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TESE DE DOUTORADO

PROCESSOS ECOLÓGICOS ENVOLVIDOS NA DISTRIBUIÇÃO DA DIVERSIDADE GENÉTICA E TAXONÔMICA DE CIANOBACTÉRIAS DE ECOSSISTEMAS AQUÁTICOS CONTINENTAIS

Porto Alegre, março de 2020

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“Life is bacterial, and those organisms that are not bacteria have evolved from organisms that were.”
(Lynn Margulis)

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RESUMO

As cianobactérias são microrganismos procariotos, capazes de realizar fotossíntese oxigênica. Ao longo de sua longa história evolutiva na Terra, adaptações genéticas, bioquímicas e fisiológicas garantiram a estes procariotos a sua perpetuação em praticamente toda a superfície terrestre. As cianobactérias se distribuem ao longo de habitats terrestres e aquáticos, em associação com outros seres vivos, e mesmo em ambientes extremos. Em ecossistemas aquáticos, especialmente, as cianobactérias são encontradas em alta abundância e são as maiores responsáveis pela produção primária. Além disso, cianobactérias podem formar florações e liberar toxinas em ambientes aquáticos, o que as torna intimamente relacionadas às condições da água e à conservação dos ecossistemas aquáticos. Estudos sobre padrões de distribuição de cianobactérias têm crescido na literatura e são importantes uma vez que podem alertar riscos de floração, e também para compreender melhor a ecologia do grupo, como habilidades de dispersão e preferências de habitat. Nesse contexto, a presente tese teve como objetivo geral explorar os fatores ecológicos que influenciam a distribuição das cianobactérias, com foco em ecossistemas de água doce. Para isso, a tese foi estruturada em três capítulos, cada qual com o objetivo central de: (I) sintetizar, através de uma ampla revisão bibliográfica, padrões biogeográficos de cianobactérias e as evidências sobre os processos ecológicos que estruturam esses padrões; (II) reconstruir as histórias filogeográficas de duas cianobactérias cosmopolitas de vida livre (*Raphidiopsis raciborskii* e *Microcystis aeruginosa*), com base na diversidade genética do gene 16S rRNA, a fim de explorar se suas distribuições atuais podem ser atribuídas aos mesmos processos ecológicos; (III) explorar os padrões de diversidade alfa e beta de cianobactérias planctônicas e perifíticas (associadas à macrófita *Scirpus californicus*) em um sistema de lagoas subtropicais e como a influência de variáveis ambientais varia entre cianobactérias de vida livre (planctônicas) e de vida associada (perifíticas). Os principais achados desta tese foram: (I) tanto fatores ambientais quanto históricos são importantes na distribuição das cianobactérias; no entanto, padrões de distribuição dependem intimamente do conceito de espécie, que ainda é debatido para procariotos, incluindo cianobactérias, escalas espaciais e ambientais, e os vieses das metodologias aplicadas nos estudos; (II) *M. aeruginosa* e *R. raciborskii* apresentaram maior diversidade genética em latitudes tropicais e evidência de expansão populacional recente. No entanto, embora ambas as espécies sejam consideram cosmopolitas, a filogeografia de *R. raciborskii* indica uma interação entre deriva e alguma limitação de dispersão, ao passo que *M. aeruginosa* parece ter uma alta frequência de dispersão intercontinental; (III) assembleias de cianobactérias planctônicas e perifíticas são distintas e estruturadas por diferentes fatores ecológicos. Enquanto a variação nas cianobactérias planctônicas foi determinada principalmente pelas condições da água, as cianobactérias perifíticas foram influenciadas apenas pela abundância de outros táxons bacterianos. Em geral, a presente tese contribuiu para o entendimento dos fatores envolvidos na distribuição das cianobactérias e como eles variam entre diferentes táxons, escalas espaciais e tipos de habitat. Ainda, forneceu perspectivas para investigações futuras nesse campo, a fim de contribuir para uma maior compreensão dos processos ecológicos que moldam a distribuição desse importante grupo de procariotos.

Palavras-chave: Biogeografia, Cyanoprokaryota, Ecologia Microbiana, Filogeografia, *Microcystis aeruginosa*, *Raphidiopsis raciborskii*

ABSTRACT

Cyanobacteria are prokaryotic microorganisms capable of performing oxygen photosynthesis. Throughout its long evolutionary history on Earth, genetic, biochemical and physiological adaptations have allowed to these prokaryotes to inhabit the entire terrestrial surface. Cyanobacteria are distributed throughout terrestrial and aquatic habitats, in association with organisms, and even in extreme environments. In aquatic ecosystems, especially, cyanobacteria are typically abundant and responsible for most of the primary production. In addition, cyanobacteria can form blooms and release toxins in aquatic environments, so that cyanobacteria are closely related to water conditions and conservation of aquatic ecosystems. Studies on cyanobacteria distribution patterns have increased in the literature and are important since they are able to alert the risks of cyanoblooms, and also to better understand the ecology of the group, such as dispersal abilities and habitat preferences. In this context, the present thesis aimed to explore the ecological factors that influence the distribution of cyanobacteria, focusing on freshwater ecosystems. For this, this thesis was structured in three chapters, with the main goals of: (I) to review the biogeographic patterns of cyanobacteria with focus on molecular data and the evidences from the published literature for the processes driving these patterns; (II) to reconstruct the phylogeographic histories of two free-living cosmopolitan cyanobacteria (*Raphidiopsis raciborskii* and *Microcystis aeruginosa*), based on the genetic diversity of the 16S rRNA gene, in order to explore whether their current distributions could be attributed to the same ecological processes; (III) to explore alpha and beta diversity patterns of planktonic and periphytic (associated to *Scirpus californicus*) cyanobacteria in a subtropical lake system, and how the influence of niche-related variables varies between free-living (planktonic) and surface-associate (periphytic) assemblages. In summary, the main findings of this thesis were that: (I) both environmental and historical factors are important in structuring cyanobacteria variation across time and space, however, distribution patterns are closely dependent on the concept of species, which is still debated for prokaryotic microorganisms, including cyanobacteria, spatial and environmental scales, and the biases of the molecular methodologies applied in the studies; (II) *R. raciborskii* and *M. aeruginosa* had greater genetic diversity in tropical latitudes and showed evidence of recent population expansion. However, although both species are considered cosmopolitan, the phylogeography of *R. raciborskii* indicates an interaction between drift and some dispersal limitation, whereas *M. aeruginosa* seems to have a high frequency of intercontinental dispersal; (III) planktonic and periphytic cyanobacteria assemblages are distinct and structured by different ecological factors. While the variation in planktonic cyanobacteria was mainly determined by water conditions, periphytic cyanobacteria were influenced only by the abundance of other bacterial taxa. In general, this thesis has contributed to the understanding of the factors involved in the distribution of cyanobacteria, and how they vary between different taxa, spatial scales and habitat types. It also provided perspectives for future research in this field, in order to contribute to a greater understanding of the ecological processes that shape the distribution of this important group of prokaryotes.

Keywords: Biogeography, Cyanoprokaryota, Microbial Ecology, *Microcystis aeruginosa*, Phylogeography, *Raphidiopsis raciborskii*

SUMÁRIO

INTRODUÇÃO GERAL

O objetivo central da ecologia e da teoria da biodiversidade é estimar padrões de distribuição da biodiversidade ao longo de táxons e escalas de espaço, tempo e abundância[1]. No entanto, esse objetivo só pode ser completamente alcançado levando em conta os organismos mais abundantes, globalmente difundidos, e diversos geneticamente, metabolicamente, taxonomicamente e funcionalmente da Terra, os microrganismos procariotos[2]. Apesar de discordâncias a respeito de estimativas globais da biomassa[3] e da real diversidade[2], é indiscutível que os procariotos representam o principal componente da biota da Terra. Além disso, embora a data ainda seja discutível, sabe-se que os primeiros seres vivos que se originaram na Terra foram procariotos, provavelmente heterotróficos, os quais deram origem a toda forma de vida que já existiu e que existe no planeta atualmente[4–6]. Como consequência, os procariotos são responsáveis pela maior parte da história evolutiva da vida na Terra[7,8] e catalisam transformações únicas e indispensáveis para o funcionamento dos ciclos biogeoquímicos[9], os quais suportam toda a vida na Terra.

Estudos sobre a distribuição da biodiversidade tradicionalmente focaram seus esforços em plantas e animais[10–12], negligenciando o mundo microbiano. Durante muito tempo, essas oportunidades inexploradas deixaram o entendimento da biodiversidade, portanto, limitado às espécies mais conspícuas de grandes eucariotos. Isso ocorreu sobretudo pela dificuldade em detectar e quantificar a diversidade microbiana em ecossistemas naturais[13,14]. Apesar de sua enorme abundância, a quantificação da diversidade bacteriana é uma tarefa que enfrenta algumas dificuldades. Em primeiro lugar, os meios de cultura, abordagem tradicional para o estudo de procariotos e construções de catálogos de cepas conhecidas, não são capazes de fornecer as condições necessárias para o crescimento da maior parte das espécies[15]. Ainda hoje, sabe-se que todos os grupos conhecidos representam uma ínfima fração da diversidade total estimada[14]. Em segundo lugar, não há consenso sobre a própria definição de espécie bacteriana, em função de sua reprodução assexuada, capacidade de transferência horizontal de genes e ausência de caracteres morfológicos facilmente visíveis[8,16,17]. A falta de uma clara definição de espécie se torna um obstáculo em estudos de distribuição microbiana, uma vez que conceitos chave, tais como amplitude de distribuição, tamanho populacional, habitat e nicho são intimamente ligados ao conceito de espécie[13,18].

Apesar das dificuldades, um número crescente de estudos publicados sobre padrões de distribuição microbiana é observado na literatura, explorando distintos grupos bacterianos em uma variedade de ecossistemas[13,18,19]. Esse crescimento é explicado pelas novas e mais sensíveis estratégias de quantificação e classificação de procariotos que estão sendo desenvolvidas e melhoradas graças à revolução do sequenciamento de DNA em larga escala e ferramentas de bioinformática[20]. Tipicamente, estudos de distribuição microbiana com abordagens moleculares utilizam dados a partir do sequenciamento do gene ribossomal 16S (16S rRNA), e classificam a diversidade bacteriana a partir da definição de OTUs (*operational taxonomic units*, em inglês)[19]. Em outras palavras, um OTU se tornou uma definição pragmática para agrupar indivíduos através da similaridade genética (geralmente >97% de similaridade do gene 16S rRNA), equivalente, mas não igual, à definição de espécie para macrorganismos[13]. A obtenção de dados moleculares é eficaz pois torna possível, além da caracterização de grupos específicos em uma resolução taxonômica mais fina e fiável, a caracterização de comunidades bacterianas inteiras, com a estimação da riqueza e abundância destes microrganismos. Dados de sequenciamento também tornam possível a determinação das relações filogenéticas entre os grupos que coexistem em comunidades e ecossistemas. Estes dados podem oferecer benefícios promissores para muitas áreas de pesquisa, como na epidemiologia de patógenos animais e vegetais, na biorremediação e, sobretudo, no entendimento da diversidade microbiana global e das forças evolutivas que a moldam[18].

Os novos dados gerados em estudos de ecologia microbiana lançaram novas ideias e hipóteses sobre a distribuição destes organismos até então não considerados, tais como a existência de padrões biogeográficos[13,21]. Tradicionalmente, o paradigma fundamental em ecologia microbiana era de que, em função da sua alta abundância, tamanho pequeno e rápida adaptação ambiental, os procariotos teriam capacidade de dispersão global e colonizariam todos os ambientes nos quais as condições ambientais fossem favoráveis[22]. Entretanto, atualmente é sabido que essa relação depende do nível de resolução taxonômica adotada. A nível do domínio, por exemplo, é indiscutível que Bacteria e Archaea sejam distribuídas globalmente[23], e a nível de classe é bem aceito que β-Proteobacteria, Cyanobacteria, Actinobacteria e Flavobacteria exibem distribuição cosmopolita em ecossistemas marinhos ou terrestres[18,24,25]. Já a nível de gênero, existe um consenso geral de que muitos procariotos têm uma distribuição cosmopolita em seus respectivos habitats[26]. Exemplos clássicos de cosmopolitismo entre gêneros bacterianos incluem *Polynucleobacter*, que foi isolado de habitats de água doce localizados em várias zonas climáticas em diferentes continentes[27], *Pseudomonas* fluorescentes[28] e *Bacillus*[29] isolados de várias regiões e países.

