VALTER LOUREIRO DE ARAUJO

Gênero e espécie novos de cianobactéria homocitada (Synechoccocales) para a costa brasileira

Salvador, Bahia

(2018)

UNIVERSIDADE FEDERAL DA BAHIA
INSTITUTO DE BIOLOGIA

CURSO DE CIÊNCIAS BIOLÓGICAS

Gênero e espécie novos de cianobactéria homocitada (Synechoccocales) para a costa brasileira

por

VALTER LOUREIRO DE ARAUJO

TCC apresentado ao Instituto de Biologia da Universidade Federal da Bahia como exigência para a obtenção do grau de Bacharel em Ciências Biológicas

Orientador: Dr. José Marcos de Castro Nunes

Coorientadora: Drª Taiara Aguiar Caires

Data da defesa: 31 de julho de 2018

Banca Examinadora

Drª. Taiara Aguiar Caires
Faculdade São Salvador
Coorientadora e Presidente da Banca

Drª Helen Michele de Jesus Affe
Jardim Botânico do Rio de Janeiro

Dr. Geraldo José Peixoto Ramos
Universidade Estadual de Feira de Santana

AGRADECIMENTOS

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa concedida durante o desenvolvimento deste trabalho, através do Acordo CAPES/CNPQ - PROTAX II (PROTAX-II).

Ao Prof. Dr. José Marcos de Castro Nunes, por ter aberto as portas do LAMAR para mim, lugar que vem sendo minha casa desde 2014. Pelos ensinamentos durante a minha iniciação científica, os quais contribuem para construir o profissional que quero ser, e por todas as oportunidades que me foram dadas e portas que foram abertas. Muito obrigado!

Aos professores Drª. Alessandra Schnadelbch, coordenadora do LAGEV/UFBA, ao Dr. Henrique Batalha, coordenador do LEBI/UFBA, à Drª. Renata Lúcia Ferreira de Lima, responsável pelo LGHM/UFBA por terem disponibilizado toda a infraestrutura para a realização das análises moleculares. Aos professores Drª Kelly Regina Leite e Dr. Lázaro Silva, coordenadores do LAVIM/UFBA, e à Drª. Moema Cortizo Bellintani, coordenadora do LCTV/UFBA. À Drª. Suzana da Cunha Lima, coordenadora no LaBBiotec/UFBA por suporte infraestrutural concedido ao meu trabalho. E ao professor Dr. Eduardo Mendes da Silva pela cessão do espaço no Laboratório MARENBA/UFBA para o estabelecimento do cultivo de cianobactérias, por todos esses anos. Muito obrigado a todos.

À minha coorientadora Drª Taiara Aguiar Caires, agradeço por toda a paciência, por todos os conselhos, puxões de orelha (todos merecidos, diga-se de passagem) e ensinamentos ao longo desse período.

À equipe do setor de microscopia eletrônica Instituto Gonçalo Muniz (FIOCRUZ-BA) especialmente a Arlene Souza Lessa e Márcio Ferreira dos Santos por todo auxilio dado nessa parte do meu trabalho.

À Drª Priscila Barreto de Jesus, minha primeira coorientadora no LAMAR, por ter me apresentado ao mundo da biologia molecular, e por toda a paciência com que estava começando. Agradeço imensamente! Desculpa eu ser uma lástima e não ter trazido uma mísera *Hypnea* da Espanha.

Aos colegas e ex-colegas LAMAR, Aaron, Adriele, Arielle, Caio, Cibele, Edilene, Fiorella, Gabriel, Kevin, Marília, Larissa, Lorena, Poline, Raquel, Tiana e Viviane! Que tornaram esse período mais agradável. Obrigado por me aturar durante todo esse tempo!

À minha família por ter me apoiado desde o começo. Por todo o suporte emocional que vocês me deram e que me fizeram passar por esses anos na universidade com muito mais tranquilidade.

RESUMO

Cyanobacteria é um grupo filogeneticamente antigo de organismos procarióticos fotossintetizantes produtores de oxigênio, tendo surgido entre 2,7 e 3,5 bilhões de anos atrás. A diversidade desse grupo é expressa através de suas propriedades morfológicas, bioquímicas e fisiológicas, possibilitando seu estabelecimento e sobrevivência em uma grande variedade de hábitats terrestres e aquáticos. O material utilizado nesse estudo foi oriundo de coleta realizada na Praia de Pirambúzios, Rio Grande do Norte, Brasil (06°00'44.62"S x 35°06'26.48"W), o qual foi mantido em cultivo em meio líquido SWBG-11, com fotoperíodo de 14:10 h (claro:escuro). Para as análises moleculares, a extração do DNA total foi realizada através do método CTAB, seguida de Reações em Cadeia da Polimerase (PCR's) para amplificar a região gênica 16S do RNA ribossomal, utilizando os *primers* 27F1/23S30R. Os produtos da PCR foram clonados usando o kit pGEM®-T Easy Vector Systems. O sequenciamento foi realizado no Centro de Energia Nuclear na Agricultura da Universidade de São Paulo (CENA/USP). Para compor a matriz de sequências para as análises filogenéticas, foi realizada busca através da ferramenta *BLAST* do *National Center of Biotechnology Information* (NCBI), totalizando 64 sequências. Foi utilizado o programa BioEdit v7.2.5 para efetuar o alinhamento, o RaxML8.2.10 para as análises de Máxima Verossimilhança (MV) e o MrBayes on XSEDE para a Inferência Bayesiana (BP). As análises ultraestruturais foram realizadas em microscopia eletrônica de transmissão na FIOCRUZ/BA. A MV e a BP evidenciaram um clado independente com 88% e 100% de suporte, respectivamente, agrupando a sequência obtida neste estudo com uma sequência denominada "Filamentous cianobacterium" oriunda dos EUA (GenBank GQ243430). As análises ultraestruturais evidenciaram a presença de tilacoides dispostos parietalmente à membrana plasmática, que, aliados à forma filamentosa, inclui esta cepa na família Pseudanabaenaceae (Synechoccocales). As características morfológicas, como a forma da célula apical, a morfometria das células, o hábitat marinho e o hábito epifítico não foram descritos para a família. Esses resultados sugerem o reconhecimento de um novo gênero, representado por duas espécies, sendo uma delas, ALCB 125750, descrita neste estudo.

Palavras-chave: Cianobacteria; ambiente marinho; taxonomia

ABSTRACT

Cyanobacteria is a phylogenetically old group of prokaryotic photosynthetic organisms, which produces oxygen, arising between 2.7 to 3.5 Bya. The diversity of this group is expressed by the morphological, biochemical and physiology properties, making possible its establishment and persistency in a wide range of terrestrial and aquatic habitats. Brazil possess one of the greatest coasts of the world, providing a big diversity of habitats that possibilities the occurrence of benthic cyanobacteria. The material analyzed in this study was collected at Pirambúzios beach (Rio Grande do Norte, Brazil) (06°00'44.62"S x 35°06'26.48"W). It was kept in liquid medium SWBG-11 for cultivation, with photoperiod of 14:10 h (light:dark). To molecular analysis, DNA extraction was carried out with CTAB method, followed by Polymerase Chain Reaction (PCR) to amplify the 16S rRNA genic region, using 27F1/23S30R primers. PCR products were cloned using the pGEM®-T Easy Vector Systems kit. Sequencing was realized at the Nuclear Center in Agriculture of São Paulo's University (CENA/USP). In order to compose the sequence matrix to the phylogenetic analysis, a search was realized through the BLAST tool of the *National Center of Biotechnology Information* (NCBI), totalizing 64 sequences. BioEdit v7.2.5 program was used to align the sequences, and RaxML8.2.10 was used to generate the Maximum Likelihood (ML) tree, and MrBayes on XSEDE to run the Bayesian Posterior Probability (PP). Ultrastructural analysis was realized on transmission electronic microscopy on FIOCRUZ/BA.MV and PP analysis showed an independent clade, grouping the sequence obtained is this study with a "Filamentous cianobacterium" (GenBank GQ243430), with 88% and 100% support values, respectively. Ultrastructural analysis highlighted the presence of thylakoids parietally arranged to the cross-wall. This data, allied with the filamentous form, put our strain inside the Order Synechococcales and at the family Pseudanabaenaceae. The morphological features such as the apical cell form, cell morphometry, the marine habitat, and the epiphytic habit are not found in any other genus to Pseudanabaenaceae. This results suggests the recognition of a new to science genus with to species, being ALCB 125750, described in this study.

Key words: Cyanobacteria; Marine Environments; Taxonomy

ÍNDICE

1 - INTRODUÇÃO GERAL

1.1 - CARACTERIZAÇÃO GERAL DAS CIANOBACTÉRIAS

As cianobactérias, também conhecidas como algas azuis ou algas azuis-esverdeadas, é um grupo filogeneticamente antigo de organismos procarióticos fotossintetizantes produtores de oxigênio, tendo surgido entre 2.7 e 3.5 bilhões de anos atrás (Brooks *et al.* 1999; Schopf & Packer, 2010; Komárek, 2016). A principal propriedade comum a todas as cianobactérias é a sua capacidade fotossintética, aliada à liberação de oxigênio molecular (fotossíntese oxigênica) (Baulina, 2012), sendo obrigatoriamente fotoautótrofas, obtendo carbono e energia por mecanismos fotossintéticos que são similares aos das plantas superiores, o que as diferem das outras bactérias (Holm-Hansen, 1968).

O principal componente do sistema fotossintético das cianobactérias é a clorofila *a*, localizada na membrana dos tilacoides, as ficobiliproteínas, clorofilas *b, d* e *f,* carotenoides e xantofilas atuam como pigmentos acessórios (Miyashita *et al.* 1996; Lee 2008; Chen *et al.* 2010; Gupta *et al.* 2013). Essa variedade de pigmentos faz com que as cianobactérias tenham uma grande amplitude na absorção do espectro de luz, sendo capazes de absorver ondas entre 400 e 750nm de comprimento (Chen & Blankenship, 2011).

A diversidade desse grupo é expressa através das suas propriedades morfológicas, bioquímicas e fisiológicas, o que possibilita o seu estabelecimento e persistência em grande variedade de hábitats terrestres e aquáticos, incluindo aqueles com abundância ou escassez de recursos, sendo um componente dominante da comunidade fotossintética do solo, em regiões quentes ou frias e áridas. As cianobactéria encontram-se: nas camadas superficiais de solos agrícolas, onde ocorrem tipicamente com muitos outros organismos fotossintetizantes; em lagos salinos, alguns dos quais são ricos em metais pesados, como o cobre; e em fontes termais (Whitton & Sinclair, 1975; Lee, 2008; Paerl & Otten, 2013; Palinska & Surosz, 2014;).

1.1.1 - CARACTERÍSTICAS MORFOLÓGICAS

A forma mais simples que uma cianobactéria pode apresentar é a unicelular, de vida livre, ou encerrada em um envelope mucilaginoso. Com o passar do tempo, a

evolução dos caracteres morfológicos do grupo resultou na formação de um conjunto de células, chamado de tricoma, que, quando cercado por uma bainha, é denominado filamento (Lee, 2008). As formas unicelulares e filamentosas podem viver isoladas ou agregar-se em colônias, onde a sobreposição das células e/ou dos filamentos também tem sido usada como caracteres taxonomicamente informativos (Brodie & Lewis, 2007).

Diversas cianobactérias unicelulares, coloniais e filamentosas possuem um envelope circundando a sua membrana externa, sendo comumente chamada de bainha, glicocálice ou cápsula, e dependendo da sua consistência, pode ser referida como gel ou mucilagem. Esta camada protege as células contra dessecação em hábitats que estão expostos à intensa radiação solar (Castenholz, 2001; Wehr & Shear, 2003; Lee 2008;). Nas cianobactérias terrestres, a bainha frequentemente se torna similar a um gel, sólida, ou lamelada, sendo frequentemente pigmentada. As algas desenvolvem bainhas com pigmentos sob alta intensidade luminosa. As bainhas incolores são desenvolvidas sob baixa intensidade luminosa, enquanto há aquelas que não produzem bainhas coloridas, não importando a intensidade da luz (Fogg, 1973).

Muitas das cianobactérias são diazotróficas, ou seja, são capazes de fixar o nitrogênio atmosférico (Bergman *et al.* 1997). Aquelas que são fixadoras podem ser divididas em duas formas, as heterocitadas e as homocitadas. As formas heterocitadas são capazes de fixar o N_2 pela ação de uma célula especializada, chamada heterocito (Bergman *et al.* 1997; Lee, 2008). Os heterocitos são fotossinteticamente inativos, ou seja, eles não fixam CO_2 nem produzem O_2. A respiração e a fosforilação oxidativa ocorrem para que a fixação do nitrogênio não dependa exclusivamente da luz, apesar de ainda possuírem o fotossistema I para suprir a energia requerida para o processo de fixação do nitrogênio (Oren, 2014). O ambiente interno de um heterocito é virtualmente anóxico, o que é ideal para a ação da enzima nitrogenase, que é sensível à presença de O_2, sendo uma especialização para a fixação do N_2 atmosférico ou dissolvido na água (Wher & Shear, 2003; Lee, 2008).

As formas filamentosas homocitadas (ou não-heterocitadas), também são capazes de fixar nitrogênio (Krumbein, 1975; Pearson, 1979; Gallon & Stal, 1992; Bergman *et al.* 1997; Stal & Wasmund *et al.* 2001). Nas cianobactérias homocitadas, a fixação do N_2 é feita através de células especializadas chamadas diazócitos, as quais produzem a enzima nitrogenase, que é sensível ao oxigênio, fazendo com que os

diazócitos realizem a fotossíntese enquanto há luz, somente fixando o nitrogênio no escuro, quando a fotossíntese cessa e o ambiente se torna anóxico (Fredriksson & Bergman, 1996; Komárek & Anagnostidis, 2005; Lee 2008).

