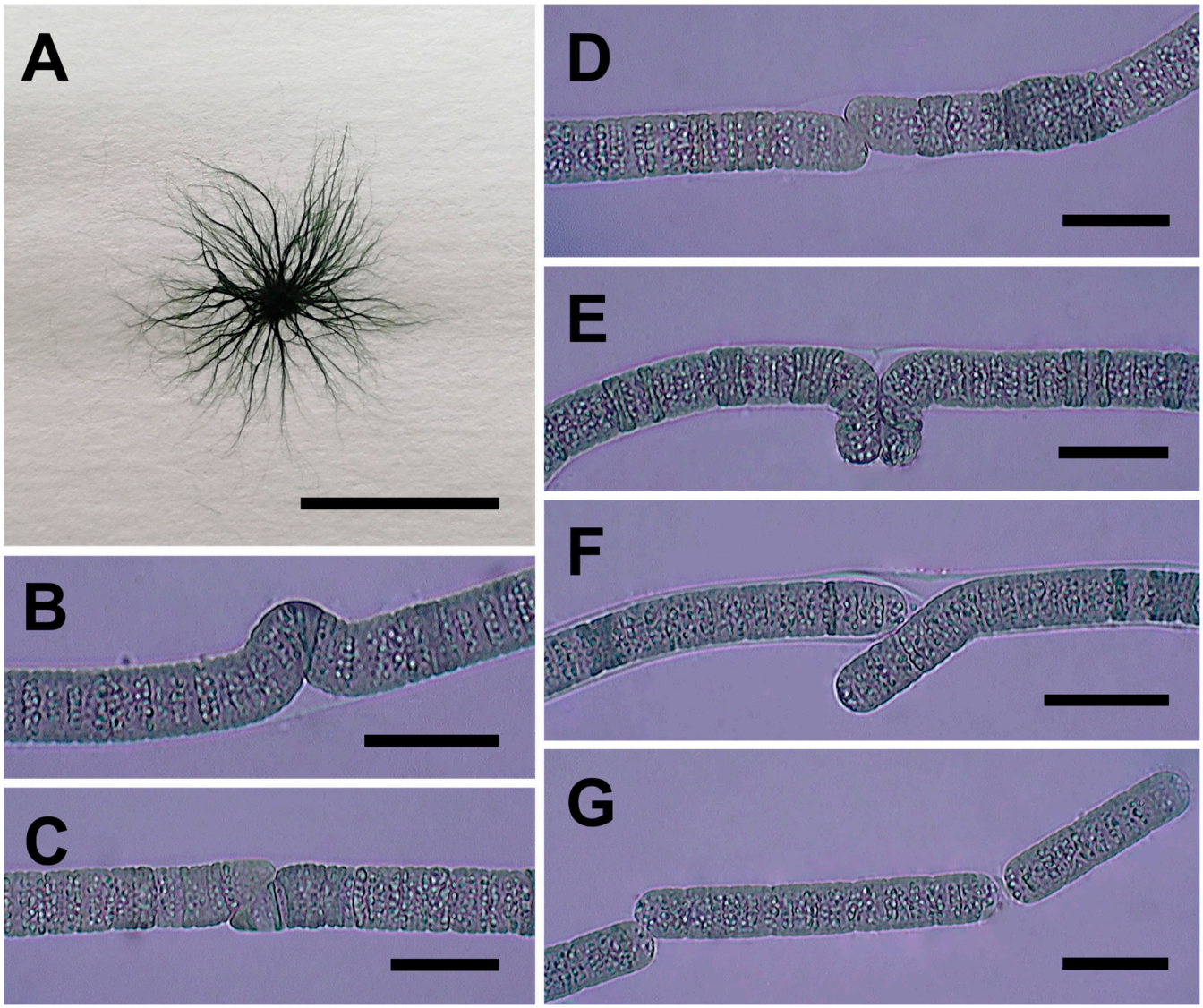


Figure 7. Morphology of *Sirenicapillaria glauca* (**A**,**B**) FFKU-RBBB 8, (**C**) FFKU-RBBB 11, (**D**,**E**) FFKU-RBBB 12, and (**F**,**G**) FFKU-RBBB 15. (**A**) Hair-like filaments radiating from a common center. (**B**) Curved filament. (**C**) Diagonal fragmentation of filament. (**D**) Filament prior to false branching. (**E**,**F**) False branching. (**G**) Hormogonia. Scale bar: (**A**) 2 cm, (**B**–**G**) 20 µm.