Embora resoluções taxonômicas acima de gênero confirmem a ideia de cosmopolitismo bacteriano em amplas escalas espaciais, resoluções taxonômicas mais finas revelam distintos padrões de distribuição microbiana. De um lado, exemplos clássicos de distribuição mundial da mesma espécie bacteriana podem ser encontrados para certos patógenos humanos, como *Escherichia coli*, por exemplo[30]. Agrupamentos filogenéticos baseados em sequências do gene 16S rRNA de bactérias de água doce também já apresentaram distribuição cosmopolita[24]. Por outro lado, observações de distribuição restrita de procariotos foram encontradas, por exemplo, na cianobactéria *Synechococcus* de fontes termais[31]. Neste estudo, embora certos morfotipos tenham sido claramente identificados em fontes termais na América do Norte, os mesmos não foram observados em outras fontes termais de outras regiões do mundo, sugerindo uma faixa restrita de dispersão devido ao isolamento geográfico ou à falta de viabilidade durante o transporte[32]. Estes padrões distintos refletem uma não-uniformidade de diferentes grupos bacterianos com relação às suas capacidades de dispersão e tolerâncias ambientais. Dentro deste contexto, portanto, o objetivo fundamental em estudos de distribuição microbiana é determinar os fatores que geram estes diferentes padrões[13], incluindo aspectos intrínsecos aos táxons bacterianos, como suas preferências de habitat, tolerâncias às condições ambientais e diferenças nas habilidades de mobilidade e dispersão, e aspectos extrínsecos, como a ação de processos históricos e estocásticos. Além disso, ainda bastante debatido é como o nível de resolução taxonômica para determinar uma espécie bacteriana interfere nos padrões de distribuição encontrados.

As cianobactérias, grupo de estudo desta Tese, são procariotos fotossintetizantes pertencentes ao filo monofilético Cyanobacteria, dentro do domínio Bacteria[33]. Apesar de existirem outros procariotos fototróficos, as cianobactérias são as únicas que realizam fotossíntese oxigênica, onde o gás carbônico (CO_2) e água (H_20) são usados para a síntese de carboidratos resultando na formação de oxigênio (O_2), que é liberado para o meio. Com base no registro fóssil, estima-se que as cianobactérias surgiram há cerca de 3,5 bilhões de anos[34], sendo os primeiros procariotos fotossintetizantes produtores de oxigênio do planeta e os responsáveis pela elevação repentina dos níveis de oxigênio na atmosfera, evento conhecido como A Grande Oxigenação (*GOE*, em inglês)[35]. Como consequência deste evento, as cianobactérias impactaram irreversivelmente a história da vida na Terra. A longo prazo, a elevação dos níveis de oxigênio na atmosfera em escala global permitiu que novos tipos de vida evoluíssem. Isso porque o oxigênio é um gás reativo, então, quando alguns organismos

descobriram como usá-lo em seu próprio metabolismo, tiveram acesso a uma nova e importante fonte de energia. Assim, ao respirar oxigênio, os organismos puderam se tornar muito mais ativos e maiores, indo além da simples multicelularidade já desenvolvida nas cianobactérias[36,37]. Além de desencadear a diversificação e a evolução de formas de vida mais complexas, as cianobactérias foram diretamente responsáveis por um dos saltos evolutivos mais importantes na história da vida na Terra, a evolução da célula eucariótica através da endossimbiose[38,39].

As cianobactérias também se destacam pela sua enorme diversidade morfológica e fisiológica. Diferentes grupos taxonômicos se distribuem entre formas unicelulares, multicelulares filamentosas, e agrupadas em colônias[40]. Em alguns grupos filamentosos, ainda, as células vegetativas podem se diferenciar em células especializadas para fixação de nitrogênio (N_2), os heterocistos. Os heterocistos são caracterizados por um envelope distinto, constituído por uma camada glicolipídica que funciona como uma barreira para o oxigênio, uma vez que a enzima nitrogenase, responsável pela redução biológica do nitrogênio, é irreversivelmente inibida pelo oxigênio[41]. Além dos heterocistos, algumas cianobactérias são capazes de formar outro tipo de célula diferenciada, os acinetos. Os acinetos são células semelhantes a esporos, frequentemente maiores que as células vegetativas, caracterizados por uma parede celular espessa e por um envelope extracelular de múltiplas camadas[42]. Essas células específicas são geralmente produzidas em resposta a condições ambientais desfavoráveis, como limitação de luz, que resultam em falta de energia celular[43]. Quando as condições ambientais se tornam adequadas novamente para o crescimento vegetativo, os acinetos podem germinar em células vegetativas[43].

Em função da sua enorme plasticidade morfológica e fisiológica, as cianobactérias podem ser encontradas em uma ampla gama de habitats. As cianobactérias se distribuem entre ecossistemas aquáticos de água doce e oceânicos, terrestres (e.g. em solos e sobre rochas), e até mesmo em ambientes extremos (e.g. hipersalinos, áridos, e nos polos), como organismos de vida livre, simbiontes e epibiontes[40]. Em ecossistemas aquáticos, particularmente, as cianobactérias são frequentemente abundantes e o principal componente responsável pela produção primária através da fotossíntese[40,44,45]. O sucesso ecológico das cianobactérias no ambiente aquático se deve às diversas características adaptativas que surgiram no decorrer da sua longa história evolutiva. Por exemplo, a presença de pigmentos fotossintéticos acessórios, como as ficocianinas, que captam energia na faixa do vermelho no espectro de luz visível e são encontradas exclusivamente em cianobactérias[46]. O comprimento de onda da faixa do vermelho é capaz de penetrar até o fundo da coluna d'água e, dessa forma, as

ficocianinas são importantes elementos adicionais da antena coletora de luz nas cianobactérias aquáticas[47]. Também pode-se mencionar a capacidade de algumas cianobactérias aquáticas em modificar sua flutuabilidade graças à presença de vesículas de gás, chamadas de aerótopos. Graças ao balanço entre a produção de aerótopos e o acúmulo de carboidratos, algumas cianobactérias são capazes de migrar verticalmente na coluna d'água, permitindo o acesso a nutrientes que costumam se acumular nas regiões mais profundas do corpo d'água[48].

Em ecossistemas aquáticos, ainda, condições ambientais particulares podem desencadear o crescimento repentino e abundante de cianobactérias, formando as notórias florações[49,50]. O aumento da temperatura e a liberação antropogênica excessiva de nutrientes no corpo d'água (com consequente eutrofização) estão entre os principais agentes causadores de florações, uma vez que provocam a rápida proliferação de algumas espécies de cianobactérias[51]. Embora as florações sejam mais comuns e frequentes em ecossistemas de água doce, alguns grupos taxonômicos também podem formar florações em águas marinhas[52]. Florações de cianobactérias causam sérios desequilíbrios nos ecossistemas aquáticos, desde a diminuição na transparência da água até alterações na cadeia trófica[53]. Além disso, florações de cianobactérias frequentemente estão associadas a espécies capazes de produzir peptídeos e alcalóides tóxicos, chamados cianotoxinas[54]. Florações de cianobactérias tóxicas em ecossistemas naturais e reservatórios de água para uso humano podem causar sérios problemas ambientais, uma vez que podem comprometer a saúde humana e de outros animais[53], e têm sido frequentemente referidas como um grave problema ambiental[49]. Entre os principais gêneros formadores de florações com espécies capazes de produzir toxinas destacam-se *Aphanizomenon*, *Dolichospermum*, *Microcystis*, *Planktothrix* e *Raphidiopsis*[55].

Associado ao aumento nos registros de florações no mundo todo, o potencial invasivo de algumas espécies de cianobactérias aquáticas também é um tema que têm chamado a atenção recentemente. Embora a invasão de bactérias seja um tanto enigmática e difícil de detectar[56], nesse sentido as cianobactérias são exceções, uma vez que possuem características morfológicas visíveis em microscopia[57]. Essa propriedade contribuiu em parte para o aumento do número de registros de invasão de algumas espécies, como *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno[58–60], *Microcystis aeruginosa* (Kützing) Kützing[61] e algumas espécies do gênero *Aphanizomenon*[57], todas com potencial de formação de florações tóxicas. Evidências sugerem que estes grupos taxonômicos são nativos de regiões tropicais, e que nas últimas duas décadas se expandiram para áreas subtropicais e temperadas[57]. Séries temporais sedimentares e registros de monitoramento em larga

escala também mostraram que tanto a expansão quanto a abundância de cianobactérias aumentaram significativamente nos últimos 200 anos, e mais rapidamente nos últimos 70 anos[62]. Características intrínsecas das espécies, como adaptação evolutiva e plasticidade ecológica, estão relacionados à capacidade invasiva das cianobactérias. A formação dos acinetos, por exemplo, pode ter um importante papel, já que torna mais provável a chegada de propágulos viáveis a um habitat favorável (além de diminuir a probabilidade de extinção diante de flutuações ambientais). Mudanças ambientais globais, como oscilações climáticas e o aumento da temperatura, também podem estar associadas às invasões de cianobactérias[60].

Apesar dos impactos negativos no ambiente e nas atividades humanas devido à formação de florações e produção de toxinas, as cianobactérias apresentam aspectos positivos em diversas áreas. Algumas cianotoxinas, por exemplo, provaram ser compostos bioativos com atividades antivirais, antitumorais e antibacterianas[63,64]. Algumas cianobactérias que vivem em ambientes marinhos, a maioria pertencente à ordem Oscillatoriales, são capazes de produzir compostos com importantes potenciais farmacêuticos, como efeitos anticarcinogênicos[65]. Além disso, associações simbióticas entre cianobactérias e plantas são muito importantes na agronomia, onde geralmente a principal contribuição da cianobactéria é a fixação do nitrogênio atmosférico aos seus hospedeiros[66]. Outro aspecto positivo derivado da produção de cianotoxinas foi sugerido por estudos sobre os papéis ecológicos de alguns desses compostos como aleloquímicos, inibindo macrófitas, algas e outros microrganismos. Estes aleloquímicos, que funcionam como uma proteção contra predação, podem ser empregados para o desenvolvimento de algicidas, herbicidas e inseticidas[67]. As cianobactérias também são consideradas fontes de compostos alimentícios saudáveis, por serem uma excelente fonte de vitaminas e proteínas[68].

Com relação à taxonomia das cianobactérias, em função da sua natureza procariótica, a identificação e classificação ainda é complexa e bastante debatida. Isso se deve principalmente a três fatores: 1) o conceito de espécie para procariotos ainda é controverso, em função da reprodução assexuada e frequente transferência horizontal de genes[8]; 2) a ocorrência de táxons crípticos, ou seja, organismos potencialmente atribuíveis ao mesmo grupo taxonômico do ponto de vista morfológico, mas que são geneticamente distantes[69]; 3) e a coexistência de dois códigos de nomenclatura distintos, o Botânico e o Bacteriológico[70]. Tradicionalmente, a diferenciação de espécies com base em aspectos morfológicos e seguindo os critérios botânicos foi amplamente utilizada, contudo, sabe-se atualmente que essa abordagem não necessariamente reflete entidades evolutiva e ecologicamente coerentes[71]. A identificação unicamente baseada em microscopia óptica

nem sempre é segura porque nem todas as características taxonômicas são distinguíveis no microscópio óptico, e porque as características morfológicas e morfométricas das cianobactérias podem variar refletindo as condições de seu crescimento[72].

A coexistência de dois códigos de nomenclatura diferentes, Botânico e Bacteriológico, tornou a situação ainda mais complexa. Por serem inicialmente consideradas algas, as cianobactérias foram classificadas com base nos princípios botânicos e sua nomenclatura foi regulamentado pelo Código Internacional de Nomenclatura Botânica (ICBN)[70]. Em 1979, após o reconhecimento da natureza procariótica das cianobactérias, Rippka et al.[73] criaram um código de classificação bacteriológica também para cianobactérias. No entanto, somente após o uso de técnicas moleculares[33], as cianobactérias foram confirmadas como membros do domínio Bacteria. Desde então, diversos esforços têm sido feitos na tentativa de criar um sistema de nomenclatura exclusivo para cianobactérias[74,75]. Nesse sentido, um primeiro esforço concreto foi a elaboração por Komárek e Golubic[76] de um guia nomenclatural especial para cianobactérias, o 'Cyano-Guide', no qual todas as principais prescrições e recomendações de ambos os códigos foram levadas em consideração. Atualmente, a abordagem polifásica, que inclui vários níveis complementares de investigação (morfológica, ultraestrutural, bioquímica, fisiológica e molecular), é o método mais aceito para caracterizar e identificar cianobactérias[77].