1.1.2 - REPRODUÇÃO

A reprodução em cianobactéria ocorre somente de maneira assexuada, usualmente por fissão binária (Fogg, 1973). Pode ocorrer também por meio de células solitárias, que são liberadas por diversos tipos de espécies filamentosas, ou por fragmentação de um filamento ou do talo.

O modo de reprodução mais frequente nas formas filamentosas é a fragmentação, formando distintos segmentos de tricomas, nomeados hormogônios (Wehr & Shear, 2003). Os hormogônios podem ser produzidos por meio de células necridiais, que são células mortas e colapsadas, cuja morte é, provavelmente, geneticamente programada, em um processo de apoptose, para a produção de hormogônios ou ramificações falsas (Graham & Wilcox, 2000). As cianobactérias cocoides, produzem baeócitos, que se dividem sucessivas vezes, em múltiplos planos de divisão, sem crescer entre tais divisões (Lee, 2008).

1.1.3 - PRODUÇÃO DE TOXINAS

Algumas cianobactérias são capazes de produzir toxinas, *i. e*. cianotoxinas, como metabólitos secundários (van Apeldoorn, 2007), as quais são classificadas de acordo com o seu mecanismo de ação, podendo ser hepatotóxicas, neurotóxicas, dermatotóxicas ou promotoras da inibição da síntese de proteínas (Carneiro, 2008). As toxinas tendem a bioacumular em diferentes níveis tróficos, levando à biomagnificação trófica, uma vez que elas persistem na água mesmo após o desaparecimento da floração (Sarma, 2013).

Gêneros formadores de *blooms* incluem membros que produzem toxinas, como, por exemplo, *Anabaena* Bory ex Bornet & Flahault, (1886), *Anabaenopsis* V.V.Miller (1923), *Aphanizomenon* A.Morren ex É.Bornet & C.Flahault (1886 '1888'), *Cylindrospermopsis* G.Seenayya & N.Subba Raju (1972), *Microcystis* Lemmermann (1907), *Nodularia* Mertens ex Bornet & Flahault (1886), *Nostoc* Vaucher ex Bornet & Flahault (1886), *Oscillatoria* Vaucher ex Gomont (1892), *Planktothrix* Anagnostidis &

Komárek (1988), *Phormidium* Kützing ex Gomont (1892) e *Raphidiopsis* F.E.Fritsch & F.Rich (1929) (Codd, 2004; Sarma 2013). Gêneros formadores de biofilme e de tapetes também possuem membros toxigênicos, como *Phormidium* (Kützing ex Gomont), *Oscillatoria* Vaucher ex Gomont e *Lyngbya* C.Agardh *ex* Gomont (Sarma, 2013).

1.1.4 - CLASSIFICAÇÃO TAXONÔMICA

A classificação taxonômica das cianobactérias era tradicionalmente realizada apenas através dos caracteres morfológicos (Gomont, 1892; Geitler, 1932; Desikachary, 1959; Komárek & Anagnostidis, 2005). Entretanto, o uso de técnicas modernas, como microscopia eletrônica e análises moleculares para a classificação taxonômica, promoveu muitas revisões taxonômicas em níveis de gênero e supragenérico (Komárek, 2008, 2010a, 2014; Komárek & Komárkova, 2004; Strunecký *et al*. 2011).

A classificação e nomenclatura das cianobactérias segue o Código Internacional de Nomenclatura Botânica (CINB), contudo, por conta da proximidade filogenética com as bactérias, as cianobactérias também têm sido classificadas de acordo com o Código de Nomenclatura das Bactérias (Rippka, 1979; Castenholz 2001), hoje denominado Código Internacional dos Procariotos (CINP). Ambos os códigos são frequentemente usados sem que haja uma coincidência entre eles, por eles possuírem regras diferentes, fazendo com que a conciliação entre eles seja problemática (Oren, 2004; Komárek, 2005).

Ao longo das últimas quatro décadas, características ecológicas, ultraestruturais e evidências moleculares têm influenciado substancialmente o conhecimento acerca das cianobactérias (Wehr & Sheath, 2003). Para garantir uma classificação taxonômica mais verossímil, Hoffman *et al*. (2005) propuseram o uso de uma abordagem polifásica, na qual são utilizadas sequências gênicas aliadas à análise de caracteres morfológicos, ultraestruturais e os dados ecofisiológicos, provê uma identificação mais precisa para a descrição de novos táxons e revisão daqueles já estabelecidos, como apresentado em Taton *et al*. (2006), Li *et al*. (2008), Komárek *et al*. (2009, 2010a, 2014), Moustaka-Gouni *et al*. (2010), Zapomělová *et al*. (2010), Vaz (2014) e Caires *et al.* (2018).

Dentre os grupos descritos a partir de uma abordagem integrativa, encontra-se a ordem Synechococcales. Essa ordem é um grande grupo não-monofilético de cianobactérias, com mais de 70 gêneros, incluindo formas unicelulares e filamentosas. Contudo, não há dados de sequências gênicas para a maioria desses táxons. (Komárek

et al. 2014). A principal característica que diferencia os membros dessa ordem para os representantes de outras ordens é o arranjo parietal dos tilacóides, junto com a forma dos filamentos. Isso por que a ordem Spirulinales também apresenta o mesmo tipo de arranjo tilacoidal, mas os filamentos são em forma de espiral (Komárek *et al.* 2014). Dentro da ordem Synechococcales encontram-se as famílias Pseudanabaenaceae Anagnostidis *&* Komárek (1988) e Leptolyngbyaceae Komárek *et al*. 2014, que representam o caráter não-monofilético dos representantes desse grupo.

Na família, Pseudanabaenaceae um dos gêneros mais representantativos é *Pseudanabaena* (Lauterborn)*,* contendo 110 espécies válidas, enquanto a família Leptolyngbyaceae é constituída por 14 gêneros com 60 espécies válidas (Komárek *et al*. 2014; Guiry & Guiry 2017). Ambas as famílias apresentam tricomas cilíndricos, unisseriados e isopolares, com variação apenas entre os diâmetros celulares dos seus representantes. Além destas características morfológicas similares, a presença de bainha facultativa e ausência de aerótopos (Komárek & Anagnostidis 2005) tornam esses grupos difíceis de distinguir somente através da morfologia.

1.2 - ESTUDOS SOBRE CIANOBACTÉRIAS MARINHAS BENTÔNICAS NA COSTA BRASILEIRA

O litoral brasileiro possui aproximadamente 9.000 km de extensão - entre as latitudes 4°N e 34°S (Short & Klein, 2016), provendo grande quantidade de habitats (e.g. recifes de corais, manguezais) (Leão & Dominguez, 2000), os quais possibilitam a ocorrência de cianobactérias bentônicas. Os morfotipos marinhos bentônicos filamentosos formam colônias macroscópicas, comumente, uniespecíficas e, algumas vezes, florações potencialmente nocivas (Suda *et al*. 2013). As cianobactérias marinhas bentônicas ocorrem largamente em ecossistemas lênticos e lóticos, com hábitos epipélicos, episâmicos e epilíticos, ou epifitando algas filamentosas, musgos ou plantas vasculares (Wehr & Shear, 2003).

As cianobactérias bentônicas do litoral brasileiro têm sido pouco estudadas e sem uma grande abrangência de estudos deste grupo, levando a um desconhecimento da real biodiversidade do grupo no Brasil (Crispino & Sant'Anna, 2006). Dentre as cianobactérias marinhas bentônicas comumente excluídas dos levantamentos florísticos para a costa brasileira, encontra-se a família Pseudanabaenaceae. Esta família apresenta

apenas cinco espécies referidas para este ambiente de acordo com o sítio *online* Flora do Brasil (2020), o que pode não condizer com a real diversidade desta família para o litoral do Brasil.

Considerando a escassa quantidade de trabalhos sobre a família Pseudanabaenaceae para o ambiente marinho brasileiro, atrelado ao tamanho da costa do país e à quantidade de hábitats que ela oferece, pressupõem-se um enorme déficit no conhecimento a respeito desse grupo potencialmente nocivo de cianobactérias.

Segundo Komárek *et al*. (2014), uma taxonomia na qual as espécies são bem caracterizadas usando uma abordagem polifásica, é fundamental para a construção de gêneros monofiléticos, como observado em Caires *et al*. (2018), na proposição do novo gênero marinho bentônico de cianobactéria *Neolyngbya*. A existência de linhagens diferentes filogeneticamente, mas que apresentam caracteres morfológicos convergentes são, algumas vezes, difíceis de serem evidenciados sem os dados moleculares, reforçando a importância de tal abordagem para a construção de relações filogenéticas verossímeis.

2 - Objetivos:

1. Caracterizar o táxon encontrado com base em aspectos morfológicos, ultraestruturais, moleculares e ecológicos; para uma delimitação específica mais precisa do mesmo;

2. Contribuir para o conhecimento da diversidade de cianobactérias marinhas bentônicas da costa brasileira

CAPÍTULO ÚNICO

Description of new genera and specie of marine homocytous cyanobacteria from the Brazilian coast

Valter Loureiro de Araujo[1]*, José Marcos de Castro Nunes[1], Taiara Aguiar Caires[1]

[1]Universidade Federal da Bahia. Laboratório de Algas Marinhas (LAMAR), Instituto de Biologia, Campus de Ondina. CEP 40210-730, Salvador, Bahia, Brasil.

*****Author to contact and adress : Email: valterloureiro@hotmail.com (Valter Loureiro de Araujo); Laboratório de Algas Marinhas, Instituto de Biologia, Universidade Federal da Bahia, Rua Barão de Jeremoabo, s/n, Salvador, BA, 40.170-115, Brasil; Telefone: 55 (71) 3283-6598 e Fax: 55 (71) 3283-6511.

Artigo formatado de acordo com as normas da revista *ALGAE* (www.e-algae.org)

ABSTRACT

Cyanobacteria is a group particularly challenging to classify because of its long and complex evolutionary history, and had its classification performed on morphological characters. Its evolutionary history is better reflected using an integrative approach combining morphological, ultrastructural and molecular data, because once the convergent evolution occurs, it often leads to taxonomical errors. This study aimed to analyze the genetic and morphological features of a Brazilian strain of cyanobacteria. The ALCB 125750 strain was collected at Pirambúzios beach (Rio Grande do Norte state, Brazil) (06°00'44.62"S x 35°06'26.48"W). The strain was put in SWBG-11 liquid medium for cultivation, under 14:10h (light:dark) photoperiod and temperature of 25±1°C. To molecular analyzes, DNA extraction were realized followed by PCR to amplify the 16S rRNA. PCR products were cloned using the pGEM®-T Easy Vector Systems. To phylogenetic analyzes, the sequence obtained was added to the sequence matrix acquired from NCBI. Ultrastructural analysis was realized on transmission electronic microscopy. ALCB 125750 strain constituted a clade with a "Filamentous cyanobacterium" sequence from USA (GenBank GQ243430). Ultrastructural analysis, highlighted the presence of thylakoids arrangement parietally to the cross-wall. This data with the filamentous form put our strain into family Pseudanabaenaceae. Morphological features, such as the apical cell morphometry, marine habitat, and epiphytic habit, aren't found in any other genus of this family, suggesting the recognition of new genus and two species, one of which obtained in this study.

Key words: Biodiversity, Cyanobacteria; Marine Environments; Phylogeny; Taxonomy

Abreviations: TEM – Transmission Eletronic Microscopy; PCR – Polimerase Chain Reaction; NCBI – National Center for Biotechnology Information.

INTRODUCTION

Cyanobacteria is a group particularly challenging to classify mostly because of its long and complex evolutionary history (having appeared between 2.7 to 3.5 Bya). Because of this, the morphological identification between individuals of this group became difficult (Schopf and Packer, 1987; Broks et al. 1999; Komárek et al. 2014).

The Brazilian tropical coast offers one of the most diverse habitats that propitiate the occurrence of many cyanobacterial eco and morphotypes (Leão and Dominguez, 2000). However this area is very poorly studied, thus very little of the diversity and distribution of these organisms are known. The cyanobacteria constitute a very important component in a variety of communities, as epiphytic forms, epilithic and also as microbial mats including coral reefs (Charpy et al. 2012).

The marine benthic morphotypes form macroscope colonies, commonly unispecific and sometimes potentially harmful blooms (Suda et al. 2013). In this kind of environment, the morphological variations are the result of the characteristic of site where they occur, such as luminosity, water temperature and substrate. Cyanobacterial classification was performed based mostly on morphological characters (Gomont, 1892; Geitler, 1932; Desikachary, 1959; Komárek and Anagnostidis, 2005), however similar morphotypes can occur under very different ecological conditions, while different morphotypes can occur under the same ecological conditions, which generates a taxonomical confusion (Kling and Watson, 2003).

The classification system of this group was radically modified with the introduction of electronic microscopy and genetic methods to cyanobacterial taxa characterization, (Komárek et al. 2014). Hoffmann et al. (2005) suggested an integration between

morphological, ultrastructural and molecular data, which they denominated *polyphasic approach*, to better reflect the evolutionary inside cyanobacteria. However, this approach hasn't been used to examine the homocyted form of marine cyanobacterial strains, however has been mostly applied to examine the filamentous heterocytous form (Genuário et al. 2017).