Collection locality: Isolated from a rock surface in Ban Bang Ben, Ranong, Thailand (09°35′50″ N, 98°30′05″ E).

Herbarium deposition: Portion of a culture of the strains (FFKU-RBBB 8, FFKU-RBBB 11, FFKU-RBBB 12, FFKU-RBBB 15) was preserved in a metabolically inactive form, placed on herbarium paper and deposited at the Thailand Natural History Museum (THNHM), Pathum Thani, Thailand with the numbers THNHM-P-2024-0078, THNHM-P-2024-0079, THNHM-P-2024-0080, THNHM-P-2024-0081.

GenBank accession number: PV210892–PV210895 (16S rRNA), PV210900–PV210903 (ITS).

Note: The rock surface was primarily inhabited by colonies of cf. *Kyrtuthrix* sp., while *S. glauca* filaments were not directly observed from environmental samples, they proliferated in enrichment cultures and were subsequently isolated through serial dilution. A detailed morphological comparison of our *Sirenicapillaria glauca* and other strains was presented in Table 6.

Table 6. Morphological comparison of *Sirenicapillaria glauca* strains.

Strains	FFKU-RBBB 8, 11, 12, 15	BLCC-M125
Thallus morphology in culture	pale to dark green, free-floating globose colony with filaments radiating from a common center	light to dark blue-green, erect, loose
Filament width (µm)	10.9–18.0	18.4–25.2(–28)
Cell length (µm)	0.9–2.2	1.3–2.8
Cell width (µm)	8.9–11.8 (12.8)	(14–)15–20(–21)
Trichome	cylindrical, rarely attenuated towards the ends, forming knot-like arrangement or two trichomes in the same sheath	cylindrical, attenuated towards the ends, cross-walls slightly constricted
Apical cell	rounded, slightly conical, no calyptra	rounded, conical, occasional calyptra
Reproduction	single cell release, straight or diagonal trichome fragmentation, hormogonia, necridia, false branching	single cell release, straight or diagonal trichome fragmentation, hormogonia, necridia
Habitat/locality	epilithic, mangrove forest, Ban Bang Ben, Ranong	benthic, growing on seagrass, Florida, USA
Reference	This study	[18]

3.4. Growth at Different Salinities

Both strains of *Picosynechococcus mangrovensis* demonstrated similar growth trends (Figure 8A,B): exponential growth in salinities ranging from 0 to 45 psu but poor growth 60 and 75 psu. Optimal growth was observed at 30 psu. Comparatively, lower growth was exhibited in 45 psu, followed by 15 and 0 psu. Additionally, *A. franciscana* FFKU-PNG 10g9 grew in salinities 0–30 psu. Our *S. glauca* isolates grew well in salinities 0–30 psu but exhibited yellowish-green filaments and poor growth in 40–65 psu.