Finalmente, estudos sobre padrões de distribuição, tema central desta Tese, têm crescido na literatura e debatido sobre fatores que causam padrões não-aleatórios de distribuição de cianobactérias, tanto temporal como espacialmente. Graças à popularização do uso de métodos moleculares, diversos estudos, tanto a nível de populações como a nível de comunidades, têm gerado e explorado dados moleculares de cianobactérias em conjunto com dados morfológicos. A maior parte destes estudos são baseados na identificação e análises filogenéticas de isolados ou amostras ambientais usando marcadores genéticos, especialmente o gene 16S rRNA, a fim de estabelecer relações entre diversidade e variáveis ambientais e espaciais[78–80]. Estudos recentes também são baseados na descoberta de novos gêneros ou espécies em novos ambientes[81], endemismos[82,83], e potenciais de dispersão e invasão[60]. Estudos sobre padrões de distribuição de cianobactérias são importantes tanto do ponto de vista da caracterização de ambientes, podendo alertar potenciais riscos de floração, por exemplo, e também para compreender melhor a ecologia de grupos taxonômicos específicos, como as habilidades de dispersão e tolerâncias ambientais de espécies potencialmente problemáticas.

ESTRUTURA DA TESE

Levando em conta a importância das cianobactérias, tanto do ponto de vista evolutivo, como protagonistas de eventos críticos ao longo da história da vida na Terra, e ambiental, como forças motrizes essenciais dos ciclos biogeoquímicos que sustentam a vida, a presente Tese nasceu da vontade de explorar os fatores ecológicos que influenciam a distribuição das cianobactérias. Padrões de distribuição de cianobactérias foram acessados tanto em escala global como em escala regional; tanto a nível de populações, acessando a diversidade genética, como a nível de assembleias, acessando a diversidade taxonômica; com foco particular em dados moleculares e ecossistemas de água doce. Para isso, a Tese foi estruturada em três capítulos, sendo os objetivos específicos de cada capítulo descritos a seguir.

CAPÍTULO I. *Everything is not everywhere: a tale on the biogeography of cyanobacteria*

(Ribeiro, K.F., Duarte, L. & Crossetti, L.O. Everything is not everywhere: a tale on the biogeography of cyanobacteria. *Hydrobiologia* 820, 23–48 (2018). DOI: 10.1007/s10750-018-3669-x)

Compreender os fatores que afetam a biogeografia de bactérias é uma das grandes questões discutidas atualmente. Estudos recentes começaram a lançar dúvidas sobre a tradicional ideia de capacidade global de dispersão, apresentando evidências de endemismo e especiação alopátrica em bactérias. Explorar padrões biogeográficos em bactérias é necessário para compreender a biogeografia como um todo e elucidar se o mundo macro e microbiano são governados pelas mesmas regras. Neste trabalho, nosso objetivo foi realizar uma ampla revisão bibliográfica sobre padrões biogeográficos de cianobactérias discutidos na literatura, levantando evidências de fatores ecológicos (ambientais e históricos) envolvidos nestes padrões. Além disso, considerações foram feitas sobre o conceito de espécie e resolução taxonômica em cianobactérias, e como estes afetam o estudo da sua biogeografia.

CAPÍTULO II. *Comparative phylogeography of two free-living cosmopolitan cyanobacteria: Insights on biogeographic and latitudinal distribution*

(Ribeiro, K.F., Ferrero, A.P., Duarte, L., Turchetto-Zolet, A.C. & Crossetti, L.O. Comparative phylogeography of two free-living cosmopolitan cyanobacteria: Insights on biogeographic and latitudinal distribution. *Journal of Biogeography* in press (2019). DOI: 10.1111/jbi.13785)

Tradicionalmente assumiu-se que bactérias de vida livre teriam pouca assinatura biogeográfica devido ao seu alto potencial de dispersão passiva. *Raphidiopsis raciborskii* e *Microcystis aeruginosa* são cianobactérias cosmopolitas de vida livre, com provável origem tropical, que frequentemente formam florações tóxicas e são consideradas espécies invasoras em latitudes médias. Apesar dessas semelhanças, seus padrões filogeográficos raramente foram diretamente comparados. Neste trabalho, nosso objetivo foi reconstruir as histórias filogeográficas de *R. raciborskii* e *M. aeruginosa*, a fim de explorar se suas distribuições atuais poderiam ser atribuídas aos mesmos fatores. A filogeografia das duas espécies foi estudada com base em dados de diversidade genética do gene 16S rRNA usando sequências distribuídas ao redor do globo.

-Este estudo contou com a colaboração da Profa. Dra. Andreia Carina Turchetto-Zolet, do Núcleo de Genômica e Evolução de Populações Naturais (GENP), Departamento de Genética da UFRGS.

CAPÍTULO III. *Alfa and beta diversity of planktonic and periphytic cyanobacteria based on 16S rRNA gene in a subtropical lake system: How similar are they?*

(Artigo a ser submetido à revista *Environmental Microbiology*)

Em ecossistemas de água doce, as cianobactérias são constituintes biogeoquimicamente significativos, sendo responsáveis pela maior parte da produção primária, e costumam ser um componente abundante tanto da comunidade algal de vida livre (fitoplâncton), como da comunidade algal associada a algum tipo de superfície, como rochas e macrófitas submersas (perifíton). Apesar disso, não é completamente entendido se cianobactérias planctônicas e perifíticas são estruturadas pelos mesmos fatores ecológicos. Neste trabalho, nosso objetivo foi explorar os padrões de diversidade alfa e beta de assembleias de cianobactérias (caracterizadas através de sequenciamento do gene 16S rRNA) planctônicas e perifíticas (associadas à macrófita *Scirpus californicus*) em um sistema de lagoas rasas subtropicais, e comparar a influência de variáveis relacionadas ao nicho (condições físicas e químicas da água e abundância de bactérias heterotróficas) sobre a distribuição das cianobactérias nos dois distintos tipos de habitat (águas abertas e associado à macrófita).

-Este estudo contou com a colaboração do Prof. Dr. Jeverson Frazzon, do Laboratório de Bioquímica de Microrganismos, Instituto de Ciência e Tecnologia de Alimentos (ICTA) da UFRGS.

CAPÍTULO I

Everything is not everywhere: a tale on the biogeography of cyanobacteria*

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Everything is not everywhere: a tale on the biogeography of cyanobacteria

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Summary
Microrganisms such as cyanobacteria have been often considered as exhibiting wide distribution mainly driven by environmental heterogeneity. Recently, however, new findings have evoked the role of previously neglected processes, such as dispersal limitation, determining the distribution of a wide range of microorganisms, including cyanobacteria. Here, we reviewed the biogeographical patterns of cyanobacteria with focus on molecular data and the evidences from the published literature for the processes driving these patterns. Also, considerations are made about concept of species, discordances in the taxonomic concepts, and level of taxonomic resolution, and how these affect the biogeographical study of cyanobacteria. From a overview, it can be observed that both environmental and historical factors are important to structure cyanobacteria diversity across time and space. Moreover, different species may exhibit significant differences in their distribution patterns, from possibly cosmopolitan species to other endemic species. However, distribution patterns are closely dependent on the concept of species, besides the taxonomic resolution, spatial and environmental scales, and the biases of the molecular methodologies applied in the studies. Thus, efforts to broaden sampling and sequencing of unknown and less-known species, as well as geographical regions and habitats poorly exploited, are crucial for a better understanding of cyanobacteria biogeography.

Keywords microbial cosmopolitanism, microbial diversity predictors, phylogeography, microbial ecology, cyanoprokaryota

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Introduction

Cyanobacteria are a diverse group of microrganisms characterized as being oxygenic photosynthetic bacteria that possess chlorophyll *a* (Castenholz, 2001). Cyanobacteria possess a high ecological plasticity, thanks to which they occur in varied, often even extreme, habitats (Whitton & Potts, 2000). This group was responsible for critical events throughout the evolutionary history of life on Earth, such as the history of Earth-surface oxygenation (Lyons et al., 2004; Rasmussen et al., 2008; Sessions et al., 2009) and the evolution of eukaryotic cell through endosymbiosis (Margulis, 1970; Woese, 1987; McFadden, 2014). In addition, cyanobacteria play key roles in the ecosystems and biogeochemical cycles (Capone et al., 1997; Liu et al., 1997). Furthermore, in aquatic ecosystems some taxa are capable of forming blooms, events characterized by the sudden increase in the abundance of one or some taxa in the environment. Also, many species from this group are able to release toxins (Carmichael, 2001; Paerl & Huisman 2009), which can affect aquatic fauna as well as human populations. However, in spite of these negative impacts on ecosystems, cyanobacteria are economically important due to their potential use in pharmaceutical, food and biotechnological industries (e.g. Singh et al., 2005; Gantar & Svircĕv, 2005; Gerwick et al., 2008; Pfeiffer et al., 2011; Zanchett & Oliveira-Filho, 2013).

Most research on cyanobacteria is guided by evolutionary, ecological, ecotoxicological, biochemical, and taxonomic concerns (for a review on common investigation on cyanobacteria see Sciuto & Moro, 2015). With the current development and improvement of molecular techniques and tools in microbial ecology, the microbial biogeography field has been deeply investigated (see Bell et al., 2005; Martiny et al., 2006, Ramette & Tiedje, 2007; Woodcock et al., 2007; Bell, 2010; Hanson et al., 2012; Naselli-Flores & Padisák, 2016; Padisák et al., 2016), including biogeography studies with focus on cyanobacteria (see Moreira et al., 2013). The methods for biogeographic studies in cyanobacteria and cyanotoxins are based on the identification and subsequent phylogenetic analyses of the isolates or environmental samples using predetermined genetic markers to establish their genetic diversity and geographic interaction (Neilan et al., 2003; Moreira et al., 2012). Thus, these studies are generally based on the discovery of new genera or species in new environments (e.g. Komárek, 1985; Couté et al., 2004; Couté & Bouvy 2004); endemisms (e.g. Sompong et al., 2005; Taton et al., 2006; Jungblut et al., 2010); potentials of dispersion and invasion (e.g. Padisák, 1997; Dyble et al., 2002; Wiedner et al., 2007; Vidal & Kruk, 2008; Sukenik et al., 2012; Wilk-Woźniak et al., 2016); and spatial distributions of species, populations and communities related to spatial and

environmental variables (e.g. Papke et al., 2003; Drakare & Liess, 2010; Chamberlain et al., 2014).

The main goal of biogeography is to describe how species are distributed and the driving forces of these distribution patterns, and *prokaryotic biogeography* has been previously defined as the scientific field that documents the spatial distribution of prokaryotic taxa (Archaea and Eubacteria) in the environment at local, regional and continental scales (Ramette & Tiedje, 2007). To detect biogeographical patterns for microorganisms is crucial for understanding the distribution of biodiversity, since microorganisms are the most abundant and diverse group of Earth, being the life-support system of the planet (Pace, 1997; Hug et al., 2016; Locey & Lennon, 2016). Despite the classical idea of pure environmental selection on microbial distributions pervading the ecological literature for a long time (Baas-Becking, 1934), recent studies have evoked the existence of geographical isolation and dispersal barriers for some microbial groups from a variety of habitats, such as heterotrophic bacteria from soils (Cho & Tiedje, 2000), thermophilic cyanobacteria (Papke et al., 2003) and archaea (Whitaker et al., 2003) from hot springs. These recent hypothesis arguing against “cosmopolitan microbial rule” indicate a still very limited understanding of the mechanisms involved in microbial biogeography and how patterns of distribution vary among taxonomic groups and habitats, the main issues currently discussed in microbial ecology.

Despite the increased interest in exploring distribution patterns of cyanobacteria, however, a key issue in biogeography challenges the research in this field: the species concept. Since biogeography is concerned with the distribution of *species*, a well-defined species concept is required. Besides the existence of more than one concept of species (Dobzhansky, 1937; Wright, 1951; Slatkin, 1987), the concept of bacterial species in particular is considerably obscure, since its definition is typically defined coarsely (e.g. as >97% similarity of 16S or 18S ribosomal RNA genes) (about bacterial species concept see Konstantinidis & Tiedje, 2005; Achtman & Wagner, 2008; Fraser et al., 2009; Caro-Quintero & Konstantinidis, 2011). Apart from these issues, cyanobacteria have a particular characteristic: the fact that they have traditionally been studied in botany and taxonomically classified in the same way as algae and plants. These issues, in addition to the scarcity of qualified professionals to identify and classify taxa, may potentially hamper studies of the group (Rejmánková et al., 2004), including biogeographical studies (as we will discuss throughout this review). This review explores the current status of the field of biogeography of cyanobacteria and highlights joint distribution/population genomics/biodiversity/ workflows that aim to overcome some of the challenges commonly found in this field.