Despite the length of the Brazilian coast, and the importance of a polyphasic characterization to study cyanobacteria, works with homocyted cyanobacteria are extremely rare, condition is not exclusive to Brazilian marine environment, to the marine environment worldwide, there is an equally poor number of published works, who deals with the homocytous form. Due the poor number of research, the size of the Brazilian coast and the quantity of habitats that's provided by them, there's a gap about the knowledge of this group, underestimating its real diversity.

This underestimated diversity highlights the necessity of further investigating cyanobacterial species using combined tools to show the real biodiversity of the homocyted cyanobacteria on the Brazilian coast. Thus, the goal of this study is describe a new genus and a new species (ALCB125750) using a polyphasic approach.

MATERIALS AND METHODS

Cyanobacterial cultivation and sampling

The sampling was realized in the tropical region of the Brazilian coast at Pirambúzios beach (Rio Grande do Norte state) (06°00'44.62"S x 35°06'26.48"W). The collecting was performed in mediolittoral zone during spring tide. Metal spatula was used to collect the epilithic samples. The refractometer (Biobrix® 211) was used to measure the water salinity. The samples was stored in seawater and put in liquid medium SWBG-11

after being transported to the laboratory (Rippka et al. 1979). To stimulate the growth of the strain, vitamin B_{12} (2 $mL \cdot L^{-1}$ of a stock 1 mg mL^{-1}, Sigma Aldrich®) was added to the medium. Filaments were isolated and put to grow under a photoperiod of 14:10 (light:dark), and irradiation of 30 µmol photons m^{-2} s^{-1} (fluorescent white bulbs), and temperature of de 25 ±1°C (Jacinavicius et al. 2012). All isolated strains are kept in cultivation chambers maintained at the Biotechnology Laboratory (LaBBiotec) at the Universidade Federal da Bahia (UFBA), Brazil. Part of the strains were preserved at formaldehyde (4%) and laid up at Alexander Leal Costa Herbarium (ALCB), at the same institution.

Morphological and ultrastructural analyzes

To morphological and ultrastructural analyzes, the strains were studied under a microscope with QImaging GO-3 digital câmera (Olympus CX31RTS5®, Tokyo, Japan). The filaments of every strain were measured using the AxioVs40 v4.8.2.0 imaging system (Carl Zeiss, Jena, Germany) program. To the Transmission Electronic Microscopy (TEM) the cyanobacteria filaments were fixed overnight in Karnovsky (final concentration: 2% of glutaraldehyde, 0,05 M of sodium cacodylate – pH 7.2, 0,001 M calcium chloride and paraformaldehyde at 2%) (Karnovsky, 1965). Biomass was washed three times with 0.01 M sodium cacodylate buffer and post-fixed with 2% osmium tetroxide in the same buffer for 2 h at room temperature. Using the same buffer, we repeated the washing, and the biomass was maintained at 4°C in 2.5% uranyl acetate overnight. We carried out with dehydration of the filaments with acetone series (30, 50, 70% for 5 min each, and 90 e 100% for 20 min each), and embedded the filaments in Poly/Bed® 812 Embedding Kit resin (Polysciences, Warrington, USA). We obtained ultrathin sections (ultramicrotome; Leica®, Mannheim, Germany), which were post-

stained with 2.5% uranyl acetate and lead citrate. We analyzed the samples with a JEOL® 1230 transmission electron microscope (JEOL, Tokyo, Japan).

The identification of genus and species was accomplished using specific literature for the group. The classification system used was the proposed by Komárek *et al.* (2014).

Molecular and phylogenetic analyzes

The strain was macerated in liquid nitrogen for the genomic DNA extraction following Fiore et al. (2000). In PCR reactions, almost complete16S rRNA and 16S-23S ITS regions was successfully amplified using the primers 27F1 (Neilan et al. 1997) and 23S30R (Taton et al. 2003), in a Veriti® 96 -Well Thermal Cycler (Applied Biosystems®, Foster City, California, USA). For PCR reactions, we used 10 ηg of genomic DNA, 0.5 µM of each primer, 200 µM of dNTPs, 2.0 mM of MgCl2, l × PCR buffer, and 1.5 U Taq DNA polymerase (Invitrogen, São Paulo, Brazil); the total volume was 25 µL. Amplifications were achieved under the following conditions: one cycle of 5 min at 94 °C; 10 cycles of 45 s at 94 °C, 45 s at 57 °C, and 2 min at 72 °C; 25 cycles of 45 s at 94 °C, 45 s at 54 °C, and 2 min at 72 °C; the final elongation step of 7 min at 72 °C. The PCR products were analyzed by running agarose gels (1 % w/v), using the Low DNA mass ladder (Invitrogen, Carlsbad, CA, USA), and they were cloned using the pGEM-T Easy vector system (Promega, Madison, WI, USA), according to the manufacturer's protocol. For blue-white colony screening, *Escherichia coli* chemo-competent DH5α cells were transformed and plated (Sambrook and Russell, 2001). Plasmids containing gene fragments were extracted using the alkaline lysis method (Birnboim and Doly, 1979). We sequenced plasmidial DNA with the plasmidial primers M13F/M13R, and the 16S rRNA with the follow internal primer sets to cover

137 the entire fragment: 357F/357R, 704F/704R and 1114F/1114R (Lane, 1991). Genetic
138 sequencing was done at Instituto Gonçalo Muniz (FIOCRUZ – Bahia State/Brazil).

139 To compose de matrix data, 63 sequences of the representatives of the orders of
140 cyanobacteria were obtained from GenBank (www.ncbi.nlm.nih.gov) following the
141 division by Komárek et al. (2014) were used to the phylogenetic analyzes. The total of
142 64 operational taxonomic unities was achieved. The bacteria *Escherichia coli* was
143 selected as the outgroup.

144 The evolutionary model (GTR+I+G) was identified by Mr.Modeltest 2.3 (Nylander,
145 2008) using the Akaike Information Criterion (AIC) (Posada and Crandall, 1998). The
146 reconstruction of the phylogenetic trees by the Maximum Likelihood (ML) algorithm
147 (Stamatakis *et al.* 2008) was done using the RaxML 8.2.10 (Stamatakis, 2006), which
148 was tested with 1.000 bootstrap. MrBayes 3.1 software (Ronquist et al. 2012) was used
149 to do the Bayesian analyzes (Mau *et al.* 1999). The results generated by this
150 phylogenetic analyze were expressed in bootstrap percentages (BP) to Maximum
151 Likelihood, and posterior probability (PP) to the Bayesian analyzes. Values less than
152 75% are not shown.

153 RESULTS

154 Based on a polyphasic characterization, we described a new genus of homocytous
155 marine cyanobacteria which is included in Pseudanabaenaceae family. This genus
156 includes two new species, one from USA and another from the Brazilian coast. Only the
157 Brazilian new species is described in this study. The new species from the USA based
158 on the "Filamentous cyanobacterium" sequence (GenBank GQ243430), is not described
159 in present study, because we could not access the sample for either morphological,

ultrastructural nor ecological analyzes, because the study that describes this "Filamentous cyanobacterium" was not published yet.

Morphological and Ultrastructural Analyzes

Genus nov. ALCB 125750 (Pseudanabaenaceae, Synechococcales) V. L. Araujo, J.M.C. Nunes, T. A. Caires

Diagnosis*:* thallus forming green mats, filaments straight to curved, unbranched, cells always short, apical cells slightly to intensively rounded.

Species nov. ALCB 125750 (Pseudanabaenaceae, Synechococcales) V. L. Araujo, J.M.C. Nunes, T. A. Caires (Fig. 1)

Description

Thallus forming mat olive-green to moss-green. Straight filaments, isopolar, unbranched. Trichomes slightly constricted at the cross-walls, 0,8-1,12 µm diam. Short cells 1,58 - 4,28 µm long, 2,5 times longer than large. Thylakoids arranged parietally. Apical cells slightly rounded.

Holotype: Brazil, Rio Grande do Norte state, Nísia Floresta city, Pirambúzios beach, 06°00'44.62"S x 35°06'26.48"W. Coll. T.A.Caires 640. 15 April 2014 (ALCB 125750).

Habit and habitat: epipsammic or epiphytic in protected areas in mediolittoral zone. This species occurred epiphyting an algae of genus *Bostrichia.*

Ultrastructural notes: thylakoids are arranged parietally to the cell wall. Cells slightly constricted at the cross-wall.

Phylogenetic Analyses

The 16S rRNA sequence obtained in this study was aligned with 63 other sequences of representatives of Cyanobacteria orders obtained from GenBank (Table 2). Our phylogenetic results show that our new genus based on ALCB 125750 strain forms an independent clade in the Maximum Likelihood (ML) and Bayesian posterior probability (BP) analyses, with 88% and 100% of support values, respectively, emerging as monophyletic group with high supports (Figure 2).

DISCUSSION

Concerning to results of the ultrastructural analysis, it showed that our strain has parietal arrangement of thylakoids, such as described for Synechococcales by Komárek *et al.* (2014). Alone, this characteristic is not very helpful to identify in which order our new genus ALCB 125750 must be included, because other genera also possess this thylakoidal arrangement, *e. g. Spirulina*, *Gloeothece* and *Synechococcus*, all three belonging to different orders, the first two belongs to the orders Spirulinales, and *Synechococcus* belongs to Synechoccocales. (Komárek and Kaštovsk, 2003; Komárek *et al.* 2014). However, the members of the order Spirulinales are known to have trichomes in coiled form, while our new genus has straight trichomes. Thus, the form of the trichome combined with the parietal arrangement of thylakoids put our new genus ALCB 125750 into the order Synechococcales.

The new genus ALCB 125750 shows some morphological features similar to the ones found inside the family Pseudanabaenaceae (Anagnostidis *et* Komárek), such as the solitary or in colony trichomes, forming mats, straight e without sheaths and the cells are longer than wider, and cylindrical (Table 1). These similar characters make the identification of *Pseudanabaena*–like morphotypes very puzzling, demonstrating that

only morphological characters are not trustworthy for generic and specific identification. We ran an identification key, based on Komárek & Anagnostidis (2005) and the result shows that ALCB 125750 present characteristics that are quite similar to species that belongs to the genus *Pseudanabaena*, notedly *P. catenata* (Lauterborn), and *P. minima* (G., S., An) Anagnostidis. However, ALCB 125750 presents morphometric characteristics that none of the known *Pseudanabaena* species possess (Table 1).

The family Pseudanabaenaceae is a clade that, according with Komárek & Anagnostidis (2005), is characterized by parietal thylakoids, trichomes without sheaths or only singly in one sheath, small trichomes (typically less than 4 µm). Within this family, individual genera are typically circumscribed based on a single morphological character (Perkerson III, 2010).

In relation to the ecological characteristics, our new species ALCB 125750 was collected in a marine benthic environment as an epiphyte on *Bostrychia* sp. red algae, while *Pseudanabaena catenata* is found in freshwater, as an epipelic, sapropelic or periphytic in freshwater. *Pseudanabaena minima* is found in ponds, moist soils, swamps or salt fields, as benthic and epipelic. None of these two species has yet been cited for the Brazilian marine benthic environment, which demonstrates the importance of ecology on the characterization of this new genus and species.

Phylogenetic Analyses

The genus ALCB 125750 formed a supported clade in the phylogenetic analyses, backing up the establishment of this new taxon (Fig 2). This clade (colored in green) showed high values of bootstrap and Posterior Probability (88% and 100%, respectively). The clade of the new genus is composed by the species: ALCB 125750

and a “Filamentous cyanobacterium” sequence (GenBank GQ243430) from the USA. The “Filamentous” cyanobacterium” was not analyzed because the type material has not been published.

Bayesian and Maximum Likelihood analysis of 64 OTUs clearly exhibited the separation of ALCB 125750 from other genera in the Synechococcales order (colored in gray), such as *Leptolyngbya* (Anagnostidis & Komárek), *Haloleptolyngbya* (P.K.Dadheech, H.Mahmoud, K.Kotut & L.Krienitz), *Halomicronema* (Abed, Garcia-Pichel & Hernández-Mariné) and *Nodosilinea* (R.B.Perkerson & D.A.Casamatta).