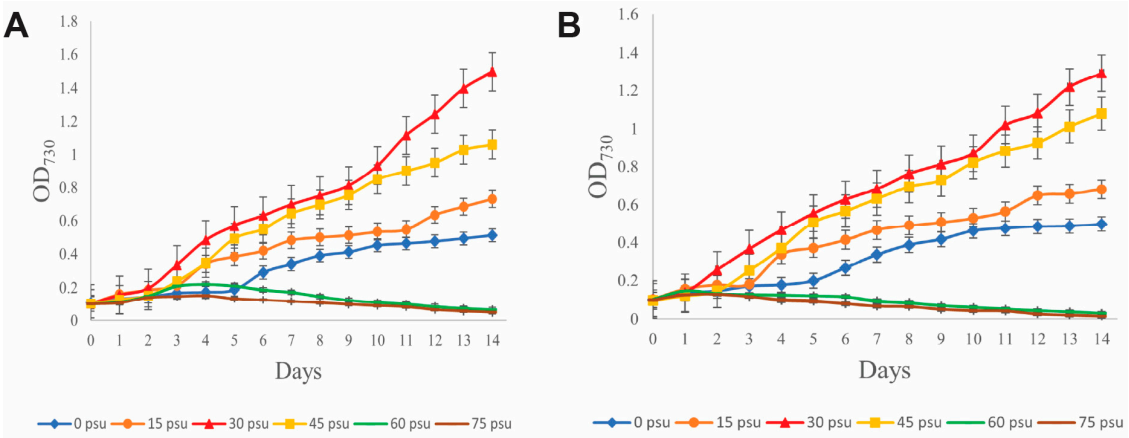


Figure 8. Growth curve of *Picosynechococcus mangrovensis* (**A**) FFKU-ACRSD 1e11 and (**B**) FFKU-HKT 2d10 in salinities 0–75 psu.

4. Discussion

4.1. Picosynechococcus mangrovensis sp. nov.

4.1.1. 16S rRNA Phylogeny and ITS Region Analyses

The 16S rRNA gene sequencing has been pivotal in confirming its phylogenetic placement and genetic distinctiveness from the type species *P. fontinalis* (Figure 2). As recommended by [35], the species threshold value of <98.7% serves as a marker for separating morphologically cryptic taxa. Interestingly, the strains of *P. mangrovensis* fall below the 98.7% limit in comparison to *P. fontinalis* (MG207959, DQ455751), *Picosynechococcus* sp. NIES 970, and "*Synechococcus*" *moorigangae* CMS01. Our analyses also revealed that "*Synechococcus*" *moorigangae* CMS01 is more closely related to the genus *Picosynechococcus* rather than *Synechococcus*. However, the lack of ITS data on *P. fontinalis* and "*Synechococcus*" *moorigangae* limits further taxonomic resolution. To address this, additional approaches from the polyphasic method were employed to support the novelty of *P. mangrovensis*.

4.1.2. Morphological Characterization

Picosynechococcus mangrovensis, though unicellular, exhibited pronounced filamentous formations of up to 160 cells in this study (Figure 5B–H). This morphological character was found to be consistent throughout the 6-month observation period. In both agar and liquid cultures, solitary cells and short pseudo-filaments of up to 10 cells were mostly present in salinities < 15 psu, whereas long pseudo-filaments of up to 160 cells were the predominant morphology at salinities > 15 psu. This salinity-dependent morphological plasticity stands in contrast to the behavior of *Crocosphaera subtropica* ATCC 51142, a unicellular cyanobacterium isolated from the intertidal area of Port Aransas, Texas, which formed short filaments of 4–16 cells under low salinity but reverted to its unicellular lifestyle as salinity or cell density increased [51]. In contrast, *P. mangrovensis* formed progressively longer filaments at higher salinities, suggesting a regulated, adaptive response to the dynamic salinity regimes due to tidal fluctuations, rather than a fixed morphological trait. Its preference for a higher salinity (Figure 8A,B) could indicate that its true ecological niche to be seaward rather than landward as found in the mangrove ecosystems. Furthermore, tidal movements likely facilitated cell/filament dispersal and its subsequent colonization of various niches beyond the core habitat of this species [52,53]. These observations underscore the broader ecological significance of morphological plasticity in cyanobacteria and the need to further investigate the links between environmental cues, morphological, and physiological responses among mangrove-associated cyanobacteria. On another note, we also consider this phenotype a unique trait to discriminate against *P. fontinalis* (few-celled) [16] and other strains including "*Synechococcus*" *moorigangae* CMS01 (4–6 cells) [46]. Additionally, thallus morphology of this new species in nature is unknown as they were not directly observed from the field. While morphological comparisons of natural and cultured populations should ideally be included in species identification [54], rare representatives of an algal consortium tend to be obscured by more dominant groups.