Cyanobacterial species concept

Although cyanobacteria have been widely studied in scientific literature, mainly with respect to aspects such as public health and bloom formation (Graham et al., 2009), little attention has been paid to their biodiversity (Nabout et al., 2013). These shortcomings in estimation of diversity and taxonomic classification are worsened by the confusion and overlap of the two taxonomic systems used to classify cyanobacteria: the Botanical Code and the Prokaryotic Code. As mentioned above, cyanobacteria were initially studied in botany, and for legacy reasons the International Code of Nomenclature for algae, fungi, and plants (Botanical Code) (McNeill et al., 2012) is still used for describing cyanobacteria in addition to the International Code of Nomenclature of Prokaryotes (Prokaryotic Code) (Parker et al., 2015). The history of cyanobacterial research in these two fields has also caused some discordance in taxonomic concepts and resulted in long discussions concerning the application of nomenclatural rules for cyanobacteria (Gaget et al., 2011; Oren, 2011; Oren & Garrity, 2014; Palinska & Surosz, 2014). Currently, the number of described cyanobacteria in the literature is controversial and the estimates range from 2,000 (Sant'Anna et al., 2006) to 8,000 species (Guiry, 2012) distributed among more than 300 genus. Nabout et al. (2013), in order to assume the underestimation of species, have estimated the number of unknown cyanobacteria species by using three models of discovery curves, and showed that the best model estimated a total of 6,280 species (the number of species already described is 43% of this total).

With the huge advances that recent technologies have brought to microbiology, especially in the field of genomics, recent studies have focused their efforts on revising the taxonomy of cyanobacteria in order to correcting mistakes caused by traditional taxonomics and clearing out the complicated evolutionary relationships of polyphyletic groups (e.g. Komárek, 2006; Komárek & Marěs, 2012; Komárek et al., 2014; Komárek, 2016). Today, the combination of microscopic data with molecular information is the most appropriate method for systematic and ecologic studies (Komárek et al., 2014; Komárek, 2016). More specifically, is considered as species within a single genus, those cyanobacterial strains or populations that belong to the "same genotype and morphotype with stable phenotypic features, and more or less stable and distinct ecological limits" (Komarek & Marěs, 2012; Komárek, 2016). Since the earliest-described species lack genetic information, previously described species are also undergoing thorough taxonomic revision (Castenholz, 2001; Komárek, 2006; Komárek, 2016). Taxonomic revisions at the suprageneric level are also underway (e.g. Marěs, 2017), and currently it seems that the best defined way how to separate cyanobacterial species using molecular markers is the use of the 16S-23S

internal transcribed spacer (ITS) region (Erwin & Thacker, 2008; Perkerson et al., 2011; Osorio-Santos et al., 2014).

It is also important to mention that, even if new efforts are made to access the genetic diversity of cyanobacteria, relating genetic diversity to a species concept is not easy. Cyanobacteria, in the same way as other prokaryotic organisms, have a set of essential genes (core genome) highly conserved and resistant to horizontal transfer (Shi & Falkowski, 2008; Larsson et al., 2011) and at same time a set of non-essential genes (accessory genome), that are more frequently subject to horizontal gene transfer and plays an important role in generating molecular diversity in cyanobacteria (Zhaxybayeva et al., 2006). Moreover, genome plasticity in cyanobacteria is evidenced by the broad distribution and hypervariability of mobile genetic elements (mainly insertion sequences), which can amount up to 10.95% of some genomes (Lin et al., 2010). Therefore, if populations of a same cyanobacterial species must have the same genotype, it is not trivial to think of which gene (or genes), and which cut-off value of nucleotide similarity, are more appropriate to consider. Phylogenetic reconstructions based on a single gene may be problematic, for instance, since they result in gene trees rather than species trees, ignoring the possibility of horizontal transfer which has been documented in prokaryotic rRNA operons (Ueda et al., 1999; Yap et al., 1999; Marěs, 2017).

Despite the genomics to be currently the most promising framework for revising the taxonomic classification of cyanobacteria, it is worth mentioning that these field has been moving forward at a pace that is relatively slower than currently observed for some of the other bacterial phyla (Alvarenga et al., 2017). Nowdays, the number of cyanobacterial genomes amounts for approximately 0.6% of all prokaryotic genomes available (Alvarenga et al., 2017). About 400 cyanobacterial genomes are available in public databases, in contrast to more than 30,000 complete genomes available for strains classified in 50 bacterial and 11 archaeal phyla (Land et al., 2015). Cyanobacteria, therefore, are still severely underrepresented in genomic databases when compared to other prokaryotes, and the currently available cyanobacterial genome databases are still lacking in taxonomic, environmental, and geographical diversity (Alvarenga et al., 2017). This scenario, in addition to providing an incomplete picture of this phylum, prevents the expansion of knowledge of the molecular biology of cyanobacteria, since sequences from neglected taxa may bring to light answers to meaningful questions (Richards, 2015).

From obtaining new genetic data of cyanobacteria in order to get closer to their real biodiversity, not only can their phylogeny be better resolved, but new approaches in ecological studies may be explored, such as in phylogenetic community ecology, phylogeography, and of course biogeography. As mentioned briefly above, biogeography is

very sensitive to the species concept and taxonomic resolution, both closely related to access to biodiversity. This is because key concepts studied in biogeography are defined from a species definition: habitats/niches are defined as a particular combination of resources and conditions necessary for a particular species (Tiedje, 1993), and both dispersion and ecological drift (including here speciation and extinction) depends on the size of the populations (Slatkin, 1987), which in turn will depend on the definition of specie adopted. Considering the processes involved in the biodiversity distribution mentioned above, in the following topics we present a synthesis of the biogeography studies on cyanobacteria, and the evidences presented in the literature of the driving factors shaping cyanobacteria distribution. At the same time, we made considerations regarding species concept, taxonomic resolution and other issues involved in the development of this research field. Finally, we discuss perspectives for this field and suggestions for future investigations that will contribute to a better understanding of the biogeography of cyanobacteria.

Environmental selection (current biotic and abiotic conditions)

One of the processes involved in the distribution of biodiversity widely studied in biogeography is the environmental selection (or environmental filtering), in which abiotic and biotic (biological interactions) conditions selects against species with different tolerances and habitat preferences. This process traditionally was thought to be a major mechanism structuring communities (Stein et al., 2014), and widely explored in ecological studies, mainly on animals and plants. In microbial ecology, the role of environmental heterogeneity also was thought to be the main factor shaping bacterial distribution, assumption known as Baas-Becking hypothesis (Baas-Becking, 1934). In this fundamental paradigm, *"everything is everywhere, but environment selects"* (Baas-Becking, 1934), the first proposition implies that microorganisms have dispersal abilities so high that the effects of past processes are suppressed; and the second one assumes that the current environmental characteristics determine the microbial distribution in the ecosystems. Although the idea of distribution purely selected by environmental conditions is currently under intense debate and review (as we will show in more detail in the following topics), a lot of evidence in the literature shows an important role of environmental heterogeneity in the biogeographical distribution of cyanobacteria.

At the same time as cyanobacteria present a broad range of metabolic capacities that enable them to deal with a range of environmental conditions (Vasas et al., 2010), this group exhibit high niche specialization (Whitton & Potts, 2000), then, the occurrence of distributions restricted to certain habitat types can highlight the role of environmental

selection. Several studies have shown that in fact, the environmental heterogeneity influence the distribution of cyanobacteria in a variety of spatial scales and ecosystems (Tables 1 and 2). At local and regional spatial scales, patterns of cyanobacteria distribution in aquatic ecosystems is commonly related to the concentration of nutrients and variables related to solar radiation, such as water transparency and water body depth (e.g. Drakare & Liess, 2010; Tian et al., 2012; Chamberlain et al., 2014; Ren et al., 2014; Harris et al., 2016). Similarly, in terrestrial environments the physical-chemical characteristics of soils and sediments appear to be important factors shaping the distribution of cyanobacteria (e.g. Garcia-Pichel et al., 2001; Thomasa & Dougil, 2006; Chamberlain et al., 2014).

Cyanobacterial blooms in aquatic ecosystems also show how changes in local and regional environmental characteristics can affect and alter the distribution patterns of these organisms. Events of cyanobacteria blooms show to what extent environmental variables (specifically the excess in nutrient concentrations and temperature increase) (Paerl et al., 2001; Paerl & Paul, 2012; Paerl & Otten, 2013) can rapidly affect and change the structure of cyanobacterial communities. Some genera in particular are widely studied because of their ability to form blooms (toxic or not) in freshwater environments, such as *Microcystis*, *Anabaena*, *Cylindrospermopsis,* and in marine ecosystems, such as *Lyngbya*, *Synechococcus*, *Trichodesmium* (Rastogi et al., 2015). On tropical blooms, for example, a meta-analysis showed that *Microcystis* blooms were more associated with higher total nitrogen concentrations, while *Cylindrospermopsis* blooms were more associated with higher maximum temperatures (Mowe et al., 2015). Also, environmental changes on a global scale (e.g. global warming) may potentially alter patterns of cyanobacterial blooms. More on this issue in the topic “Invasions and Global Climate Change”.

In extreme environments, cyanobacteria exhibit remarkable adaptability, notwithstanding the adverse environmental conditions of most inhospitable ecosystems in the Earth, including the frozen regions of the poles (e.g. Taton et al., 2006; Wood et al., 2008; Namsaraev et al., 2010), hypersaline environments (see Oren, 2015), and, on the other extreme, the high temperatures of hot springs (e.g. Papke et al., 2003; Miller et al., 2007; Ionescu et al., 2010). These commonly isolated ecosystems provide appropriate scenarios for assessing biogeographical patterns of distribution of highly specialized groups. In the polar environments, the distribution of cyanobacteria has been correlated with aeolian processes (Michaud et al., 2011), salinity (Jungblut et al., 2005) and soil chemical characteristics (Wood et al., 2008), whereas in thermal springs areas cyanobacteria distribution shows a relation with water temperature (Sompong et al., 2005).

Biotic variables (such as the abundance and richness of other organisms) may indicate that biological interactions are acting as important factors shaping cyanobacteria distribution. This relationship has already been studied and evidenced both in studies with experimental approach as well as through field data (e.g. Sullivan et al., 2003; Muhling et al., 2005; Agawin et al., 2007; Van Wichelen et al., 2010; Apple et al., 2011; Sønstebø & Rohrlack, 2011). A study specifically evaluating the diversity of *Synechococcus* in marine ecosystems (Muhling et al., 2005), for example, found evidence that viral infection may play an important role in determining the success of different *Synechococcus* strains. Positive and negative interactions with herbivores also influence cyanobacterial distribution patterns. For instance, copepods may facilitate cyanobacteria (Hong et al., 2013) and high abundances of generalist herbivores (e.g., *Daphnia*) can control cyanobacterial blooms when released from planktivorous fish predation (Sarnelle, 2007). Also, cyanobacterial blooms occurring in response to eutrophic conditions in water bodies may also be followed by associations with heterotrophic bacteria, several being capable of enhancing cyanobacterial growth (Berg et al., 2009).

In extreme environments such as poles and hot springs, biological interactions also play an important role in cyanobacteria distribution patterns. In the Antarctic continent, a study has observed an increase in the diversity of cyanobacteria from sub-Antarctic to continental Antarctica (Namsaraev et al., 2010), a pattern that could be explained by the disappearance of the vegetation cover since plants and mosses limit the amount of resources (nutrients available and light) for cyanobacteria. At the other extreme, in the alkaline hot springs of Yellowstone National Park, a strong negative association between the relative abundances of cyanobacteria and *Chloroflexi* (non-sulfur green bacteria) shows a likely competitive interaction between these two groups, possibly by habitat and/or limiting resources (Miller et al., 2009).

Thinking about continental and global distribution of cyanobacterial species, some taxa are studied because of their worldwide distributions. Before highlighting some of these studies, however, some terms used need to be clear. In biogeography, species considered as *cosmopolitan* are characterized by global distribution or distribution spanning several biogeographic provinces (Dijoux et al., 2014). It is important to mention here that this definition, which will be used henceforward, does not automatically imply in species that have highly efficient means of dispersal by being carried by wind or water (Fenchel & Finlay, 2004), but only concerns the distribution pattern seen today (dispersal abilities will be covered in the next topic). Also, we will use the *subcosmopolitan* term, which was suggested by Padisák (2003) in order to distinguish from those cosmopolitan, species

whose global occurrence is related to certain environments corresponding to species-specific adaptations.