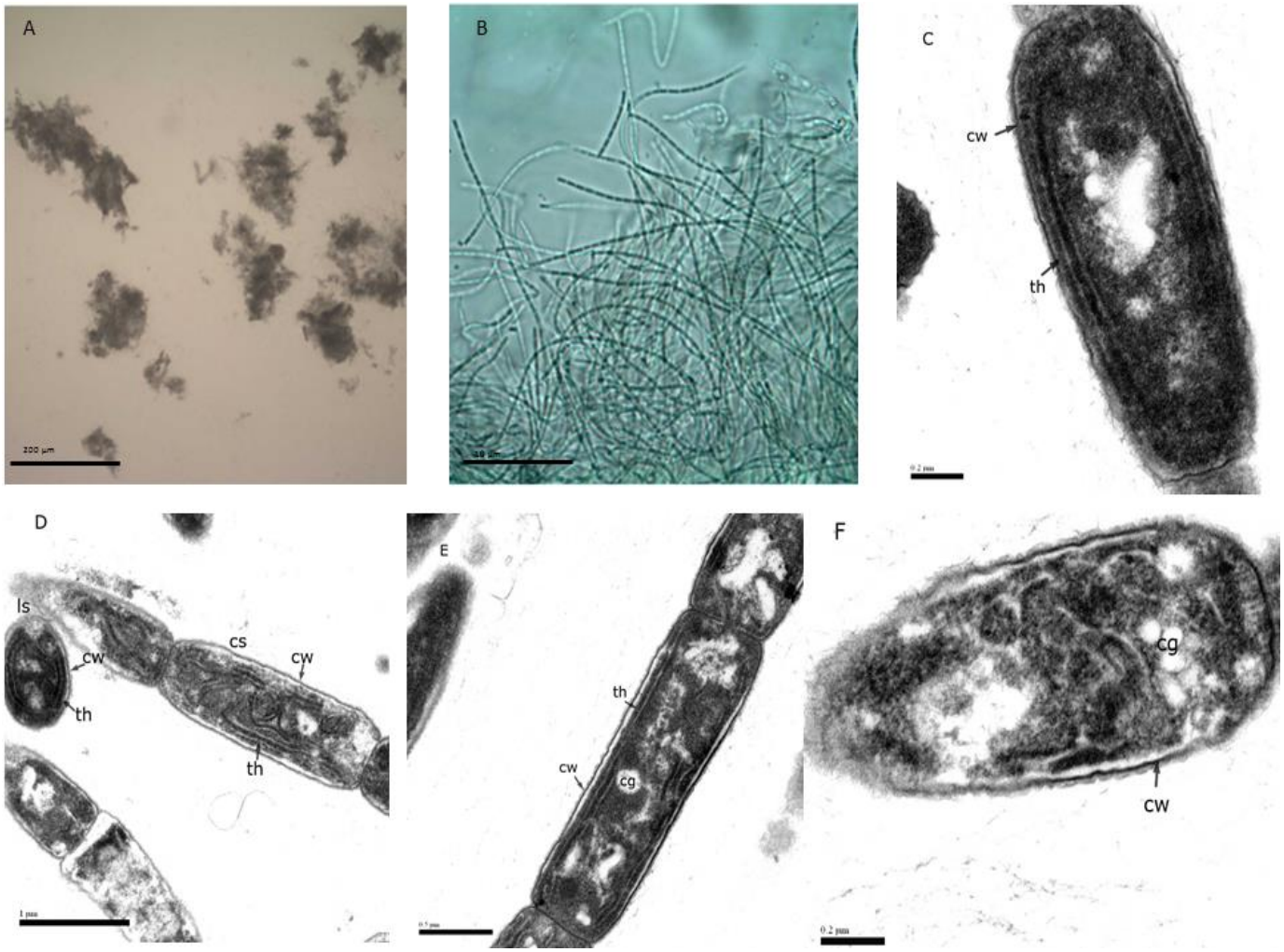
The presence of members of the genera *Leptolyngbya* and *Pseudanabaena* in distinct clades throughout the tree, including as the sister group (colored in brown) of ALCB 125750, corroborate the polyphyletic condition of those groups (Castenholz, 2001; Komárek and Anagnostidis, 2005), showing that unrelated lineages possess highly comparable morphological features, which can be the result of convergent evolution, and not common descendance (Engene et al. 2011). According to Perkerson III et al. (2010), the systematic and taxonomic problems within cyanobacteria occur due to convergent evolution and the lack of genera-level autapomorphies. Moreover, Komárek et al. (2014) put a light over both groups regarding their polyphyletic condition and the lack of revision of them.

CONCLUSION

The combination of genetic, ultrastructural, and morphological features, it’s a very useful way to provide different evidence that allowed us to highlight differences infra and interspecific. The use of polyphasic approach allowed us to recognize and describe a well-supported genus and species ALCB 125750, with two species. The species

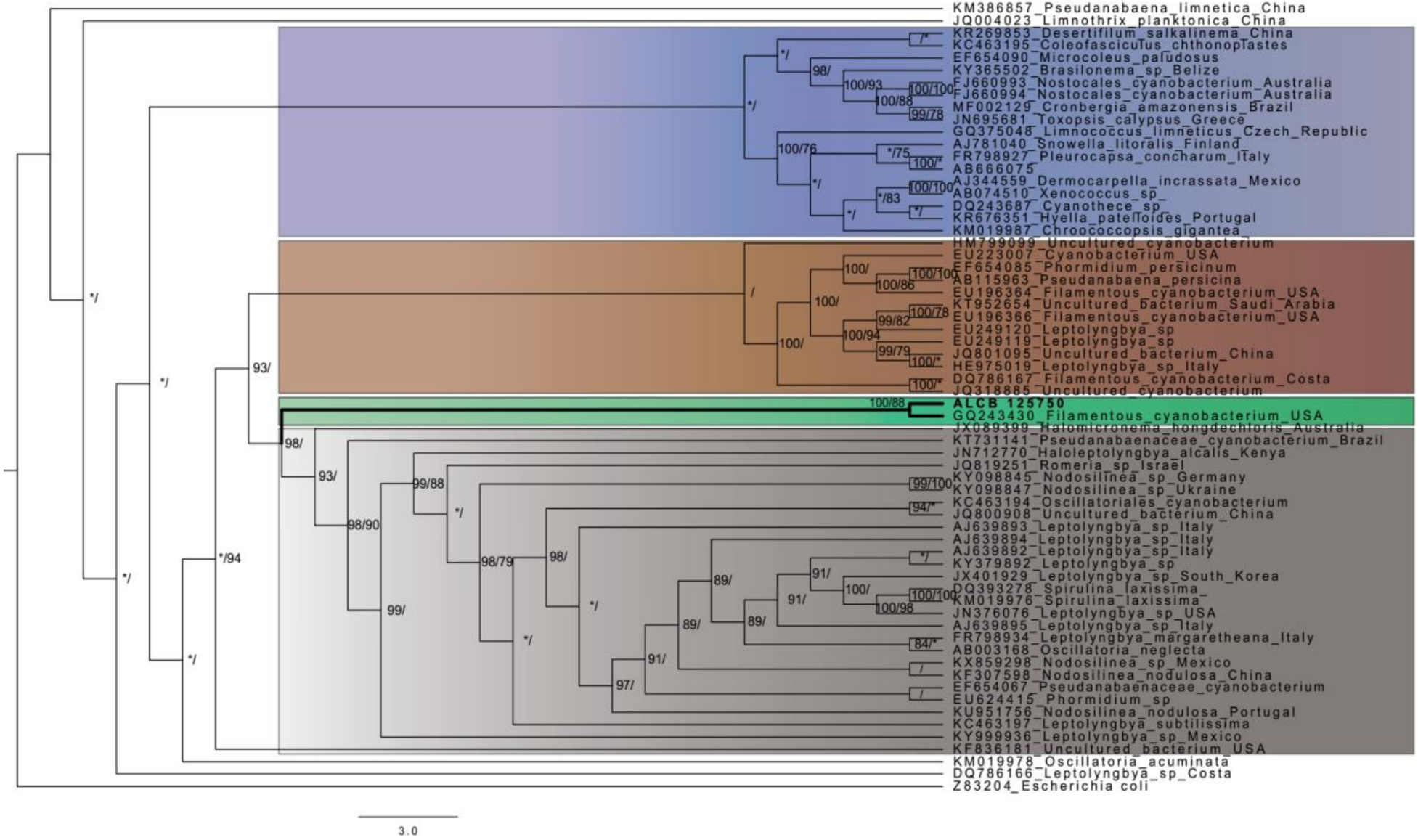
ALCB 125750 is characterized by its small cell size, its marine benthic habit and the disposition of thylakoids. We also corroborate the polyphyly of the genus *Leptolyngbya*, that is present throughout the phylogenetic tree, except in the clade in brown. Molecular, ecological and ultrastructural data about the homocytous cyanobacteria are not abundant due the lack of studies to unveil the biodiversity of this cyanobacterial group. This setting makes the taxonomy of cyanobacteria very challenging.

Acknowledgments To CNPq/CAPES (National Council for Scientific and Technological Development/Coordination of Personal Enhancing in Superior Education) for the scholarship granted to the first author.



259

260 **Fig 1.** Characterization of the new genus and species ALCB 125750. **A**.
261 General aspects of the mat. **B**. Detail of the trichomes.**C-F**. Ultrastructural
262 sections (TEM). **C**. arrangement of the thylakoids (th), parietally to the cross-
263 wall (cw). **D**. Cross section (cs) and longitudinal sections (ls) of filaments. **E**.
264 Detail of the arrangement of the thylakoids (th), parietally to the cross-wall (cw).
265 **F**. The arrow indicates the cyanophycin granules (cg). Cell walls (cw);
266 thylakoids (th); cyanophycin granules (cg). Scale bars: A= 200 µm; B= 10 µm;
267 C=0,2 µm; D= 1 µm; E=0.5 µm; F= 0,2 µm



268 **Fig 2**. Maximum likelihood (ML) phylogeny based on the 16S rRNA gene sequences of Cyanobacteria (ntax = 64). Values are ML bootstrap values
269 (right) and Bayesian posterior probabilities (PP) converted to percentages (left). Values under 75% are not shown. New sequence obtained in this study
270 is indicated in bold; other sequences from Genbank, in which the access numbers are followed by names. * = clade not supported. Blue= Clade formed
271 by representants of the order Nostocales. Brown= Sister group of the genus ALCB 125750. Green= Genus generated in this study. Gray =
272 Representants of the order Synechococcales.

Table 1. Comparison between ALCB 125750 and *Pseudanabaena* species who related to it (Komárek & Anagnostidis, 2005). NI=Not informed

Characteristics	*P. catenata* Lauterborn 1915	*P. minima* (G.S. An.) Anagnostidis 2005	ALCB 125750 sp. nov
Filament	Trichome solitary or aggregated in small mats	Trichome solitary, or crowded in clusters, straight or almost straight,	
Filament width (µm)	1,2-2 (2.2) wide	1.3-2,5 wide	0,8-1,12 wide
Sheath		NI	
Trichom	Strongly constricted; ungranulated;	Intensely constrict	Slightley constrict
Trichom width (µm)	40-200 long	1.5-4 long	1,58-4,28 long
Cells: form and lenght (µm)	Cylindrical, 1.5x3xlonger than wide; rarely isodiametric; (1.5) 2-6 (8-10?)	1.5x longer than wide,	2,5x longer than wide
Apicall cell	*Rounded or slightly conic*	Widely rounded	Slightly rounded
Environment	Freshwater, benthic,	Moist soils, benthic and epipelic in ponds, springs, swamps and salty fields	Marine, benthic

286 **Table 2**. Taxa included in the analysis, with locality and/or environment information, reference publication, and GenBank
287 accession numbers. The taxa sequenced in this study is presented in bold.

Taxon	Locality &/or Environment	Voucher/strain	Reference publication	GenBank accessions
Outgroup				
Escherichia coli		562/	Ridell *et al.* (1995)	Z83204
Ingroup				AB666075
640	**Pirabúzios beach, Nísia Floresta city, Rio Gr&e do Norte state, BRazil**	**ALCB 125750**	**This Study**	
Snowella litoralis (Häyrén) Komárek & Hindák	Finland	371757/0tu37s04	Rajaniemi-Wacklin,P. *et al*. (2006)	AJ781040

Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O.Komárek & Zapomelová	Czech Republic: Svet	665911/Svet 06	Komarkova,J. *et al*. (2010)	GQ375048
Microcoleus paludosus Gomont		450472/SAG 1449-1a	Siegesmund,M.A. *et al*. (2008)	EF654090
Chroococcopsis gigantea Geitler		1521635/SAG 12.99/	Friedl,T. Unpublished	KM019987
Coleofasciculus chthonoplastes		1352644/EcFYyyy500	Dojani,S. *et al*. (2013)	KC463195

(Thuret ex Gomont) M.Siegesmund, J.R.Johansen & T.Friedl				
Toxopsis calypsus	Greece: Peloponnese	1108047/PLF	Lamprinou, V. *et al*. (2012)	JN695681
Lamprinou, Skaraki, Kotoulas, Economou-Amili & Pantazidou				
Cronbergia amazonensis	Brazil	1983329/CMAA1598	Genuario, D., B. (2018)	MF002129
Genuário, Sant'Anna & I.S.Melo				

Hyella patelloides Ramos, Brito *et* Kaštovsky	Portugal: Moledo do Minho beach	945734/LEGE 07179	Brito, A. *et al*. (2017)	KR676351
Dermocarpella incrassata Lemmermann	Mexico	54303/SAG 29.84; PCC 7326	Fewer, D. *et al*. (2002)	AJ344559
Haloleptolyngbya álcalis P.K.Dadheech, H.Mahmoud, K.Kotut & L.Krienitz	Kenya: Lake Nakuru	1169407/KR 2005/106	Dadheech, P. K. *et al.* (2012)	JN712770
Brasilonema sp.	Belize	1980447/BZ	Johansen, J., R. *et*	KY365502

		-HDL-007	*al*, (2017)	
Desertifilum salkalinema	China	1736058/CH AB7200	Cai, F., -F. *et al*. (2017)	KR269853
F.-F.Cai & Li, nom. inval (holotype = *Desertifilum tharense* Dadheech & Krienitz).				
Pleurocapsa concharum Hansgirg	Italy:Firenze, Villa La Pietra	980868/VP3 -02	Cuzman, O., A. *et al*, (2010)	FR798927
Cyanothece sp.		354330/	Zhang, Y. Unpublished	DQ243687

Xenococcus sp.		179406/PCC 7307	Seo,P. & Yokota,A. Unpublished	AB074510
Limnothrix planctonica (Woloszynska) Meffert	China	1131003/CHAB753	Zhu,M.L. *et al*. (2012)	JQ004023
Pseudanabaena limnetica (Lemmermann) Komárek	West Lake, Hangzhou	1572394/CHAB774	Yu,G. Unpublished	KM386857
Pseudanabaenaceae cyanobacterium		450516/isolate=DPG1-KK5	Siegesmund, M. A., *et al*. 2008	EF654067