4.1.3. Ecological Adaptation and Geographical Distribution

Picosynechococcus presented a wide ecological range, such as those reported in *Nodosilinea* [41], *Haloleptolyngbya* [55], and *Halomicronema* [56]. The genus was originally documented inhabiting freshwater mineral springs (*P. fontinalis*), but their occurrences have since been reported in caves (EscA1.13a, EscA1.4.6), decaying wood (BDU 141741), sand (BDU 70542), low salinity brine pond (PCC 7117), hypersaline (L21-BG-1), marine (PCC 7002, PCC 7003, PCC 73109, BDU 140431, sy1), and brackish habitats (PCC 11901, BDU 10144, PH40, NIES 970, CMS01) [16,46,47]. In the case of *P. mangrovensis*, strains were isolated from tree bark and stump in the intertidal zone of mangrove environments. Inter-

estingly, it exhibited a slight halophilic nature [9] with favorable growth ranging from 30 to 45 psu, while the growth was reduced with increasing salt concentrations. The genetics, morphology, and ecology of this taxon not only warrants its classification as a new *Picosynechococcus* species but also highlights the plasticity and ability of cyanobacteria to thrive and adapt to a variety of habitats. Moreover, *Picosynechococcus* predominantly occurred in India (KM350242–KM350245, OP237032, NR_177752), Europe (AY946243, PP849317, PP849319, MG207959), North America (AB015059, DQ455751), the Hawaiian archipelago (AF513475, GCA001521855), and South America (GCA001693275), with records also available from Kiribati (KJ206338), Gabon (GCA001693295), Western Australia (GCA000019485), East Asia (Japan, GCA002356215; China, MH791327), and Southeast Asia (Singapore, GCA005577135). Our new taxon represents the first record of *Picosynechococcus* in Thailand, and the second sighting of the genus in Southeast Asia.

4.2. Allocoleopsis franciscana

4.2.1. 16S rRNA Phylogeny and ITS Region Analyses

Our strain FFKU-PNG 10g9 was embedded in a subclade containing the type strain *Allocoleopsis franciscana* PCC 7113, with a 16S rRNA genetic similarity of 100%, ITS dissimilarity of 0.4%, and identical D1-D1′ and Box-B secondary structures, thereby confirming the identity of our strain as *Allocoleopsis franciscana*. However, we could not compare the ITS sequences of the former two taxa with other *Allocoleopsis* strains as they had different operon types [57]. Even so, based on the ITS dissimilarity analysis of the sequences lacking tRNAs, it is very likely that there are at least two cryptic species in the genus. Nevertheless, this would require additional polyphasic studies of *Allocoleopsis* and a greater taxon sampling to characterize and diagnose them.

4.2.2. Morphological Characterization

The key feature that differentiates our strain from other *Allocoleopsis* strains YJ-1, LSB78, and LSB38 is the cell width. FFKU-PNG 10g9 displayed a length range of 3.4–5.0 μm, narrower than YJ-1 (5–7 μm), LSB78 (5.3–7.3 μm), and LSB38 (4.4–8.4 μm) (despite a minor overlap) (Table 5). The cell width of *Allocoleopsis franciscana* FFKU-PNG 10g9 spanned 3.4–5.0 μm, significantly narrower from the type strain PCC 7113 (5–7 μm), while the cell length of our strain achieved a broader range of 3.4–8.7 μm, in contrast to the length range 6.0–7.5 μm of the latter strain (Table 5). These observations highlight the morphological plasticity of cyanobacteria in different environments [3]. Additionally, we also provided the thallus morphology of *Allocoleopsis franciscana* in nature (Figure 6A) and in cultures (Figure 6B,C), which was not reported in previous literature. We are hopeful that it will aid in the identification of other *Allocoleopsis* specimens in future floristic surveys.

4.2.3. Ecological Adaptation and Geographical Distribution

Whilst the terrestrial *Allocoleopsis* has been generally described in greenhouse soil crusts [19], urban soils [49], and desert soils [44,50], they also grew on gravel submerged in freshwater [48]. The occurrence of *Allocoleopsis franciscana* in Thailand mangroves highlights the ecological flexibility and its potential role in the ecological dynamics of mangrove ecosystems. The genus has also been predominantly documented in the tropical regions of the USA [19,44] and some temperate regions of South Korea [48], China [49], and Algeria [50]. Our study expands the previously known geographic distribution of the genus to the Southeast Asian region.