Temperature is one of the majors studied factors shaping cyanobacteria distribution at global scales, and some subcosmopolitan species are restricted to the warmest or coldest climatic regions. Pantropical species are those that occur only roughly between the two Tropics and some cyanobacteria species found in this group are all *Cylindrospermopsis* species (except *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju; more about this species in the subsequent topics), *Arthrospira fusiformis* (Voronikhin) Komárek & J.W.G. Lund, *Anabaena fuellebornii* Schmidle, *Anabaena iyengarii* Bharadwaja, *Anabaena leonardii* Compère, *Anabaena oblonga* De Wildeman and *Anabaenopsis tanganyikae* (G.S. West) Woloszynska & V.V. Miller (Padisák, 2003). Well-known temperate species, on the other hand, appear to be restricted to temperate zones (Hoffmann, 1996; Vyverman, 1996), like *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek, *Limnothrix redekei* (Goor) Meffert, *Dolichospermum solitarium* (Klebahn) Wacklin, Hoffmann et Komárek, *Dolichospermum flos-aquae* Lyngbye Brébisson ex Bornet et Flahault) Wacklin, Hoffmann et Komárek, *Dolichospermum lemmermannii* (Richter in Lemmermann) Wacklin, Hoffmann et Komárek, *Anabaenopsis arnoldii* Aptekar, *Anabaenopsis milleri* Voronichin, *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault and *Cuspidothrix issatschenkoi* (Usachev) P.Rajaniemi, Komárek, R.Willame, P. Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen.

Global distribution of cyanobacteria in the extreme cold environments, or cryoenvironments, have also received attention recently (e.g. Taton et al., 2006; Wood et al., 2008; Namsaraev et al., 2010). Based on clone-library and phylogenetic analysis (16S rRNA), Jungblut et al. (2010) evaluated the global distribution of cyanobacteria by comparing communities from the North America (High Arctic Canada) with those from analogous sites in Antarctica. This study has showed that several of the High Arctic ribotypes were found to be >99% similar to Antarctic and alpine sequences. Moreover, more than 68% of all identified ribotypes at each site matched only cyanobacterial sequences from perennially cold terrestrial ecosystems, and were <97.5% similar to sequences from warmer environments. These results show a subcosmopolitan distribution of ecotypes from different cyanobacterial taxa that, although they have a global distribution, they are highly specialized and adapted to the extreme cold conditions of the poles.

Some species of cyanobacteria that present subcosmopolitan distribution are of particular interest since specific strains are capable of forming harmful blooms. Among these, *Microcystis aeruginosa* (Kützing) Kützing is a bloom-forming freshwater cyanobacteria that abounds in eutrophic and hypertrophic freshwater bodies worldwide

(Chorus 1999; de Figueiredo 2006; Kardinaal 2007; Vareli 2009). This species has already been studied in biogeography and phylogeography and a huge of evidences of a subcosmopolitan distribution is found in the literature (Haande et al., 2007; Van Gremberghe et al., 2011). Studies analyzing populations from distinct geographic locations have revealed a high degree of intra-specific genetic similarity, indicating a robust species definition for this cyanobacterium, and this outlook was corroborated in studies using distinct molecular markers and taxonomic resolutions, such as the 16SrRNA marker (Neilan et al., 1997), cpcBA-IGS marker (Bittencourt-Oliveira et al., 2001), 16S-23S ITS marker (Haande et al., 2007; Van Gremberghe et al., 2011) and PC-IGS marker (Haande et al., 2007).

Van Gremberghe et al. (2011) studying several *Microcystis* ITS sequences from worldwide suggest a truly cosmopolitan distribution for this cyanobacterium, and interestingly, no genetic structuring according to climate conditions was found (several *Microcystis* ITS types were detected in a wide range of climates, indicating a broad tolerance or capacity for rapid local adaptation). Van Gremberghe et al. (2011) argue that is more plausible that the genetic structure of *Microcystis* populations is driven by a recent global expansion and founder effects that arise whenever new habitat patches are created (Boileau et al., 1992), which are then colonized by a random selection of strains from regional or possibly global sources. The rapid local adaptation in a wide array of environmental conditions may be a result of the genome plasticity of *M. aeruginosa* (Frangeul et al., 2008), allowing new genetic interactions and higher variance on which natural selection can act. Also, *de novo* mutations might be involved in rapid adaptation to novel environments given the large population sizes and short generation time of *M. aeruginosa* (van Gremberghe et al., 2011). Indeed, the distribution of non-neutral genes of *M. aeruginosa* has already been related to habitat specificity, for example to land use (Marmen et al., 2006, using the *mcyD* and *mcyA* genes), and an evidence of local expansion of *M. aeruginosa* as response to environmental adaptation was recently found (using a multilocus approach) (Tanabe & Watanabe, 2011).

Two groups of well-studied *picocyanobacteria, Synechococcus* and *Prochlorococcus*, also provide some evidences about processes that might structure populations on a global scale. *Synechococcus* and *Prochlorococcus* are marine cosmopolitan genera of cyanobacteria and the major primary producers in the world's oceans (Li, 1994). The two genera are distinguishable by their possession of dissimilar light-harvesting apparatus (Ting et al., 2002) and even based on 16S rRNA gene (a very conservative genome region), several lineages within *Synechococcus* have been described (Fuller et al., 2003), while other potential lineages have been designated based on others

genomic regions, such as ITS and *ntcA* sequences (Ahlgren & Rocap, 2006a; Penno et al., 2006). It is widely known that different environmental pressures act not only between these two taxa, but also within each taxa, structuring well-known ecotypes (populations genetically different) in space according to different habitat preferences (e.g. Ernst et al., 2003; Zwirglmaier et al., 2008; Martiny et al., 2009; Ahlgren & Rocap 2012; Huang et al., 2012; Mazard et al., 2012; Shibl et al., 2014) (Table 2). In a study on a global scale (Zwirglmaier et al., 2008), for instance, the distribution lineages (based on 16S rRNA) of these two cyanobacterial groups was related to environmental variables and, interestingly, *Prochlorococcus* appeared to be more influenced by the physical parameters (temperature and depth) while *Synechococcus* by chemical parameters (nutrients). Genetically distinct clades based on ITS sequences with very broad distributions have also been detected (Rocap et al., 2002; Chen et al., 2006), and for these two taxa, ITS diversity was comparable with *Microcystis*. However, *Synechococcus* and *Prochlorococcus* have presented a genetic structure clearly more pronounced when comparable with *Microcystis* populations (Van Gremberghe et al., 2011).

Although the role of environmental selection on the distribution of several cyanobacterial taxa and habitats is well known, some factors must be taken into account. First, the spatial scale is an important factor that must be considered. Increasing environmental heterogeneity with the area along with the specificities of taxa in different habitats is the most common explanation for taxa-area patterns (Rosenzweig, 1995), thus, it is expected that environmental selection to be stronger at finer spatial scales than at broader ones. Moreover, this is certainly a taxon-dependent pattern. For example, in an environment where increasing the area leads to an increased environmental variation, the distribution of groups with greater environmental tolerance would tend to be less dependent on spatial scale and vice versa. In addition, this relationship can be found not only among species, but also among populations of the same taxon with different habitat preferences, or ecotypes, a possible approach to be explored in future studies with other cyanobacteria taxa, mainly species not yet, or little, explored.

The second issue to be taken into account is the taxonomic resolution adopted in the study. As mentioned earlier, when microbial taxa are defined from molecular approaches and classified into operational taxonomic units (OTUs), they are defined grossly through similarity in the sequence of one or more regions of the genome. Thus, since habitats are defined as a particular combination of resources and conditions necessary for a particular taxon (Tiedje, 1993), turnover habitat estimative are sensitive to the taxonomic definition, and thus, some biogeographical patterns of microorganisms may be more detectable at fine resolutions (detecting more compositional variation) than the

coarsest (Hanson et al., 2012). For example, in a scenario where "all cyanobacteria" were defined as a single taxon, one would observe a cosmopolitan distribution group with an enormous niche amplitude, and the influence of environmental selection would not be detectable. Horner-Devine et al. (2004) described taxa-area relationships for non-photosynthetic bacteria from salt marsh sediments using different OTU definitions (95%, 97% and 99% 16S rDNA sequence similarity) at scales from centimeters to hundreds of meters. This study showed that the taxa varied in the space mainly due to the environmental heterogeneity, but the turnover rate of the taxa was dependent on the bacterial lineage and the taxonomic resolution adopted: the turnover was higher with the increase of the taxonomic resolution. That is to say that finer taxonomic resolutions (e.g., 99% of sequence similarity) actually tend to detect greater compositional variation and consequently have greater power of environmental selection detection.

Regarding Cyanobacteria, a study analyzing the spatial variation of *Synechococcus* marine populations (Mazard et al., 2012) showed that in all cut-off values (88%, 91%, 94%, 97% and 99% of similarity of multi-locus sequences) the environmental factors showed a correlation with the distribution of the populations. However, the OTU definition using the cut-off value of 94% provided the best separation of the OTUs in relation to the sampled sites and the environmental parameters and, therefore, the best resolution for detecting possible ecotypes. Similarly, Martiny et al. (2009) have showed that *Prochlorococcus* distribution was dependent on the degree of sequence identity used to define a taxon (using the 16S-23SrRNA ITS region): light correlates with broad-scale diversity (90% cut-off), whereas temperature with intermediate scale (95% cut-off). These approaches not only identifies ecological differences at the population level, but also allows the analysis of the biogeographical distribution of ecotypes as a function of environmental variation and evolutionary processes, an feasible approach to be adopted in future studies with other species of cyanobacteria from many other ecosystems.

Finally, it is important to mention that genetic diversity and morphological diversity do not automatically imply the existence of ecotypes (or even different species). One case that may be mentioned is the cyanobacterium *M. aeruginosa*. Although on the one hand a high level of genetic diversity in a high number of genotypes was detected in the genus (Neilan et al., 1995; Kondo et al., 2000; Bittencourt-Oliveira et al., 2001; Wilson et al., 2005; El Herry et al., 2008; Yoshida et al., 2008; Tanabe et al., 2009; Fathalli et al., 2011; Gaevsky et al., 2011), on the other hand a number of morphospecies previously described (based on colony morphology) are not supported by molecular data forming a clade of nearly identical 16S rDNA sequences (Lepère et al., 2000; Litvaitis, 2002). Based on this extremely low 16S sequence divergence, along with DNA-DNA hybridisation data, Otsuka

et al. (2001) suggested, under the rules of the Bacteriological Code, merging all morphospecies into a single species. This example shows how the concatenated sequencing of several regions of the genome is important so that issues tangible to the ecological, evolution, and obviously taxonomy, are fully understood.

Historical processes (dispersion, past environmental selection and drift)

In this section we discuss the first statement of the Baas-Becking hypothesis, *'everything is everywhere*', which implies that microorganisms have so great dispersion capacity as to quickly remove the effects of past processes. Historical processes that may influence the current distribution of organisms include dispersal, past environmental selection and drift, which may lead to genetic divergence between populations and compositional variation between communities (Martiny et al., 2006). One of the main arguments behind the 'everything is everywhere' is that the small size, large population size, and consequently the high abundance of propagules increases the dispersion rate to levels where the dispersal limitation essentially does not exist. The high dispersal rate increases the similarity in the composition of communities and decreases rates of intra- and inter-population differentiation through increased gene flow, in the case of cyanobacteria and other prokaryotes, through horizontal gene transfer (HGT) (Martiny et al., 2006; Hanson et al., 2012).

One commonly used way to assess the role of the dispersal limitation in structuring biological communities is through distance-decay relationship: how communities become more dissimilar as the distance between them increases (taking away the effect of environmental variation on space) (Nekola & White, 1999; Morlon et al., 2008). Thus, the effect of geographic distance should be relatively weak in habitats where the dispersal rate is high and vice versa. Hillebrand et al. (2001) were one of the first to record this relation for microbial taxa. This study showed that in all taxonomic groups analyzed (diatoms, ciliate, corals and polychaetes) the species similarity declined significantly with distance, being one of the first evidence of dispersal limitation for microorganisms, which contradicts the classical hypothesis about the cosmopolitan dispersion of microorganisms.

Isolated and extreme environments (that is, island-like habitats) can act as barriers to gene flow, lead to the isolation of microbial groups and consequently genetic divergence, and thus facilitate speciation processes. An study based on temporal phylogenetic approach calibrated using microfossil data from the extremophile cyanobacteria *Chroococcidiopsis* (Bahl et al., 2010) from cold and hot deserts showed that there was no relation between the genetic differentiation of the lineages (based on 16S-ITS-23S rRNA) of this taxon with the geographic distance, nor on a global scale or for each

phylogenetically defined cluster. However, the common ancestry time of the lineages precedes the estimates for contemporary aridity in the desert regions where this taxon occurs, indicating that the distribution of *Chroococcidiopsis* was, at least in part, limited by barriers and/or invasive colonization. Another global scale study with *Synechococcus* from hot springs (Papke et al., 2003) showed a negative correlation between geographic distance and genetic similarity (based on 16S-ITS-23S rRNA) among the lineages, suggesting that the non-random and non-cosmopolitan distribution of *Synechococcus* populations is influenced, at least in part, by geographic isolation and dispersal limitation. Similarly, phylogeographic patterns of thermophilic cyanobacteria *Mastigocladus laminosus* Cohn ex Kirchner from thermal areas shows a significant positive correlation between genetic differentiation (based on 16S rRNA and nitrogen metabolism loci) and geographic distance, providing evidence for this species of a distribution pattern influenced by geographic isolation (Miller et al., 2007).