Nodosilinea nodulosa (Z.Li & J.Brand) Perkerson & Casamatta	China: Hong Kong, South China Sea	416010/UTEX B 2910	Muhlsteinova, R. *et al.* (2014)	KF307598
Leptolyngbya sp.	Italy:Emilia-Romagna, Imola, Bubano Basin	271630/0BB30S02″	Castiglioni, B. *et al*. (2005)	AJ639892
Uncultured bacterium	Saudi Arabia	77133/clone=AG2_B08	Miyake, S. *et al*. (2016)	KT952654
Filamentous cyanobacterium	USA: NN Dry Rocks Reef, Northern Florida Keys	492282/73-2	Myers, J., L. & Richardson, L., L. (2009)	EU196366
Uncultured cyanobacterium	Puerto Rico Trench	1211/clone=PRTBB8722	Eloe, E., A., *et al*. (2011)	HM799099
Filamentous	Costa Rica	397329/LLi7	Finsinger, K. &	DQ786167

cyanobacterium		1	Hess,W., R. Unpublished	
Leptolyngbya sp.	South Korea	1229172/KIOST-1	Kim, J., H., Unpublished	JX401929
Leptolyngbya sp.	Italy:Formentera	1221563/CCAP 1442/1	Gachon, C., M., M. Unpublished	HE975019
Filamentous cyanobacterium	USA: Great Salt Lake, Utah	658598/GSL035	Beer, L., L. Unpublished	GQ243430
Leptolyngbya sp.	Mexico: Cuatrocienegas	1983256/CCM 4″	Barrera-Rojas, J. Unpublished	KY999936
Nostocales cyanobacterium	Australia: Heron Isl&	994809/HI15	Dominguez-Escobar, J., (2011)	FJ660993
Leptolyngbya subtilíssima		1352645/EcFYyyy700	Dojani, S. *et al*. (2013)	KC463197

(Hansgirg) Komárek				
Uncultured bacterium	China: Yellow River Delta	77133/clone =BJGMM-3s-467	Jia,F. *et al*. Unpublished	JQ801095
Phormidium sp.		545217/SA G 61.90	Siegesmund, M., A. *et al*, (2008)	EU624415
Leptolyngbya sp.	Bahamian marine stromatolite	487779/HB C1	Foster, J., S. *et al*. (2009)	EU249120
Leptolyngbya sp.	Italy:Emilia-Romagna, Imola, Bubano Basin	271632/0BB 32S02	Castiglioni, B. *et al*, (2005)	AJ639894
Uncultured cyanobacterium		1211/clone= T-Pc3	Jin, H. *et al*. Unpublished	JQ318885

Pseudanabaenaceae cyanobacterium	Brazil: Bertioga/SP	1716595/CENA320	Alvarenga, D., O., *et al*, (2016)	KT731141
Filamentous cyanobacterium	USA: Horseshoe Reef, Northern Florida Keys	492280/FLK9	Myers, J., L. *et al*. (2009)	EU196364
Nodosilinea sp.	USA: California, Imperial Valley	1115757/BL0902	Taton, A. *et al*. (2012)	JN376076
Cyanobacterium	USA: Florida, Northern Florida Keys, Conch Shallow Reef	481519/62-2	Myers, J., L. *et al*. (2009)	EU223007
Nodosilinea sp.		1963213/isolate=Prim-5-5	Mikhailyuk, T. *et al*. Unpublished	KY098847
Leptolyngbya		1930147/RK	Albrecht, M., *et al*.	KY379892

sp.		_2_3	(2017)	
Nodosilinea sp.	Mexico	1902582/PC 471	Vazquez-Martinez, J. Unpublished	KX859298
Uncultured bacterium	China: Yellow River Delta	77133/clone =BJGMM-3s-118	Jia, F. *et al*. Unpublished	JQ800908
Leptolyngbya sp.	Italy:Emilia-Romagna, Imola, Bubano Basin	271633/0BB 19S12	Castiglioni, B. *et al*. (2005)	AJ639895
Uncultured bacterium	USA	77133/ clone=INDI_ S_SPR_11H _(2)	Reihle, J. Unpublished	KF836181
Spirulina laxíssima G.S.West		1236702/ SAG 256.80	Friedl, T. Unpublished	KM019976

Halomicronema hongdechloris Chen, Li, Birch & Willows	Australia: Shark Bay	1641165/C2206	Chen, M. (2012)	JX089399
Leptolyngbya sp.		487783/HBC8	Foster, J., S. *et al*. (2009)	EU249119
Pseudanabaena persicina (Reinke ex Gomont) Anagnostidis		402770/SAG 80.79	Siegesmund, M. A., *et al*, (2008)	EF654085
Romeria sp.	Israel: Hula Nature Reserve	1192302/ KLL-H-201	Alster, A. Unpublished	JQ819251
Nostocales cyanobacterium	Australia: Heron Isl&	994810/ HI14	Dominguez-Escobar, J. *et al.* (2011)	FJ660994

Leptolyngbya sp.	Costa Rica	397324/LLi18	Finsinger, K. & Hess,W.R. Unpublished	DQ786166
Oscillatoriales cyanobacterium		1352668/EcFYyyy400	Dojani, S. *et al.* (2013)	KC463194
Leptolyngbya margaretheana (G.Schmid) Anagnostidis & Komárek	Italy:Firenze, Piazza SS. Annunziata	980873/1T12	Cuzman, O., A. *et al.* (2010)	FR798934
Nodosilinea sp.	Germany: Warnemuende, Mecklenburg-Vorpommern, coast of Baltic Sea	1963211/isolate=WD-4-2	Mikhailyuk, T. *et al.* Unpublished	KY098845
Leptolyngbya	Italy:Emilia-Romagna,	271631/0BB	Castiglioni, B. *et*	AJ639893

sp.	Imola, Bubano Basin	24S04	*al.* (2005)	
Oscillatoria neglecta Lemmermann		454132/ IAM M-82	Ishida, T. *et al.* Unpublished	AB003168
Nodosilinea nodulosa (Z.Li & J.Brand) Perkerson & Casamatta	Portugal: Praia da Luz	1828761/LE GE 06101	Ramos, V. *et al,* Unpublished	KU951756
Spirulina laxíssima G.S.West		1828761/SA G 256.80	Choi, G.,-G. *et al.* Unpublished	DQ393278
Oscillatoria acuminata PCC		56110/SAG 1449-3	Friedl, T. *et al.*	KM019978

6304		Unpublished	
Pseudanabaena persicina (Reinke ex Gomont) Anagnostidis	945773/SAG 80.79	Chaw, S. *et al.* Unpublished	AB115963

REFERENCES

Birnboim, H.C. & Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research 7:1513-1518.

Bohunická M., Johansen, J., R and Fučíková, K.. 2011. *Tapinothrix clintonii* sp. nov. (Pseudanabaenaceae, Cyanobacteria), a new species at the nexus of five genera. Fottea 11:127–140.

Brocks, J.J.; Logan,G.A.; Buick, R.; Summons, R.E. 1999. Archean Molecular Fossils and the Early Rise of Eukaryotes. Science 285:1033-1036.

Caires, T., A. 2013. Cianobactérias Marinhas Bentônicas Filamentosas Do Litoral Do Estado Da Bahia, Brasil. Dissertação de Mestrado em Ciências – Instituto de Biologia da Universidade Estadual de Feira de Santana.

Caires, T., A. 2017. Caracterização polifásica e avaliação do potencial biotecnológico do complexo *Lyngbya* (cyanobacteria) em ambientes marinhos costeiros do brasil. Tese de doutorado em Botânica –Universidade Estadual de Feira de Santana.

Caires, T. A., de Mattos Lyra, G., Hentschke, G. S., de Gusmão Pedrini, A., Sant'Anna, C. L., & de Castro Nunes, J. M. 2018. Neolyngbya gen. nov.(Cyanobacteria, Oscillatoriaceae): A new filamentous benthic marine taxon widely distributed along the Brazilian coast. Mol. Phylo. Evol 120:196-211.

Charpy, L., Casareto, B., E., Langlade, M. J., Yoshimi. S. 2012. Cyanobacteria in coral reef ecosystems: a review. J. Mar. Biol 9:1-9.

Desikachary. T.V. 1959. Cyanophyta. ICAR Monographs, Nova Deli. 686 pp.

Fiore, M.F., Moon, D.H., Tsai, S.M., Lee, H. & Trevors, J.T. 2000. Miniprep DNA isolation from unicellular and filamentous cyanobacteria. Journal of Microbiological Methods 39:159-169.

Geitler, L. 1932. Cyanophyceae. In: Rabenhorst, L. (ed). Kryptogammenflora von Deutschland, Osterreich, under de Sweitz. Leipzig: Akademische Verlagsgesellschaft. 673-1056.

Genuário, D.B., Vaz, M.G.M.V., Melo, I., S. 2017. Phylogenetic insights into the diversity of homocytous cyanobacteria from Amazonian rivers Molecular Phylogenetics and Evolution 116: 120–135

Genuário, D., B., Souza, W., R., Monteiro, R., T., R., Sant´Anna C., L., Melo, I., S. 2018. *Amazoninema* gen. nov., (Synechococcales, Pseudanabaenaceae) a novel cyanobacteria genus from Brazilian Amazonian rivers. International Journal of Systematic and Evolutionary Microbiology 68:2249-2257.

Gomont, M. 1892. Monographie des Oscillariées (Nostocacées homocystées). Annales Sciencias Naturales Botany 15: 263-368, 16: 91-264.

Jacinavicius, F.R., Gama Júnior, W.A., Azevedo, M.T.P. & Sant'Anna, C.L. 2012. *Manual para cultivo de cianobactérias*. 1st ed. Publicações Online do Instituto de Botânica de São Paulo, São Paulo, 32 pp.

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol. 27:137.

Komárek, J. and Kaštovsk, J. 2003. Coincidences of structural and molecular characters in evolutionary lines of cyanobacteria. Algological Studies 109: 305–325.

Komárek, J.; Anagnostidis, K. 2005. Cyanoprocaryota. 2. Teil: Oscillatorialles. Spektrum, Akademische Verlag (Süβwasserflora von Mitteleuropa; Band 19/2), Berlim. 759 p.

Komárek, J., Kaštovský, J., Mareš, J., Johansen, J., R. 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86:295–335.

Mau, B., Newton, M.A. & Larget, B. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. Biometrics 55:1–12.

Neilan, B.A., Jacobs, D., DelDot, T., Blackall, L.L., Hawkins, P.R., Cox, P.T. & Goodman, A.E. 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. Int. J. Syst. Bacteriol. 47:693–697.

Nylander, J.A.A. 2008. *MrModeltest 2.3*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Pearson, H., W., Howsley, R., Kjeldsen, C., K., Walsby, A., E. 1979. Aerobic nitrogenase activity associated with a non-heterocystous filamentous cyanobacterium. FEMS Microbiol Lett 5:163–167

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol 111:1-61.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61:539-847.

Sambrook, J. & Russell, D.W. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York.

Schopf, J., W., & Packer, B., M. 1987. Early Archean (3.3-Billion to 3.5-Billion-Year-Old) Microfossils from Warrawoona Group, Australia. Science 3:237:70-3.

Stal, L., J. & Krumbein, W., E. 1985. Nitrogenase activity in the non-heterocystous cyanobacterium *Oscillatoria* sp. grown under alternating light-dark cycles. Arch. Microbiol. 143:67.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688-2690.

Suda, S., Moriya, R., Sumimoto, S., Ohno, O., Suenaga, K. 2013. Genetic diversity of filamentous cyanobacteria from shore regions of Okinawa. Journal of Marine Science and Technology 21:175-180.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R. & Wilmotte, A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl. Environ. Microb 69: 5157–5169.

Wasmund, N., Voss, M., Lochte, K. 2001. Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and re-calculation of a budget of nitrogen fixation. Mar Ecol Prog Ser 214:1–14.

Zuker, M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31: 3406-3415.

References cited exclusively in Table S1

Albrecht, M., Proschold, T. & Schumann, R. 2017. Identification of Cyanobacteria in a Eutrophic Coastal Lagoon on the Southern Baltic Coast. Front Microbiol 8:923.

Alvarenga, D., O., Rigonato, J., Branco, L., H., Melo, I., S. & Fiore, M., F. 2016. *Phyllonema aviceniicola* gen. *et* sp. nov. and *Foliisarcina bertiogensis* gen. et. sp. nov., novel epiphyllic cyanobacteria associated with *Avicennia schaueriana* leaves. Int. J. Syst. Evol. Microbiol 66:689-700.

Brito, A., Ramos, V., Mota, R., Lima, S., Santos, A., Vieira, J., Vieira, C., P., Kastovsky, J., Vasconcelos, V., M. & Tamagnini, P. 2017. Description of new genera and species of marine cyanobacteria from the Portuguese Atlantic coast. Mol. Phylogenet. Evol. 111:18-34.

Cai,F.-F., Chen,Y.-X., Zhu,M.-L., Li,X.-C. & Li,R.-H. 2017. Desertifilum salkalinema sp. nov. (Oscillatoriales, Cyanobacteria) from an alkaline pool in China. Phytotaxa 292:262-270.

Castiglioni, B., Rizzi, E., Frosini, A., Sivonen, K., Rajaniemi, P., Rantala, A., Mugnai, M., Ventura, S., Wilmotte, A., Boutte, C., Grubisic, S., Balthasart, P., Consol&i, C., Bordoni, R., Mezzelani, A., Battaglia, C. & De Bellis, G. 2005. Development of a universal microarray based on the ligation detection reaction & 16S rRNA gene polymorphism to target diversity of cyanobacteria. Microbiology 70;7161-7172.

Chen, M., Li, Y., Birch, D. & Willows, R., D. 2012. A cyanobacterium that contains chlorophyll f - a red-absorbing Photopigment. FEBS Lett. 586:3249-3254.