4.3. Sirenicapillaria glauca

4.3.1. 16S rRNA Phylogeny and ITS Region Analyses

The morphological resemblance of our *Lyngbya*-like strains to *Sirenicapillaria* species (*S. glauca*, *S. rigida*, and *S. stauglerae*) is problematic for taxonomical evaluation [18], in addition to their high 16S rRNA genetic similarity to *S. stauglerae* and *S. glauca* (>98.7%). However, they are genetically distant enough to assign our strains to *S. glauca* on the basis of 16S rRNA phylogeny (Figure 2), ITS secondary structures (Figures 3B and 4K), ITS dissimilarity values (Table 6), as seen in other works on cryptic taxa [18,40,58]. This work also represents the first report of an ITS operon type containing both tRNA genes for *S. glauca* (FFKU-RBBB 11 and 12).

4.3.2. Morphological Characterization

A comparison between *S. glauca* collected from Thailand (in this study) and the USA [18] revealed notable differences in cell size and reproduction (Table 6). Although the filament width and cell width of the type strain BLCC-M125 ranged from 18.4 to 25.2(–28) µm and (14–)15–20(–21) µm, our strains demonstrated a narrower range of 10.9–18.0 µm and 8.9–11.8 (12.8) µm, respectively (Table 6). Furthermore, what caught our attention was the frequent occurrence of *Scytonema*-like (Figure 7E) and *Tolypothrix*-like (Figure 7F) false branching in cultured materials [59]. This particular morphological trait was stable and consistent throughout the 6-month long light microscopy observation on *Sirenicapillaria glauca* strains, which has ultimately expanded our knowledge of the morphological diversity in the genus.

4.3.3. Ecological Adaptation and Geographical Distribution

S. glauca was originally described as epipsammic, epiphytic, planktonic, and benthic organisms, living on seagrass beds in the marine coasts of the USA [18]. We consider our strains of the species to be epilithic, albeit not directly observed on the surface of the rock sample from which they were isolated. Notably, the occurrence of *S. glauca* in Thailand mangroves represents a notable geographic extension for the genus, as well as its first documentation in the Andaman Sea of Thailand.

4.4. Current Status and Future Surveys of Cyanobacterial Diversity in Thailand Mangroves

The world of mangrove-associated cyanobacteria provides vast room for new discoveries. Despite the prevalent mangrove conservation efforts in Thailand, the diversity of cyanobacteria in the Andaman region remains unknown. The first documentation of *Picosynechococcus*, *Sirenicapillaria*, and *Allocoleopsis* from this study highlights the importance of investigating cyanobacterial diversity in less explored mangrove habitats (e.g., subaerial, benthic, epilithic) of the southern regions of Thailand. Moreover, our findings from this polyphasic study serve as a baseline for establishing a reliable and comprehensive checklist based on updated identification keys from the tropical region.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050319/s1, Table S1: Percent similarity of 16S rRNA gene sequences among strains of *Picosynechococcus*, *Sirenicapillaria*, *Allocoleopsis,* and other closely related genera.

Author Contributions: Conceptualization, N.M.; methodology, B.L.C.G., F.M., J.P., S.M. and N.M.; software, B.L.C.G. and N.M.; validation, B.L.C.G., F.M. and N.M.; formal analysis, B.L.C.G., F.M. and N.M.; investigation, B.L.C.G., J.P., S.M. and N.M.; resources, B.L.C.G., J.P., S.M. and N.M.; data curation, B.L.C.G. and S.M.; writing—original draft preparation, B.L.C.G. and N.M.; writing—review and editing, B.L.C.G. and N.M.; visualization, B.L.C.G.; supervision, J.P. and N.M.; project

administration, N.M.; funding acquisition, N.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Data Availability Statement: All nucleotide sequences used in this study are available from NCBI, with their accession numbers provided in the main text.

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Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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