Although dispersal limitation seems to be an important factor in the distribution of some cyanobacterial groups, as shown above, other groups appear to have broad dispersal abilities and (sub)cosmopolitan distributions. A study in Jordanian hot springs (Ionescu et al., 2010), for instance, showed that some cyanobacterial isolates presented high similarity (>99% 16S rRNA) with others from hot springs of other regions of the globe. Similarly, the aforementioned study by Jungblut et al. (2010) showed that many ribotypes from Arctic showed >99% similarity (16S rRNA) with Antarctica and Alps sequences. Among these, further, an Arctic sequence showed 99.8% similarity to the sequence of *Leptolyngbya antarctica* (West & G.S.West) Anagnostidis & Komárek sequenced from Antarctica, indicating a high dispersion capacity for this species and thus a subcosmopolitan distribution pattern. However, as will be discussed below, these subcosmopolitan distributions records have used taxon definitions based on the 16S rRNA gene sequence similarity, a very conservative taxonomic definition.

Among the examples of global dispersion, the best-known example is *Cylindrospermopsis raciborskii*. This species is one of the most notorious cylindrospermopsin (CYN) producers that can be found in freshwater habitats in the temperate, tropical, subtropical regions of the world (Moreira et al., 2011). It is known that to make a definite determination of the native range of microbial species is extremely difficult: *C. raciborskii* was found for the first time in 1887 in the Nile, reported as *C. kaufmannii* (Schmidle) Huber-Pestalozzi, (Wołoszyńska, 1912), however its *Locus typicus* is, indeed, the Rava Demangan pond in Java, Indonesia (Wołoszyńska, 1912). *C. raciborskii* expanded rapidly to Europe in the last century and is considered an invasive alien species of the temperate zone (Padisák, 1997).

Phylogeographic studies have showed that populations of *C. raciborskii* are geographically grouped. Neilan et al. (2003) based on 16S rRNA and cyanobacterium-specific short tandem repeat sequence (HIP1) clustered: (1) strains from the USA and Brazil; (2) European strains (Germany, Hungary, and Portugal); (3) Australia strains. Gugger et al. (2005) based on 16S–23S internally transcribed spacer (ITS1) revealed the same continental cluster distribution and suggested that the current expansion of *C. raciborskii* in Europe and in Central – and North America did not result from recent invasion and colonization by African or Australian strains (Padisák, 1997), but rather represent local strains that maintained "cryptic" populations over time and only recently proliferated due to climate change and variations in other environmental conditions (Padisák, 2016). Also, Moreira et al. (2012) based on multilocus sequences recently showed that *C. raciborskii* strains grouped into three well-supported distinct clusters: (1) European, (2) African/American, and (3) Asian/Australian and also suggested the recent invasion of *C. raciborskii* in Portuguese and other European temperate environments.

C. raciborskii has been probably the only cyanobacterial species for which dispersal routes could be reconstructed and the speed of the dispersal could be estimated: it was estimated that less than a century was needed to colonize appropriate habitats all over the world (Padisák, 2016). The successful dispersion of *C. raciborskii* was largely attributed to its ability to tolerate travel along river courses (Padisák, 1997). Moreover, Piccini et al. (2011) proposed that phenotypic and genetic variability of *C. raciborskii* populations is linked to the existence of different ecotypes whose success is subject to the local environmental conditions. It is also speculated that unique physiological traits of *C. raciborskii* enable their proliferation in newly colonized ecosystems, currently exposed to greater environmental and temperature disturbances (Padisák, 2016). Further considerations about the invasive potencial of *C. raciborskii* and the possible traits related to its invasiveness in the topic "Invasions and Global Climate Change".

Phylogeographic studies with other cyanobacterial taxa also show how historical processes, such as dispersal limitation, adaptive radiation and allopatric speciation, can affect the global distributions of populations (Table 2). Another example that can be mentioned is the bloom-forming and nitrogen fixing filamentous cyanobacterium *Dolichospermum (Anabaena) circinalis* (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek. Worldwide blooms of *D. circinalis* are well known due to their production of neurotoxins as anatoxin-a and paralytic shellfish poisons (PSPs) (Padisák, 2016). Beltran & Neilan (2000) identified a geographic segregation of neurotoxin production in this cyanobacterium: American and European isolates of *D. circinalis* produce only anatoxin-a, while Australian isolates produce exclusively PSPs. Moreover, the phylogenetic

structure of *D. circinalis* (based on 16S rRNA) suggested a monophyletic group with worldwide distribution, and the PSP- and non-PSP-producing *D. circinalis* formed two distinct 16S rRNA gene clusters (Beltran & Neilan, 2000). Although the phylogeographic structure of this cyanobacterium can not yet be fully understood, these results may suggest a truly (sub)cosmopolitan distribution pattern followed by local environmental adaptations.

Restricted and/or endemic distributions are interesting in biogeography because a combination of factors is required to emerge them, such as geographic isolation, temporal continuity for local adaptations to accumulate, and mechanisms to reduce the intensity of HGT (Souza et al., 2008). Overall, endemism can have two different kinds of origin: the most pure form of endemism is when a species evolves at a certain location and remains exclusive to that location; and the so-called relict endemism occurs as a result of habitat fragmentation or destruction and a subsequent extinction from all localities except one (Padisák, 2016). Regarding cyanobacteria, some examples can be mentioned: *Aphanizomenon manguinii* Bourrelly and *Trichormus subtropicus* (N.L. Gardner) Komárek & Anagnostidis, so far been recorded only on several islands in the Caribbean region (Komárek, 1985). Also many recently described species of *Cylindrospermopsis* which have so far been recorded only in Central America: *C. acuminatocrispa* Couté & M.Bouvy in a reservoir in NE Brazil (Couté & Bouvy 2004), *C. catemaco* Komárková-Legnerová & R.Tavera in Lake Catemaco, Mexico (Komárková-Legnerová & Tavera, 1996) and *C. taverae* Komárek & Komárková-Legnerová in Central Mexico (Komárek & Komárková-Legnerová, 2002). And also a newly described species from Europe: *C. sinuosa* Couté, M.Leitão & H.Sarmento (Couté et al., 2004).

Polar regions are ideal locations for evaluating microbial endemism, since they contain parallel environments separated by large geographic distances and potential barriers to dispersal (Staley & Gosink, 1999), and indeed many possibly endemic cyanobacteria from Antarctica have been identified already (Taton et al., 2006; Comte et al., 2007; Jungblut et al., 2010; Michaud et al., 2011) (Table 1). In hot springs, another island-like habitat, distinct cyanobacterial populations were also characterized as possibly endemic (Papke et al., 2003; Sompong et al., 2005). Despite the importance of knowing endemic distributions, however, it is obvious that the detection of microbial endemism has some difficulties: the relation between the detection of endemism and the taxonomic resolution (as will be discussed below); the difficulty of confirming an endemic distribution because many cyanobacteria species (as well as other microorganisms) can inhabit an aquatic ecosystem unnoticed, since it may remain at a rather low biomass concentration and it does not form a conspicuous fraction of the community (Sukenik et al., 2012); and finally the difficulty of distinguishing whether a present taxon is in fact an active member of

the community or a dormant transient (Curtis et al., 2002; Hanson et al., 2012). In addition, there several species that, for a long time, have been known only from the type locality (local endemics), which cannot be considered as *real endemics* as long as more surveys in the same habitat-types in other continents are missing (Padisák, 2016).

As for environmental selection, the influence of historical processes on the current distribution of microorganisms is dependent on the spatial scale and taxonomic resolution adopted in the study. Here, dispersion capacity is expected to decrease with increasing distance. One study with the archaeal *Sulfolobus* (Whitaker et al., 2003) have shown this relationship, in which in spatial scales ranging from meters to tens of thousands of kilometers, the magnitude of the dispersion rate is in fact associated with the spatial scale (migration is greater between closer sites). Again, it is clear that this relationship is taxon-dependent, since different taxonomic groups have different rates of dispersion and/or establishment success, and more or less capacity to cover long distances.

Regarding taxonomic resolution, both dispersion and drift (including here speciation and extinction) depends on the size of the populations, which in turn will depend on the definition of species adopted. Again, using the definition of a taxon as "all cyanobacteria", for example, would result in a large estimate of population size, high potential for dispersion, and a high probability of cosmopolitanism, and thus the influence of historical processes would be completely neglected. This relationship has already been evidenced in a study with marine populations of *Prochlorococcus* (Martiny et al., 2009), in which the distribution of the populations was correlated with the dispersal rate only in the finer taxonomic resolution examined (99.5% sequence similarity using the 16S-23S ITS region). This is a possible approach to be tested in future researches with other cyanobacterial taxonomic groups.

Finally, the relation between the detection of endemism and the taxonomic resolution is obvious. On the one hand, studies using a wide range of molecular techniques to evaluate spatial patterns of prokaryotes, such as 16S rDNA sequencing and DNA/DNA pair hybridization, indeed suggest that genera of prokaryotes are widely distributed (e.g. Hagstrom et al., 2000; Brandao et al., 2002; Zwart et al., 2002; Hedlund & Staley 2004). In addition, cosmopolitan distributions have already been recorded using a taxonomy definition based on the 16S rRNA gene (e.g. Garcia-Pichel et al., 1996; Ionescu et al., 2010; Jungblut et al., 2010). Since 1% divergence in the 16S rRNA gene being equal to isolation or reduced genetic exchange for <10 million years (Jungblut et al., 2010), this gene is highly conserved and thought to underestimate the number of endemic species in a given habitat (Cho & Tiedje, 2000). On the other hand, when methods that offer a fine resolution are employed, bacteria appear to have more restricted and endemic distributions

(e.g. Cho & Tiedje, 2000; Whitaker et al., 2003). This same pattern occurs in studies with cyanobacteria, where endemism and restricted distributions (suggesting low dispersion capacity and/or high niche specificity) are more detected at fine taxonomic resolutions than coarser (e.g. Papke et al., 2003; Miller et al., 2007; Mazard et al., 2012).

Invasions and Global Climate Change

The entry, establishment, and spread of non-native species to a new ecosystem, is frequently described as biological invasion (Vitousek et al., 1997; Ricciardi & Cohen, 2007). Biological invasion is considered as an extension of normal colonization processes such as succession (Elton, 1958), and it was proposed that the term *invasive species* will be restricted to describe non-native species that expanded their geographic range, became abundant and have environmental and economic impact (Colautti & Macisaac, 2004). Invasions also may threaten global biodiversity by altering the structure and function of ecosystems and disrupting key biological interactions (Traveset & Richardson, 2006). Indeed, when invading new areas, phytoplankton species (including cyanobacteria) are able to cause irreversible environmental changes by outcompeting native species, changing food-web structures (Dufour et al., 2006), or reduce diversity (Borics et al., 2000, 2012). Moreover, biological invasions are important in biogeography since invasion involve dispersal ability, environmental adaptation, unique physiological traits (like dormant stages as dispersal units) and ecological plasticity.

The invasion of free-living microorganisms to new aquatic habitats is rather cryptic and difficult to detect therefore invasions of these “invisible invaders” have been rarely reported (Litchman, 2010). In that sense, algae and cyanobacteria are exceptions as they have visible characteristic of spectral signature and microscopic morphological features (Sukenik et al., 2012). This property partly contributed to the increased number of records on invasion of cyanobacteria taxa, such as *C. raciborskii* (Padisák, 1997; Dyble et al., 2002; Wiedner et al., 2007; Vidal & Kruk, 2008; Sukenik et al., 2012; Wilk-Woźniak et al., 2016), *Raphidiopsis mediterranea* Skuja (Wilk-Woźniak et al., 2016), and some species of the genus *Aphanizomenon* (Sukenik et al., 2012). Sedimentary time series and large-scale monitoring records show that both the expansion and abundance of cyanobacteria has increased significantly in the last 200 years, and more rapidly in the last 70 years (Taranu et al., 2015). Among the well-studied cyanobacteria due to their invasion potential, the most important freshwater invasive cyanobacteria are the species *C. raciborskii* (already mentioned in the topic “Historical processes”) and to a lesser extent *Aphanizomenon ovalisporum* Forti, which are native to tropical regions and in the last two decades have

expanded to subtropical and temperate areas (Kokociński et al., 2009; Kaštovský et al., 2010; for a detailed review on the invasiveness of these two taxa see Sukenik et al., 2012).