Cuzman, O., A., Ventura, S., Sili, C., Mascalchi, C., Turchetti, T., D'Acqui, L., P. & Tiano, P. 2010. Biodiversity of phototrophic biofilms dwelling on monumental fountains. J. Microb. Ecol 60:81-95.

Dadheech, P., K., Mahmoud, H., Kotut,K. & Krienitz,L. 2012. *Haloleptolyngbya alcalis* gen. et sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya. Hydrobiologia 691:269-283.

Dojani, S., Kauff, F., Weber, B. & Buedel, B. 2013. Genotypic & Phenotypic Diversity of Cyanobacteria in Biological Soil Crusts of the Succulent Karoo and Nama Karoo of Southern Africa J. Microb. Ecol. In press.

Dominguez-Escobar, J., Beltran, Y., Bergman, B., Diez, B., Ininbergs, K., Souza, V. & Falcon, L., I. 2011. Phylogenetic and molecular clock inferences of cyanobacterial strains within Rivulariaceae from distant environments. FEMS Microbiol. Lett 316:90-99.

Eloe, E., A., Shulse, C., N., Fadrosh, D., W., Williamson, S., J., Allen, E., E., & Bartlett, D., H. 2011. Compositional differences in particle-associated and free-living microbial assemblages from an extreme deep-ocean environment. Environ Microbiol Rep 3:449-458.

Fewer, D., Friedl, T. & Budel, B. 2002. Chroococcidiopsis and heterocyst-differentiating cyanobacteria are each other's closest living relatives. Mol. Phylogenet. Evol. 23:82-90.

Foster, J., S., Green, S., J., Ahrendt, S., R., Golubic, S., Reid, R., P., Hetherington, K., L. & Bebout, L. 2009. Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. ISME J 3:573-587.

Genuario, D., B., Sant'Anna, C., L. & Melo, I., S. 2018. Elucidating the Cronbergia (cyanobacteria) dilemma with the description of Cronbergia amazonensis sp. nov. isolated from Solimoes river (Amazonia, Brazil). Algal Res 29:233-241.

Johansen, J., R., Mares, J., Pietrasiak, N., Bohunicka, M., Zima, J., Jr., Stenclova, L. & Hauer, T. 2017. Highly divergent 16S rRNA sequences in ribosomal operons of *Scytonema hyalinum* (Cyanobacteria) PLoS ONE 12: e0186393. https://doi.org/ 10.1371/journal.pone.0186393.

Komárková, J., Jezberová, J., Komárek, O. & Zapomělová, E. 2010. Variability of Chroococcus (Cyanobacteria) morphospecies with regard to phylogenetic relationships. Hydrobiologia 639:69-83.

Lamprinou, V., Skaraki, K., Kotoulas, G., Economou-Amilli, A., & Pantazidou, A., I. 2012 Toxopsis calypsus gen. nov., sp. nov. (Cyanobacteria, Nostocales) from cave 'Francthi', Peloponnese, Greece: a morphological and molecular evaluation. Int. J. Syst. Evol. Microbiol. 62:2870-2877.

Miyake, S., Ngugi, D., K., & Stingl, U. 2016. Phylogenetic Diversity, Distribution, and Cophylogeny of Giant Bacteria (Epulopiscium) with their Surgeonfish Hosts in the Red Sea. Front Microbiol 7:285.

Myers, J., L. & Richardson, L., L. 2009. Adaptation of cyanobacteria to the sulfide-rich microenvironment of black band disease of coral. FEMS Microbiol. Ecol. 67:242-251.

Muhlsteinova, R., Johansen, J., R., Pietrasiak, N., Martin, M., P., Osorio-Santos, K., & Warren, S., D. 2014. Polyphasic characterization of *Trichocoleus desertorum* sp. nov. (Pseudanabaenales, Cyanobacteria) from desert soils and phylogenetic placement of the genus Trichocoleus. Phytotaxa 163:241-261.

Perkerson III, R., B., Perkerson, E., A. & Casamatta, D., A. 2010. Phylogenetic examination of the cyanobacterial genera Geitlerinema and Limnothrix (Pseudanabaenaceae) using 16S rDNA gene sequence data. Algological Studies 134:1–16.

Rajaniemi-Wacklin, P., Rantala, A., Mugnai,M. A., Turicchia, S., Ventura, S., Komarkova, J., Lepisto, L. & Sivonen, K. 2006. Correspondence between phylogeny and morphology of *Snowella* spp. and *Woronichinia naegeliana*, Cyanobacteria commonly occurring in lakes. J. Phycol. 42:226-232.

Ridell, J., Siitonen, A., Paulin, L., Mattila, L., Korkeala, H., & Albert, M., J. 1994. Characterization of Hafnia alvei by biochemical tests, random amplified polymorphic DNA PCR, and partial sequencing of 16S rRNA gene. J. Clin. Microbiol 32:2335-2337.

Siegesmund, M., A., Johansen, J., R., Karsten, U., & Friedl, T. 2008. Coleofasciculus Gen. Nov. (Cyanobacteria): Morphological and Molecular Criteria For Revision Of The Genus Microcoleus Gomont(1) . J. Phycol 44:1572-1585.

Taton, A., Lis, E., Adin, D., M., Dong, G., Cookson, S., Kay, S., A., Golden, S., S. & Golden, J., W. 2012. Gene Transfer in Leptolyngbya sp. Strain BL0902, a Cyanobacterium Suitable for Production of Biomass & Bioproducts. 7: e30901. doi:10.1371/journal.pone.0030901

Zhu, M., L., Yu, G., L., Li, X., C., Tan, W. & Li, R., H. 2012. Taxonomic and phylogenetic evaluation of Limnothrix strains (Oscillatoriales, Cyanobacteria) by adding Limnothrix planktonica strains isolated from central China. Hydrobiologia 698:367-374.

5 – ANEXOS

Normas da Revista ALGAE

INSTRUCTIONS FOR AUTHORS

ALGAE

EDITORIAL POLICY

Manuscripts may be submitted in the form of Research Articles. Research Notes describing new techniques or equipment are welcome; they should include data that illustrate the usefulness of the technique. Reviews are generally solicited, but interested authors are encouraged to contact the Editor-in-Chief. Authors should make appropriate depositions of materials to accession services (e.g., culture collections, herbaria, GenBank, etc.) to permit future work by the scientific community.

Manuscripts submitted for publication are independently reviewed by at least two referees. However, manuscripts that clearly do not meet the criteria for publication may be rejected by the Editor-in-Chief and/or the Associate Editor concerned without being sent for review. The Editor-in-Chief is responsible for all decisions regarding publication. In most cases, a decision will be made in consultation with an Associate Editor. The manuscript must be prepared according to the instructions that follow; those that do not conform or are incomplete may be returned for correction prior to review.

Copyright

The acceptance regulations of a manuscript for publication automatically include the consent of the author(s) to transfer the copyright or license to the Korean Society of Phycology. Authors will complete a Copyright Agreement Form (CAF) at the time of proofreading. The corresponding author can sign on behalf of co-authors. The CAF can be downloaded from the Manuscripts Center (http://www.e-algae.org/), or contact the editorial office (ALGAE Editorial Office, Department of Oceanography, Chonnam National University, Gwangju 500-757, Korea) to obtain a form. Acceptance of the agreement will ensure full copyright protection, and disseminate the article to the widest possible readership in print and electronic formats as appropriate. Authors are themselves responsible for obtaining permission to reproduce copyright material from other sources.

Disclaimer

Publisher, editors, reviewers and authors do not accept any legal responsibility for errors, omissions or claims, nor do they provide any warranty, express or implied, with respect to information published in ALGAE.

http://www.e-algae.org

ETHICS

Research published in ALGAE must have been conducted in accordance with institutional, national and international guidelines concerning the use of animals in research and/or the sampling of endangered species. For the policies on research and publication ethics that are not stated in these instructions, the *Guidelines on Good Publication Practice* can be applied (available from: http://publicationethics.org/resources/guidelines).

Authorship

All authors must have materially participated in the research and/or article preparation. Individuals listed as authors must: (1) agree to be listed; (2) have contributed to the research reported; (3) approve the submitted version of the manuscript. The statement that all authors have approved the final article should be true and included in the disclosure.

Conflict of Interest Statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to pharmaceutical companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

Originality and Duplicate Publication

All submitted manuscripts should be original and should not be under consideration by other scientific journals for publication at the same time. No part of the accepted manuscript should be duplicated in any other scientific journal without the permission of the Editorial Board. If duplicate publication related to the papers of this journal is detected, the authors will be announced in the journal their institutes will be informed, and there will be penalties for the authors.

MANUSCRIPT SUBMISSION

Submissions must be original in that the information is not copyrighted, published or submitted elsewhere, except in abstract form, i.e. they have not published before; must have been approved by all parties immediately involved, e.g. authors or institutional authorities; and must meet ethics guidelines (see ethics). If the manuscript has previously been submitted to any publication outlet, this must be disclosed and a rationale for its submission to ALGAE provided. Submission of a manuscript implies agreement to ALGAE editorial terms of publication, including the transfer of copyright to the Korean Society of Phycology and the online posting of a prepress abstract. Authors are encouraged to submit new manuscripts, and revisions, electronically. Acceptable electronic formats are MS-Word and HWP. Pages and lines must be numbered. All fonts must be embedded in the file, which must not contain any security settings.

All manuscripts must be submitted via the ALGAE editorial office, either online, by email, or by post. Submission of manuscripts to ALGAE is normally done through our online manuscript submission and review system, ALGAE Manuscript Center, provided by the Korean Society of Phycology. Manuscripts will be handled by the Editor-in-Chief or one of the Associate Editors. Authors are invited to specify their preference and (in their cover letter) to identify 3-5 suitable referees.

Online Manuscript Submission System

Please submit your article via ALGAE Manuscript Center.
New users should first create an account. Once a user is logged on to the site, the submission should be made via the Manuscript Center. Full instructions are provided, and additional support is available online. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission, these source files are needed for further processing after acceptance. Each manuscript should be accompanied by a cover letter containing a brief statement by the authors as to the novelty upon which they base their request for publication in ALGAE. The authors may indicate the names, full postal addresses, telephone and fax numbers, and e-mail addresses of three to five impartial potential peer reviewers. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used. There are no special instructions for formatting letters to the editor, except that they must be prepared in MS Word for online submission. Authors without high speed internet access, please contact the Editorial Office for immediate assistance. Authors can track the progress of their manuscripts and will be notified when referee and editor comments are available online. Use of the ALGAE Manuscript Center will facilitate the transfer of manuscripts between the Editor-in-Chief or Associate Editors and reviewers.

E-mail Submission

Send the article to the Editor-in-Chief (kykim@chonnam.ac.kr). Attach two files – 1) the cover letter, which must include the response to reviews if the submission is a revision; and 2) the manuscript – and send the email to the above address. Hard copies are not required unless electronic submission is impossible. In that case, manuscripts may be submitted – as one hard copy and one electronic file on disc – by post.

By Post

Send to ALGAE Editorial Office, Department of Oceanography, Chonnam National University, Gwangju 61186, Korea. Tel: +82 62 530 3465 Fax: +82 62 530 0065

MANUSCRIPT PREPARATION

General Requirements

The target length of Research Articles is approximately 10 journal pages (note: estimate 3 typed pages of text for every printed journal page; about 4,000 words). Authors preparing papers in excess of 15 journal pages should contact the Editor-in-Chief first. Research Notes of 1–3 printed pages are acceptable. Use the same general format as that of Research Articles. Review Articles can be up to 20 printed pages and can include several levels of headings.

Manuscripts must be prepared in a 12-point font size and double-spaced throughout (including references, tables and legends) with margins of at least 3 cm all round. Times New Roman, Courier or Helvetica fonts are preferred. All pages should be numbered serially, with the first page a title page, the second an abstract, followed by the text, references, tables, and legends for figures, in that order. It is also suggested that you number the lines of your manuscript to facilitate the review/editing process. Do not right justify or divide words at the ends of lines. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced when the article is processed. In particular, do not embed "graphically designed" equations or tables, but prepare these using the word processor alone.

The Abstract and Results should be written in past tense, except in the case of descriptions of new species. Latin names of algal species studied should have the taxonomic author(s) of the name listed when first used (but not other species cited from the reference). To find the taxonomic author(s), check the Algaebase Web site (http://www.algaebase.org). To see the accepted form of the author's name, please consult with the International Plant Names Index (http://www.ipni.org/index.html). Consult the latest issue of the journal for other questions regarding style. New taxa must be established in accordance with the latest International Code of Botanical Nomenclature (for more information on this, see below).

Research Articles

Research Articles should be subdivided into six sections:

INTRODUCTION, MATERIALS AND METHODS, RESULTS (or OBSERVATIONS), DISCUSSION, ACKNOWLEDGEMENTS, and REFERENCES, although this may not be appropriate for some articles (such as some taxonomic papers). To avoid unnecessary errors, authors are strongly advised to use the "spell-check" and "grammar-check" functions of their word processor. Do your very best to use correct English grammar, spelling and punctuation; if you are not a native English speaker, you should have the text edited by someone, as the editors cannot always be expected to carry out major linguistic revision.

Research Articles should be prepared according to the following format:

1. The title page should contain a concise title, the name(s) and address(es) of the author(s), any necessary footnotes, and a short running title suitable for page headings.

• *Title.* Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. The title should have not more than 100 characters (ca. 15 words, 2 lines in print), and 150 characters at most. If the name of an organism is used in the title, an indication of its taxonomic position must be given. Nomenclatural authorities should only be used in titles when nomenclatural changes are being proposed.