As already mentioned, *C. raciborskii* was an originally Pantropical species and since the first comprehensive review on its distributional area and dispersal was published (Padisák, 1997), this cyanobacterium was documented in most parts of the globe (Padisák, 2016). According Wilk-Woźniak (2016), the distribution of the records of this species suggests the following possible migration scenarios: (1) from the centre of Africa towards the north of the continent; (2) from North Africa to Europe; (3) from Java to eastern Australia; (4) from Java through South Asia to Europe and (5) from Australia to Southern Africa and on to the eastern parts of North, Central and South America. Kokocinski et al. (2017) have showed that, in Europe, the recent invasion of *C. raciborskii* in the East-Central regions may be related to environmental factors, mainly temperature-related variables. Today, *C. raciborskii* is find in many areas from Europe, such as Germany (Mischke, 2001; Stuken et al., 2006), France (Briand et al., 2002; Druart & Briand, 2002; Cellamare et al., 2010), and Poland (Stefaniak & Kokocinski, 2005; Kokocinski & Soininen, 2012; Kobos et al., 2013); and others Mediterranean/subtropical regions including Portugal (Saker et al., 2004), Algeria (Bouaicha & Nasri, 2004), Italy (Mugnai et al., 2008; Barone et al., 2010), Tunisia (Fathalli et al., 2010), Egypt (Hamed, 2005; Mohamed, 2007), and Israel (Zohary & Shlichter, 2009; Alster et al., 2010). Recent expansion of the species in South America (Vidal & Kruk, 2008; Fabre et al., 2010) and Africa (van Vuuren & Kriel, 2008) toward higher latitudes is also documented (Padisák, 2016). Several studies published in the literature concerns its invasiveness potencial (e.g. Padisák 1997; Sukenik et al., 2012; Wilk-Woźniak et al. 2016), and part of this effort is due to the fact *C. raciborskii* is a potentially toxic and bloom-forming cyanobacterium. Australian strains, for example, are known to produce cylindrospermopsin, and Brazilian strains have been reported to produce paralytic shellfish poisoning toxins (Neilan et al., 2003).

Since the invasion must be initiated with dispersion to new zones (by aeolian transport, migrating animals, and also facilitated by human activities) and the subsequent establishment to the new environment, the invader needs a set of traits that support its establishment and proliferation (Sukenik et al., 2012). Regarding *C. raciborskii*, and also other Nostocales cyanobacteria, can be mentioned: the ability to form dormant cells (akinetes) that may survive long and extreme dispersion routes and survive on unfavorable conditions; and the ability to fix atmospheric nitrogen in the absence of combined inorganic sources, thus extending the spectrum of ecosystems to which they can invade (Sukenik et a., 2012). This species may also utilize other limiting resources, such as phosphorus, more efficiently than other cyanobacteria due to high affinity and P storage capacity (Isvánovics

et al., 2000; Wu et al., 2011). Moreover, an important feature of *Cylindrospermopsis* is its wide thermal tolerance (Briand et al., 2004), which is essential to maintain the populations during cold winters. Padisák (1997), for instance, have reported that akinetes of *C. raciborskii* germinate at temperatures <24˚C. Finally, allelopathy (ability to synthesizes allelo-chemicals that inhibit other phytoplankton species or deter and reduce grazing) was suggested as a beneficial trait of *C. raciborskii* that contributes to its stable dominance and geographic expansion (Fastner et al., 2007; Figueredo et al., 2007; Paerl et al., 2011).

Besides *C. raciborskii*, some other potentially invasive species of cyanobacteria are mentioned in the literature. For instance, dispersal of halophilic species in temperate waters has been facilitated by winter de-icing of roads and improper treatment of industrial sewage (Kaštovský et al., 2010). In that sense, it can be mentioned the cyanobacterium *Cuspidothrix issatschenkoi* (Usachev) P.Rajaniemi, Komárek, R.Willame, P. Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen (Padisák, 2016), whose expansion in the twentieth century might be enhanced by gradual adaptation to typical freshwater environments and the ongoing climate warming (Kaštovský et al., 2010). Also, *Planktothrix rubescens* is another cyanobacterium whose invasive potential has been described in the literature (Kaštovský et al., 2010; Padisák, 2016). This species had its original distribution area covering southern central Norway and the western alpine area, especially large lakes in Switzerland, Austria Italy, Germany, Slovenia, and France (e.g. Barco et al., 2004; Jann-Para et al., 2004; Jacquet et al., 2005; Legnani et al., 2005; Ernst et al., 2009). In addition to appears to proliferate within its original area (Jacquet et al., 2005), increasing number of reports provide evidence for its dispersal both southward and eastward to Spain (Almodóvar et al., 2004; Barco et al., 2004), Portugal (Paulino et al., 2009), Central Italy (Messineo et al., 2006), Sicily (Naselli-Flores et al., 2007; Naselli-Flores, 2014), Greece (Vareli et al., 2009), Turkey (Albay et al., 2003; Akcaalan et al., 2007), Poland (Krupa & Czernas, 2003; Lenard, 2009), and Hungary (Vasas et al., 2014) (Padisák, 2016).

One can not fail to mention the role of global climate change in cyanobacterial invasion patterns. This is because global warming leads to worldwide proliferation of cyanobacterial species, thus increasing its invasiveness potencial, and consequently increasing blooms events. In addition to local environmental changes that can potencially increase cyanobacteria proliferation (e.g. nutrient overenrichment of waters), climate change is a potent catalyst for the further expansion of cyanobacterial blooms (Paerl & Huisman, 2008; Paerl & Huisman, 2010; Häder & Gao, 2015). Rising temperatures favor cyanobacteria in several ways, thus increasing its potential for invasiveness: (1) cyanobacteria generally grow better at higher temperatures (often above 25°C), giving a competitive advantage at elevated temperatures (Elliott et al., 2006; Jöhnk et al., 2008); (2)

global warming causes lakes to stratify earlier in spring and destratify later in autumn, which lengthens optimal growth periods; and finally, (3) global warming affects patterns of precipitation and drought, for example, more intense precipitation will increase surface and groundwater nutrient discharge into water bodies (Paerl & Huisman, 2010).

Harmful blooms of toxic cyanobacteria are of particular interest and concern because of their economic and health consequences (Rastogi et al., 2015). Harmful blooms due to excessive growth of certain cyanobacteria followed by the production of toxic compounds have been reported in many eutrophic to hypertrophic lakes, ponds, and rivers throughout the world (Rastogi et al., 2015) Many of these bloom events, for instance, are of toxic *Microcystis* species and strains that proliferate under current environmental alterations, including nutrient enrichment, global warming, and regional hydrologic changes (Paerl & Paul, 2012). Microcystin is among the most commonly occurring toxin produced by cyanobacteria in natural waters (Babica et al., 2006; Rastogi et al., 2015), and can cause liver complications and damage to the nervous system if ingested (Bláha et al., 2009). Moreover, it has been suggested that UV-B radiation may significantly influence strain composition of cyanobacterial blooms in favor of microcystin (MC) producers (Ding et al., 2013).

Another cyanobacterium potentially harmful-bloom forming is the already mentioned *C. raciborskii*. In a modeling effort, for instance, Mehnert et al. (2010) have demonstrated that under a scenario of climate change with an increase of 4˚C in the water temperature, *C. raciborskii* would outcompete a native species (*Aphanizomenon gracile* Lemmermann). Also, temperature-dependent release of cylindrospermopsin and microcystin has already been reported for *A. ovalisporum* (Cirés et al., 2011), *Planktothrix agardhii* (Walls et al., 2017) and *Microcystis* (Dziallas & Grossart, 2011), implicating management complications associated with global change scenarios. However, it is worth to mention that not all harmful blooms are associated with an invasion process, since many cyanobacterial species are of broad geographic distribution and rapidly respond to current environmental changes. Furthermore, although temperature (and global warming) are recognized as important factors leading to the proliferation of cyanobacteria, the exact mechanisms and the role of environmental factors regulating harmful blooms are disputable and yet to be understood, requiring more efforts both through field data and through experiments and modeling approaches.

Concluding remarks and future research perspectives

The central and global importance of microorganisms in the natural ecosystems throughout the world is obvious, however, despite the increase of studies and data, the

application of ecological and evolutionary theories in microbial systems is still very limited. From the body of evidence collected by that time, it is known that a variety of non-mutually exclusive factors influence the distribution of microbial diversity on the planet, such as environmental selection (e.g. Mazard et al., 2012), allopatric speciation (e.g. Dvorák et al., 2012), and dispersal limitation (e.g. Miller et al., 2007), which during a long time was assumed did not exist for the microorganisms (Baas-Becking, 1934; Finlay, 2002; Fenchel & Finlay, 2004). Regarding cyanobacteria, there is also an increase of interest in phylogeographic and biogeographic studies, from approaches that go beyond ecological and taxonomic traditional studies from a solely microscopic approach. However, the studies still comprise a very small variety of habitats and taxonomic groups explored (Tables 1 and 2). Due to the highly diverse character of these group, from unicellular to multicellular organisms with differentiated cells (heterocyst and akinetes), with free living and associated/symbiotic life forms, and present in the most diverse habitats of the Earth, interesting perspectives for future biogeographical studies emerge.

As expected from the Baas-Becking hypothesis, environmental selection is indeed found to have some influence on cyanobacterial distribution patterns of various taxonomic groups and habitats (Tables 1 and 2). The studies reported here show that both in global scales (e.g. Zwirglmaier et al., 2008) and at local scales (e.g., Chamberlain et al., 2014), environmental characteristics influence, at least in some degree, the distribution of cyanobacteria. The key question here is how distinct taxa, and how distinct taxonomic resolutions adopted to define a "taxon", respond to environmental variation. We think, therefore, that future biogeographic studies within this topic will benefit from exploring issues such as: i) what is the ecological relevance of different levels of taxonomic resolution adopted for a given taxon?; ii) what level of taxonomic resolution is required for the detection of ecotypes of a given species and how important are the environmental variables that potentially structure the distribution of ecotypes?; iii) how do different taxonomic and trait-based groups respond to environmental variation?; iv) how the cyanobacterial traits are distributed and responding to environmental variation?

On the influence of historical processes, recent studies with contradictory evidences about microbial cosmopolitism have been debating this issue, including studies with cyanobacteria taxa (Table 2). Thanks to previous studies, it is clear that dispersal capacity is dependent on several factors, such as the traits and dispersion strategies of give taxon may influence your establishment success (Hanson et al., 2012 and cited references). Akinetes-forming cyanobacteria, for instance, may have a greater dispersion capacity than those that do not form these structures because it is more likely to remain viable until arrival in a favorable habitat (in addition to decreasing the likelihood of extinction in front to

environmental fluctuations) (see about *C. raciborskii* in the topics above). In addition, the type of habitat may have some influence on the microbial dispersion. For example, taxa from sediment may have a lower dispersion capacity relative to taxa from surface soil in a same ecosystem or geographic area.

In the context of historical processes, therefore, interesting questions still need to be explored in the future, such as: i) how different taxonomic groups (defined at different levels of taxonomic resolution) and trait-based present patterns of dispersion and gene flow? ii) in fluid/continuous habitats (e.g. lakes, oceans) the rate of dispersion is greater than in island-like/poorly flowing habitats (e.g. periphyton, soil crusts)?; iii) what is the role of population isolation, HGT and genetic drift in cyanobacterial distribution patterns at large spatial scales and at fine spatial scales (using target molecular markers for each purpose)?; iv) what are the dispersive routes of different taxa and how do they vary over time? (that is, to apply molecular clocks in order to put the spatial distribution of different cyanobacteria taxa into a temporal framework).

Finally, to answer all these questions, in addition to studies with field data, both manipulative experimental tests and temporal studies are needed. Experimental manipulations have the potential to be useful tools for understanding how environmental and spatial factors influence the distribution of microbial taxa because microorganisms provide much better controlled and more flexible experimental systems for testing ecological theory than larger organisms (Bell, 2010). For instance, one experimental study has identified a specific temporal window during which the effects of dispersal limitation were apparent, although a subtle environmental variation operating over time scales of a few days can quickly overcome this dispersal limitation (Bell, 2010). Also, another experimental study showed that the slope of the taxa-area relationship for natural bacterial communities inhabiting small aquatic islands is comparable to that found for larger organisms (Bell et al., 2005). These approaches can provide important insights into biogeography of cyanobacteria, providing the opportunity of analyzing dispersion, HGT and environmental selection more precisely. For instance, testing taxa-area and distance-decay relationships, correlations among abundance, genetic diversity and ecotypes diversification, and the comparison of these patterns among different species and clades of cyanobacteria on controlled environmental conditions.