• *Author names and affiliations.* Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after the author's name and in front of the appropriate address for identification. Provide a valid address with ZIP or postal number for each affiliation, including the country name.

• *Corresponding author.* Clearly indicate who will handle correspondence at all stages of refereeing and publication, as well as post-publication. Ensure that telephone and fax numbers (with country and area code) are provided, in addition to the e-mail address and the complete postal address.

• *Present/permanent address.* If an author has moved since the work described in the article was done, or was visiting at the time, a "Present address" (or "Permanent address") may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main affiliation address. Superscript letters are used for such footnotes.

• *Running title.* Provide a condensed running head with 3 to 6 words.

2. The ABSTRACT should not be more than 250 words and should be concise, informative and intelligible without reference to the main text. It should indicate the objectives, main results and conclusions of the paper. Do not repeat information in the title or make reference to the literature. Authorities for species names should be included in the abstract only for primarily taxonomic papers. Begin the abstract by stating the scientific question of concern. Explain the methods used to tackle the question. The results should be outlined briefly and put into a concise, broad perspective. Immediately after the abstract, list 5 to 8 **Key Words** (arranged alphabetically and separated by semicolons), using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and" or "of"). Include a section of **Abbreviations** after the keywords. List the abbreviation, followed by its meaning written out in full (e.g., DIN, dissolved inorganic nitrogen). Common abbreviations (e.g., DNA) do not need to be listed. The journal accepts standard abbreviations from the *Journal of Biological Chemistry*. All non-standard abbreviations should be listed alphabetically. The abbreviation is spelled out at first mention in the main text, and thereafter, only the abbreviation/acronym is used.

3. The INTRODUCTION must define the problem within the context of existing knowledge, state the objectives of the work and provide an adequate background; avoid a detailed literature survey or a summary of the results. It should not be a general review of the field, but it should provide essential background for those who are not experts in the particular area.

4. MATERIALS AND METHODS

Describe the methodology used in the study in sufficient detail to allow the work to be reproduced by another scientist. Methods already published should be indicated by a reference, and only relevant modifications should be described. Whenever possible, give sources of materials in detail. If cultures are used, indicate the strain or clone number and the source. If study sites are mentioned, provide latitudes/longitudes. For materials and supplies (including software), indicate the source (company name, city, state, country) on first reference. In addition, list the model number for equipment used, as appropriate. Use metric units (*Systéme International d'Unités*, SI) and SI style (e.g., μmol photons m^{-2} s^{-1}, μg L^{-1}). For description of laboratory procedures, the terms liter (abbreviated to L) and milliliter (mL) may be used. Do not use dots or full-stops between parts of the term. For more information on this see below.

5. RESULTS

Only results pertinent to the subject may be included. Data must not be repeated in figures and tables. Rationale for undertaking certain aspects of the investigation, methods, techniques, and so on, must be excluded. As far as possible, cite each figure and table in the text in order of presentation (e.g., Figure 1 before Figure 2). When tables or figures are presented, cite only the significant results in the text. Tables of specimens studied or gene sequences used should be placed in supplementary materials. Photographs should only be included if necessary to illustrate results. Include statistical analyses or other indicators to enable assessment of the variance of replicates of the experiments. Names of new taxa must be followed immediately by the Latin description or, preferably, diagnosis (using only essential characters), with citation of holotype. For new combinations, basionyms should be cited in full.

For further guidance on taxonomic papers, please see the web site (http://www.algae.kr/layout_taxonomy).

6. DISCUSSION

This should explore the significance of the results in relation to the problem outlined in the introduction - not repeat them. Reference to illustrative material should be minimal and should be provided only when necessary to emphasize a specific interpretation. A **CONCLUSION** should be added if results and discussion are combined.

7. ACKNOWLEDGEMENTS

This is to be used as necessary to acknowledge the source of financial grants in completing the study. The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

8. REFERENCES

In the text, refer to the author's name (without initial) and year of publication. When reference is made to a work by two authors, both names should be given using "and" (e.g., Lee and Kang 1986); for three or more author names, give the first author followed by "et al." and the year (e.g., Kim et al. 2010). Multiple references must be arranged in chronological order (e.g., Lee and Kang 1986, Kim 1990, Kim et al. 2000*a*, 2000*b*). Only cite articles or books already published or in press, not unpublished work "in preparation". The author is responsible for verifying the accuracy of unpublished citations.

In the list at the end of the paper, references in the **REFERENCES** section should be typed, double-spaced and in alphabetical order, with multiple references by the same author(s) arranged chronologically. If an author's name in the list is also mentioned with co-authors, the following order should be used: publications of the single author, arranged according to publication dates; publications of the same author with one co-author; publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 2000*a*, 2000*b*, etc. For Volume (Vol.) and Bulletin (Bull.), Arabic numerals should be used; the full number of pages should be given in the form of pp. 123-128. Type references flush left with an extra space between citations; they will

be formatted properly at the press. The name of the journal should be abbreviated according to the *World List of Scientific Periodicals* and the selection according to *Abbreviated Titles of Biological Journals* issued by the Biological Council or the *Biosis Serial Sources*, which is published each December and is available in most libraries (or go to http://www.library.uq.edu.au/faqs/endnote/biosciences.txt). If in doubt about any journal abbreviation, write out the journal title in full. The number of a fascicle in brackets after the volume number should be given only if the volume is not paginated consecutively. The titles of publications in non-Latin alphabets should be transliterated, and a notation such as "(in Korean)" or "(in Greek, with English abstract)" should be added. Work accepted for publication but not yet published should be referred to as "in press". References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text. All publications cited in the text should be presented in a list of references following the text of the manuscript. The manuscript should be carefully checked to ensure that the spelling of author's names and dates are exactly the same in the text as in the reference list.

The style to be used for references is as follows:

Journal articles

Kim, K. H. & Lee, I. K. 2000. Mixed-phase reproduction in *Antithamnion sparsum* Tokida (Ceramiaceae, Rhodophyta) from Korea. Algae 15:183-193.

Mann, H., Mann, S. & Fyfe, W. S. 1987. Aragonite crystals in *Spirogyra* sp. (Chlorophyta). J. Phycol. 23:506–509.

Articles from books, conference reports, symposium proceedings, etc.

Give the title of the chapter, the editor(s) and title of the volume, the publisher and place of the publisher (not the location where the conference was held), and the pages of the chapter. The date cited must be the year of publication (not the year in which the conference was held).

Example :

Sheath, R. G. & Hambrook, J. A. 1990. Freshwater ecology. *In* Cole, K. M. & Sheath, R. G. (Eds.) *Biology of the Red Algae*. Cambridge University Press, Cambridge, pp. 423-453.

Conover, J. T. & Sieburth, J. McN. 1966. Effect of tannins excreted from Phaeophyta on planktonic animal survival in tide pools. *In* Young, E. G. & McLachlan, J. L. (Eds.) Proc. 5th Int. Seaweed Symp., Pergamon Press, Oxford, pp. 99–100.

Book

Write the title of the book in lower case, and give the publisher, place of publication and pages. In the case of a book series, give the series editor as well.

Example :

van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995. *Algae: an introduction to phycology*. Cambridge University Press, Cambridge, 623 pp.

Dissertations

Write the title in lower case, 'MS / PhD thesis / dissertation', and give the university and its location.

Example :

Boo, S. M. 1985. A systematic study on six tribes of eramiaceae (Rhodophyta, Ceramiales) in Korea. Ph.D. dissertation, Seoul National University, Seoul, Korea, 446 pp.

9. Tables

Authors should take notice of the limitations set by the size and layout of the journal. Large tables should be avoided. Reversing columns and rows will often reduce the dimensions of a table. If many data are to be presented, an attempt should be made to divide them over two or more tables. Tables should be created using the Table function in the word processor (rather than using tabs). Tables should have a descriptive title at the top of each table. The title and table of contents must be double-spaced throughout, in 12-point font, and on a separate numbered page. Tables should be numbered consecutively with Arabic numerals according to their sequence in the text. The text should include references to all tables. Tables should not include vertical lines or shading; if either is essential, the material must be submitted as a figure for direct reproduction. Column headings should be brief, with units in parentheses. Tables and their captions should be self-explanatory; e.g., abbreviations and acronyms must be defined again. Any explanation essential to understanding the table should be given as a footnote at the bottom of the table, not in the heading. Include in the footnotes all non-standard abbreviations used and enough information for the table to be understood without undue recourse to the text. For table footnotes, use superscripted lower case letters (^{a}, ^{b}, ^{c}) rather than symbols or numbers; asterisks (*, **, ***) can be used to indicate statistical significance. These can be applied in the case of figures. Statistical measures such as SD or SEM should be identified in the headings. If a table

provides data on biological species, its legend should begin with the full Latin name of that species.

10. Figures

Line drawings, diagrams and photographs should be planned so that after reduction they will fit within either the width of one column (8 cm) or two columns (17 cm), and be no more than 25 cm in length. Normally, only previously unpublished illustrations are acceptable. Figures should be numbered in Arabic numerals consecutively as they are mentioned in the text (Fig. 1), (Figs 2 & 3), (Figs 1-4), etc. Each figure should be submitted as a separate Tagged Image Format (TIFF), Encapsulated PostScript (EPS) or MS Office files created at a resolution of 300 dpi (or 600 dpi for combination images). In the case of very large files, Joint Photographic Experts Group (JPEG) or Graphics Interchange Format (GIF) files may be submitted initially. Do not import the figures into the text file, but, instead, indicate their approximate locations directly on the manuscript. Combination images, or images that contain both line artwork (vector graphics) and halftones or photographs (bitmap graphics), must be supplied at a higher resolution to prevent image quality loss in the bitmap graphics, such as in the symbol keys. Please do not attempt to increase the resolution of a lower resolution figure by resaving it at 300 dpi or higher. The result may be pixilated or grainy images and poor text quality. Photographs (halftones) will not be reduced or enlarged; thus, they must be printed to fit in one column or two columns in width and be no more than 18–20 cm (8 inches) in length so that the legend will fit on the same page as the illustration. Plan line drawings and graphs to fit these dimensions after reduction, and with all lines, symbols and lettering bold enough to permit 1/2 to 2/3 reduction in size; many line drawings are reduced to the width of a single column. All terms, abbreviations, and symbols should correspond to those used in the text of the paper. It is preferable to include a symbol key on line drawings. Component figures within a plate should be labeled with letters, not numbers and should not be encircled (e.g., a, b, c, not 1, 2, 3, ①, ②, ③). Only use the following fonts in your illustrations: Arial, Courier, Times, Symbol. Do not use two shades of gray in histograms.

Color photographs may be printed at the expense of the author after consultation with the Editor-in-Chief. A color plate fund is available to assist the publication of color plates where these are considered essential. This is based on scientific need for color, as established during peer review. Authors should consult the editorial office with respect to color reproduction at the submission stage. Any figure submitted as a color original may appear in color within the journal's online edition. For color reproduction in print, you will receive information regarding the costs from the editorial office after receipt of your accepted article. Please indicate your preference for color in print or on the Web only.

Legends must be typed double-spaced, listed or in paragraph form, and placed together on a separate sheet in the manuscript document you submit. Each legend should comprise a brief title (not on the figure itself) and enough information for interpretation of the figure, with all abbreviations used. All terms, abbreviations, and symbols should correspond with those used in the text. List scales at the end of the legend for each plate as follows: Scale bars represent: Fig. 1, 20 mm; Figs 2-4, 50 nm.

11. Electronic Supplements

Additional material of importance to an article may be included for possible publication on the ALGAE website (http://www.e-algae.org) as an electronic supplement. This might include overlong tables or appendices and will be linked from the online article. Materials should be submitted as a file(s) separate from the main manuscript document. Tables and figures should be numbered S1, S2, etc.

Online Version

Manuscripts will appear simultaneously on the online version of the journal at http://www.e-algae.org/. Abstracts are accessible online to the public; individual members and subscribing libraries can view the full contents of the journal through a password system. See subscription information at http://www.e-algae.org/ for details.

Digital Object Identifier

The digital object identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. When you use the DOI to create URL hyperlinks to documents on the web, they are guaranteed never to change. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (example taken from an article in Algae 26(1): 1-15): http://dx.doi.org/10.4490.algae.2016.31.00.00.

NOMENCLATURE AND UNITS

Genus and Species Names

Genus and species names must be in italics. Generic names should only be abbreviated when immediately preceded in the text by the mention of the same species or another of the same genus. When referring to a species, do not use the genus name alone, unless you have previously defined it that way; be precise when using 'sp.' (singular) and 'spp.' (plural). All Latin binominals should be followed by the authorities in full, e.g. (J.E. Smith) Setchell & N.L. Gardner, when first used in the title or text (but not in the abstract), unless a large number of names with authorities are grouped in a table. To find the taxonomic author(s), check the Algaebase Web site (http://www.algaebase.org).

Equations and Units

Use standard SI units. Compound units are given with the proper exponent without a period (e.g., gO_2 g^{-1} dw h^{-1}). Relations or concentrations (e.g., mg per l) must be given as 'mg l^{-1}' (not mg/l). Variables are usually italicized (except for Greek letters). Italicization should be consistent in normal, superscript and subscripted text. Leave one blank space on either side of '=', '>', ± etc. where these denote equalities or inequalities. SI (metric) units must be used. Leave a space between numerals and their units (e.g., 10 mm). Example: '$p < 0.05$, $r^2 = 0.879$' (not '$p<0.05$, $r^2=0.879$'); but: 'we studied organisms of size <0.5 µm'.

COVER PHOTOGRAPHS

Particularly good photographs that pertain to a paper being submitted will be considered for use on the cover page. Submit the image file(s) (300 dpi or better resolution, TIFF or EPS format) with the final copy of the manuscript, preferably scaleable to a 12.7 x 17.8 cm (5 x 7 inch) format. Include a brief legend for the inside front cover (send as a separate Word document). See the inside cover of a recent issue for the legend format.

REFERÊNCIAS

Acinas, S., G., Haverkamp T., H.,A., Huisman, J and Stal, L., J. 2009. **Phenotypic and genetic diversification of Pseudanabaena spp. (cyanobacteria)**. International Society for Microbial Ecology. v. 3. p.31–46

Baulina, O., I. 2012. **Ultrastructural plasticity of cyanobacteria**. Springer, Berlin

Bergman, B., Gallon, J.R., Rai, A.N., Stal, L.J. 1997. **N_2 Fixation by non-heterocystous cyanobacteria**. FEMS Microbiology Reviews. v.19. p.139–185.

Brodie & Lewis, 2007 Brodie J, Lewis J (2007) **Unravelling the algae: the past, present, and future of algal systematics**. Systematics Association special volumes, 75. CRC Press, London.

Brooks, J., J., Logan, G., A., Buick, R., Summons, R., E. 1999. **Archean Molecular Fossils and the Early Rise of Eukaryotes**. Science. v. 285, p.1033-1036.

Caires, T., A. 2013. **Cianobactérias Marinhas Bentônicas Filamentosas Do Litoral Do Estado Da Bahia, Brasil**. Dissertação de Mestrado em Ciências – Instituto de Biologia da Universidade Estadual de Feira de Santana.

Caires, T., A. 2017. **Caracterização polifásica e avaliação do potencial biotecnológico do complexo *Lyngbya* (cyanobacteria) em ambientes marinhos costeiros do brasil**. Tese de doutorado em Botânica –Universidade Estadual de Feira de Santana.

Caires, T.A., Sant'anna, C.L., Nunes, J., M., C. 2013**. A new species of marine benthic cyanobacteria from the infralittoral of Brazil: *Symploca infralitoralis* sp. nov.** Brazilian Journal of Botany 36: 159-163.

Caires, T. A., Mattos, G., L., Hentschke, G. S., Pedrini, A., G., Sant'Anna, C. L., & Nunes, J., M., C. 2018. **Neolyngbya gen. nov.(Cyanobacteria, Oscillatoriaceae): A new filamentous benthic marine taxon widely distributed along the Brazilian coast**. Mol. Phylo. Evol 120:196-211

Carneiro, T. G.; Leite, F. **Cianobactérias e suas toxinas**. Revista Analytica. Campinas, v. 32, n. 1, p. 36-41, jan. 2008.

Castenholz, R. W. 2001. Phylum BX. **Cyanobacteria**. In: Boone, D.R.; Castenholz, R.W. (eds). Bergey's Manual of Systematic Bacteriology. New York: Springer. p.: 473–487.

Chen, M., Schliep, M., Willows, R.D., Cai, Z.-L., Neilan, B.A., Scheer, H., 2010. **A redshifted chlorophyll**. Science v.329. p.1318–1319.

Chen, M., Blankenship, R.E., 2011. **Expanding the solar spectrum used by photosynthesis. Trends in Plant** Science. v.16. p.427–431.

Codd, G., A., Morrison L., F., Metcalf, J., S. 2005. **Cyanobacterial toxins: risk management for health protection**. Toxicology and Applied Pharmacology. v.203. p.264–272.

Desikachary, 1959; Desikachary. T.V. 1959. **Cyanophyta**. ICAR Monographs, Nova Deli. 686 pp.

Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. Disponível em: <http://floradobrasil.jbrj.gov.br/>. Acesso em: 31 Out. 2017.

Fredriksson, C., & Bergman, B. 1997. **Ultrastructural characterisation of cells specialised for nitrogen fixation in a non-heterocystous cyanobacterium *Trichodesmium* spp**. Protoplasma. v.197. p.76.

Fogg, G. E. Stewart, W., D., P., Fay, P., Walsby, A., E. 1973. **The Blue-Green Algae**. Academic Press, New York. v.8, p.460.

Gallon J., R., & Stal L., J.1992. **N2 fixation in non-heterocystous cyanobacteria: An overview**. In: Carpenter EJ, Capone DG & Rueter JG (Eds) Marine Pelagic Cyanobacteria: *Trichodesmium* and other Diazotrophs (pp 115–139). Kluwer Academic Publishers, The Netherlands.

Garcia-Baptista, M.; Baptista, L. R. M. 1991. **Algas psâmicas de Jardim BeiraMar, Capão da Canoa, Rio Grande do Sul**. Revista Brasileira de Biologia. v.2. p.325- 342.

Geitler, L. 1932. **Cyanophyceae**. In: Rabenhorst, L. (ed). Kryptogammenflora von Deutschland, Osterreich, under de Sweitz. Leipzig: Akademische Verlagsgesellschaft. 673-1056.

Gomont, M. 1892. **Monographie des Oscillariées (Nostocacées homocystées)**. Annales Sciencias Naturales Botany 15: 263-368, 16: 91-264.

Graham, L., E. & Wilcox, L., W. 2000. **Algae**. Upper Saddle River: Prentice-Hall. 640p.

Guiry, M.D in Guiry, M.D. & Guiry, G.M. 2017. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Acesso em 14 Abr. 2018.

Gupta, V., Ratha S K, Sood, A., Chaudhary, V., Prasanna, R. 2013. **New insights into the biodiversity and applications of cyanobacteria (blue-green algae)—Prospects and challenges**. Algal Research 2, 79–97.

Hoffmann, L., Komárek, J.; Kaštovský, J. 2005. **System of cyanoprokaryotes (Cyanobacteria) – state in 2004**. Algological Studies. v. 117. p.95-115.

Holm-Hansen O. 1968. **Ecology, physiology, and biochemistry of blue-green algae**. Annu Rev Microbiol. 22:47–70.

Kling, H. J., Laughinghouse IV H. D, Šmarda, J., Komárek, J., Acreman, J., Bruun, K., Watson, S., B., & Chen, F. 2012 **A new red colonial *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) from North American large lakes**. Fottea, Olomouc, 12(2): 327–339

Komárek, J. 2005. **The Modern Classification of Cyanoprokaryotes (Cyanobacteria).** Oceanological and Hydrobiological Studies. v. 34. p.05-17

Komárek, J., 2009. **Modern taxonomic revision of planktic nostocacean cyanobacteria: a short review of genera**. Hydrobiologia (2010) 639:231–243

Komárek, J. 2010a. **Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept)**. Hydrobiologia. v.639: p.245–259.

Komárek, J. 2010b. **Modern taxonomic revision of planktic nostocacean cyanobacteria: a short review of genera**. Hydrobiologia. v.639. p.231–243

Komárek, J. 2016. **A polyphasic approach for the taxonomy of cyanobacteria: principles and applications**. European Journal of Phycology, DOI: 10.1080/09670262.2016.1163738

Komárek, J., & Anagnostidis, K., 2005. **Cyanoprokaryota-2. Teil/2nd part: Oscillatoriales**, in: Büdel, B., Krienitz, L., Gärtner, G., Schagerl, M. (Eds.), **Süsswasserflora von Mitteleuropa**. Elsevier/Spektrum, Heidelberg. p. 759.

Komárek, J., Kaštovský, J., Mareš, J., Johansen, J., R. 2014. **Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach**. Preslia. v. 86. p.295–335.

Lagesen K, Hallin PF, Rødland E, Stærfeldt HH, Rognes T Ussery DW. **RNammer: consistent annotation of rRNA genes in genomic sequences**. Nucleic Acids Res. 2007 Apr 22. RNAMMER.

Leão, Z., M., A., N., & Dominguez, J., M., L. 2000. **Tropical Coast of Brazil**. Marine Pollution. Bulletin 41. p.112-122.

Lee, R. E. 2008. **Phycology**. 4ª ed. Cambridge; Cambridge University Press. 561 p.

Li, R., Wilhelm, S., W, Carmichael, W., W., Watanabe, M., M. 2008. **Polyphasic characterization of water bloom forming Raphidiopsis species (cyanobacteria) from central China**. Harmful Algae. v 7. p.146–153.

Miyashita H., Ikemoto H., Kurano N., Adachi K., Chihara M., Miyachi S. 1996. **Chlorophyll d as a major pigment**. Nature (London). v. 383. p.402.

Molica, R.; Azevedo, S. 2009. **Ecofisiologia de cianobactérias produtoras de cianotoxinas**. Oecol. Bras. 13, 229–246.

Moustaka-Gouni, M., Vardaka, E., Tryfon, E., 2007. **Phytoplankton species succession in a shallow Mediterranean lake (L. Kastoria, Greece): steady-state dominance of *Limnothrix redekei*, *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii***. Hydrobiologia v. 575: p 129–140.

Moustaka-Gouni M, Kormas KA, Polykarpou P, Gkelis S, Bobori DC & Vardaka E. 2010. **Polyphasic evaluation of *Aphanizomenon issatschenkoi* and *Raphidiopsis mediterranea* in a Mediterranean lake**. J Plankton Res. v.32. p.927–936.

Nunes, J. M. C., Santos, A. C. C.; Santana, L. C. 2005. **Novas Ocorrências de Algas Marinhas Bentônicas para o Estado da Bahia, Brasil**. Iheringia, Sér. Bot., Porto Alegre, v. 60. p.99-106.

Oren, A. 2004. **A proposal for further integration of the cyanobacteria under the Bacteriological Code**. International Journal of Systematic and Evolutionary Microbiology. v. 54. p.1895–1902

Oren., A. 2014**. Cyanobacteria: biology, ecology and evolution in: Cyanobacteria: An Economic Perspective**. Eds. Naveen K. Sharma, Ashwani K. Rai and Lucas J. Stal. First Edition. John Wiley & Sons, Ltd.

Paerl, H., Otten, T. 2013. **Harmful cyanobacterial blooms: causes, consequences, and controls** Microb. Ecol., 65, pp. 995-1010

Palinska, K., A., & Surosz, W. 2014. **Taxonomy of cyanobacteria: a contribution to consensus approach**. Hydrobiologia. v.740. p.1–11.

Pearson, H., W., Howsley, R., Kjeldsen, C., K., Walsby, A., E. 1979**. Aerobic nitrogenase activity associated with a non-heterocystous filamentous cyanobacterium**. FEMS Microbiol Lett. v.5. p.163–167

Rippka, R. 1979. **Isolation and purification of Cyanobacteria**. In: Pacher, L.; Glazer, A.N. (eds). **Cyanobacteria Methods in Enzymology**. Blackwell. v.167. p.3-27.

Sarma, T. 2013. **Handbook of Cyanobacteria**. Boca Raton: CRC Press.

Schopf, J., W., & Packer, B., M. 1987. **Early Archean (3.3-Billion to 3.5-Billion-Year-Old) Microfossils from Warrawoona Group**, Australia. Science. v.237. p70-73.

Short, A. D.; Klein, A. H. F. 2016. **Brazilian Beach Systems**: Introduction. In: Short, A. D., Klein, A. H. F. (eds.). Brazilian Beach System, Coastal Research Library, 17:1-35.

Stal, L., J. & Krumbein, W., E. 1985. **Nitrogenase activity in the non-heterocystous cyanobacterium *Oscillatoria* sp. grown under alternating light-dark cycles**. Arch. Microbiol. v.143: p.67-71.

Strunecký, O., Elster, J., komárek, J. 2011 **Taxonomic revision of the freshwater cyanobacterium „*Phormidium" murrayi* = *Wilmottia murrayi***. Fottea 11(1): 57–71

Suda, S., Moriya, R., Sumimoto, S., Ohno, O., Suenaga, K. 2013. **Genetic diversity of filamentous cyanobacteria from shore regions of Okinawa**. Journal of Marine Science and Technology. v.21. p.175-180.

Taton A, Grubisic, S., Ertz, D., Hodgson, D., A., Piccardi, R., Biondi, N., Tredici, M., R., Mainini, M., Losi, D., Marinelli, F., Wilmotte, A. 2006. **Polyphasic study of Antarctic cyanobacterial strains**. J Phycol. v.42. p.1257–1270.

van Apeldoorn M., E, Egmond HPV, Speijers GJA, Bakker GJI. 2007. Toxins of cyanobacteria. Molecular Nutrition & Food Research 51:7-60

Vaz, M.G.M.V., Genuário, D.B., Andreote, A.P.D., Malone, C.F.S., Sant'Anna, C.L., Barbiero, L., Fiore, M.F., 2014. ***Pantanalinema* gen. nov. and *Alkalinema* gen. nov.: novel pseudanabaenacean genera (Cyanobacteria) isolated from saline– alkaline lakes**. Int. J. Syst. Evol. Microbiol. v.65. p.298–308.

Wasmund, N., Voss, M., Lochte, K. 2001. **Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and re-calculation of a budget of nitrogen fixation**. Mar Ecol Prog Ser. v.214: p.1–14.

Wehr, J., D., & Sheath, R., G. 2003. **Freshwater Algae of North America**: Ecology and Classification. Academic Press. 1ª Ed. p 918.

Whitton, B., A., & Potts, M. 2000. **Introduction to the cyanobacteria, In Ecology of Cyanobacteria: Their Diversity in Time and Space** (Eds B. A. Whitton, M. Potts). p 1-11.

Whitton, B. K. & Sinclair, C. 1975. **Ecology of blue-green algae**. ScL Prog. Oxf, 62: 429-446.

Zapomělová, E., K. Řeháková, J. Jezberová & J. Komárková, 2010. **Polyphasic characterization of eight planktonic *Anabaena* strains (cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field**. Hydrobiologia. v.639: p.99–11.