Conclusions

Among prokaryotic organisms, cyanobacteria are one of the most morpho-physiologically distinct groups (from unicellular to multicellular life forms; with or not differenciate cells; with toxic, invasive and bloom-forming capacities; etc.) and this feature

provides interesting approaches for biogeographic studies that are still little explored. In a overview, biogeography of cyanobacteria is still in its infancy and generalizations can not yet be made. On the one hand, considered (sub)cosmopolitan species may actually represent ecotypes with restricted distributions; on the other hand, species with restricted or endemic distributions may actually be the result of an underestimation of occurrence due to insufficient sampling effort. Due to the ecological, sanitary and economic importance, bloom-forming and invasive toxic species are still the most studied and of which there are more data, both ecological and genomic, in the literature. Thus, efforts to know and map the real diversity of cyanobacteria in the most diverse ecosystems is the first step to leverage the study of biogeography of this group. The exploration of little-known geographic areas (e.g. subtropical environments) and habitats (e.g. terrestrial, microbial mats), as well as sampling and sequencing genomes from cyanobacteria of lesser known taxa, are quite needed to improve our understanding on biogeography of cyanobacteria.

So far, the fact that there are some cyanobacteria species with broad distributions and others with possible endemic distributions implies in the non-uniformity of the response of different taxa to ecological/evolutionary processes. To what extent these distribution patterns are dependent on species concept, level of taxonomic resolution, spatial and environmental scale, and the biases of the molecular methodologies applied in the studies, are key issues to be explored in future studies. We suggest that to answer these questions adequately, it is necessary to use multiple taxonomic definitions based on a variety of genetic markers (and biochemical and morphological characteristics, more easily accessible for cyanobacteria), and subsequent testing of hypothesis in the light of ecological and evolutionary theories. It should also be noted that taxonomic identification based on molecular methods, although more detectable of variation in biodiversity, actually provides data for the investigation of biogeographical patterns of microbial genes, not whole organisms. Therefore, another possible approach is to compare biogeographic patterns of cyanobacterial genes with whole genomes (already available for some cyanobacterial species) and microscopic-based definition, testing how distribution patterns depend on the definitions of cyanobacterial species adopted.

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TABLES

Table 1 Examples of 16S-23S rRNA gene target and microscopy-based studies that have found non-random distributions of cyanobacterial communities.

Approximate scale (km)	Habitat	Identification method	Correlated with	Endemism	Reference
3,500	Antarctic free-ice terrestrial sites	Molecular	Vegetation cover	✓	Namsaraev et al. (2010)
900	Swedish lakes	Molecular	Environmental variables (total organic carbon, biomass of eukaryotic phytoplankton, pH and conductivity)		Drakare and Liess (2010)
260	Hot springs, Thailand	Microscopy	Temperature and barriers to the dispersal of some taxa	✓	Sompong et al. (2005)
140	Microbial mats in lakes of Eastern Antarctica	Microscopy and molecular	Environmental variables (salinity, light intensity and depth)	✓	Taton et al. (2006)
80	Nansi Lake, China	Microscopy	Environmental variables (temperature and phosphorus)		Tian et al. (2012)
55	Aquatic habitats, High Arctic Canada	Molecular	Global distribution of low-temperature cyanobacterial ecotypes throughout the cold terrestrial biosphere		Jungblut et al. (2010)
40	Hongze Lake, China	Microscopy	Environmental variables (water temperature, dissolved oxygen, nitrate, chemical oxygen demand, transparency and total nitrogen)		Ren et al. (2014)
40	Coastal waters and sediments of the Big Island, Hawaii	Molecular	Habitat type (sediments and water) and environmental variables (temperature, salinity, dissolved oxygen, chlorophyll a, pH, total suspended matter, nitrogen stable isotope, organic matter and mean sediment size)		Chamberlain et al. (2014)
35	Batticaloa Lagoon, Sri Lanka	Microscopy	Environmental variables (nitrate, total phosphorus and turbidity levels)		Harris et al. (2016)
20	Habitats across Taylor Valley, Antarctica	Molecular	Eolic processes	✓	Michaud et al. (2011)
10	Dongping Lake, China	Microscopy	Environmental variables (temperature, chemical oxygen demand and concentration of inorganic nitrogen)		Lu et al. (2013)

Table 2 Examples of molecular-based studies that have found non-random distributions of cyanobacterial populations or ecotypes.

Habitat	Taxon	Taxon characterization	Identification method	Correlated with	Reference
Hot and cold deserts	*Chroococcidiopsis*	extremophile cyanobacteria	multi-locus sequences	Ancient evolutionary legacy	Bahl et al. (2010)
Strains of four continents from different habitats	*Cylindrospermopsis raciborskii*	filamentous cyanobacteria; heterocyst and akinetes-forming; potentially forming harmful blooms	16S-23S ITS-1	Recent spread across the American and European continents from restricted warm refuge areas/recent colonization of Australia by African strains	Gugger et al. (2005)
Isolates from Africa and Europe			ITS1, PC-IGS, nifH and rpoC1	Geographical cluster distribution	Haande et al. (2008)
Aquatic samples from all continents			16S rRNA, 16S-23S ITS-L, 16S-23S ITS-S and rpoC1	Clustering of the strains due to geographic origin/recent invasion in the European temperate environments	Moreira et al. (2011)
Freshwater systems from different continents			16S rRNA and HIP1 PCR profiles	Geographical cluster distribution	Neilan et al. (2003)
Thermal areas	*Mastigocladus laminosus*	thermophilic filamentous cyanobacteria; heterocyst and akinetes-forming	multi-locus sequences	Dispersal barriers and geographical isolation by distance	Miller et al. (2007)
Marine benthic microbial mats	*Microcoleus chthonoplastes*	filamentous cyanobacteria	16S rRNA	No dispersal barriers/cosmopolitan distribution	Garcia-Pichel et al. (1996)
Different habitats from three continents	*Microcoleus vaginatus*	filamentous cyanobacteria	16S rRNA and 16S-23S ITS	Dispersal barriers and allopatric speciation	Dvorak et al. (2012)
East-African water bodies	*Microcystis aeruginosa*	colonial cyanobacteria; potentially forming harmful blooms	PC-IGS and ITS1 rDNA	Clustering of the strains due to geographic origin	Haande et al. (2007)
Strains from five continents			mcy gene cluster (mcyA, mcyD and mcyG)	Geographical cluster distribution/evidence of positive selection in mcy genes	Moreira et al. (2013)
Water bodies from Israel			mcyD and mcyA genes	Environmental, geospatial and land use factors	Marmen et al. (2016)

Samples from all continents			rDNA ITS	Intercontinental dispersal/cosmopolitan distribution	van Gremberghe et al. (2011)
Arctic ecosystems from Europe and Canada	*Phormidium autumnale*	filamentous cyanobacteria	16S rDNA and 16S−23S rDNA ITS	Rate of colonization relatively high/cluster of genotypes in the phylogenetic tree according to their occurrence in similar habitats	Strunecký et al. (2012)
European freshwater habitats	*Planktothrix*	filamentous cyanobacteria; gas vacuoles present	mcy gene cluster	Spatial isolation favoring the genetic divergence	Kurmayer and Gumpenberger (2006)
Atlantic and Pacific oceans	*Prochlorococcus*	unicellular picocyanobacteria	16S-23S rRNA ITS	Environmental selection and dispersal limitation	Martiny et al. (2009)
Seawaters around the world	*Synechococcus*	unicellular picocyanobacteria	multi-locus sequences	Environmental selection	Mazard et al. (2012)
Gulf of Aqaba, Red Sea			rpoC1 gene	Cyanophage infection	Mühling et al. (2005)
Hot springs			16S rRNA and 16S-23S ITS	Genetic drift by geographical isolation	Papke et al. (2003)
Temperate-zone lakes and the brackish Baltic Sea	*Synechococcus and Prochlorococcus*	unicellular picocyanobacteria	16S rRNA and ITS-1	Ecosystem-dependent adaptive radiation	Ernst et al. (2003)
Diverse marine ecosystems			16S-23S rRNA ITS	Environmental selection	Huang et al. (2012)

CAPÍTULO II

Comparative phylogeography of two free-living cosmopolitan cyanobacteria: Insights on biogeographic and latitudinal distribution*

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Comparative phylogeography of two free-living cosmopolitan cyanobacteria: Insights on biogeographic and latitudinal distribution

Biogeography of two freshwater cyanobacteria

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Abstract

Aim: Free-living bacteria have long been assumed to have little biogeographic signature because of their high potential for passive dispersal. *Raphidiopsis raciborskii* and *Microcystis aeruginosa* are free-living cosmopolitan cyanobacteria with a probable tropical origin, that frequently form toxic blooms and are considered invasive species in middle latitudes. Despite these similarities, their phylogeographic patterns have seldom been directly compared. Our aim was to reconstruct the phylogeographic histories of *R. raciborskii* and *M. aeruginosa*, in order to explore whether their current distributions could be attributed to the same biogeographic events.

Location: Global.

Taxa: *Raphidiopsis raciborskii* (Nostocales) and *Microcystis aeruginosa* (Chroococcales).

Methods: The phylogeography of these species was studied based on global genetic diversity patterns from a dataset of worldwide 16S rRNA gene sequences. Genetic diversity indices were measured globally and by latitude. Analyses based on distance matrices were performed to evaluate the correlation between genetic divergence, geographic distance and climatic conditions. Demographic history was investigated through neutrality tests and Bayesian Skyline Plot analysis.

Results: For both species, the genetic diversity is highest in tropical latitudes, and the data provided evidence of recent population expansions (in the last 2,500 years). *R. raciborskii* showed much lower genetic diversity than *M. aeruginosa*. A significant phylogeographic structure was found for *R. raciborskii* but not for *M. aeruginosa*. Climatic conditions had a significant influence on the genetic structure of both species, but this influence was stronger and varied according to latitude only for *R. raciborskii*.

Main conclusions: Supporting the hypothesis of a tropical origin and recent dispersal to temperate habitats, both species had higher genetic diversity in tropical latitudes and showed evidence of recent population expansions. *R. raciborskii* populations showed a significant decline in genetic similarity with increasing geographic distance, indicating an interaction between drift and some dispersal limitation on its phylogeography. In contrast, *M. aeruginosa* seemed to have a high frequency of intercontinental dispersal. Finally, the particularities of each species, such as the ability to form akinetes in *R. raciborskii* and high genome plasticity and niche specialization in *M. aeruginosa*, may be associated with the lower genetic diversity of *R. raciborskii* when compared to *M. aeruginosa*.

Keywords

biogeography, cosmopolitanism, cyanoprokaryota, *Raphidiopsis raciborskii*, dispersal limitation, microbial ecology, *Microcystis aeruginosa*, phylogeography

Introduction

Microbial biogeography has received much attention in recent decades (Hanson, Fuhrman, Horner-Devine, & Martiny, 2012; Martiny et al., 2006; Ramette & Tiedje, 2007). From this recent interest, biogeographic patterns hitherto unknown in microbial taxa, such as endemism (e.g. Taton et al., 2006) and allopatric speciation (e.g. Dvořák, Hašler, & Poulíčková, 2012; Whitaker, 2006) have been documented. At the same time, the traditional idea that only current environmental conditions affect microbial distributions (Baas-Becking, 1934) has been refuted in several studies (e.g. Ribeiro, Duarte, & Crossetti, 2018). To understand how microbial diversity is distributed and its causes is crucial, since microorganisms, especially prokaryotes, are an essential component of the Earth's biota and comprise the majority of biomass and genetic diversity (Whitman, Coleman, & Wiebe, 1998).

Cyanobacteria are distinguished among prokaryotes by their ability to perform oxygenic photosynthesis, which is responsible for the largest portion of global primary productivity (Li, 1994). Cyanobacteria show particular characteristics such as the existence of multicellular species, capacity to form differentiated cells, and high ecological plasticity that allow them to inhabit temperate, tropical and even extreme ecosystems, performing crucial roles in global biogeochemical cycles (Whitton & Potts, 2007). The potential invasiveness of some species (e.g. Sukenik, Hadas, Kaplan, & Quesada, 2012), together with the ability to form blooms and to release toxins in aquatic ecosystems (Christoffersen, 1996), has important implications in the context of global climate change, in which the transformation of pristine aquatic environments into "green soups" is an often-reported concern (Paerl & Huisman, 2008).

Research on cyanobacteria has explored aspects of public health and bloom formation, but little attention has been paid to their biodiversity (Nabout, da Silva Rocha, Carneiro, & Sant'Anna, 2013). The traditional classification and studies of cyanobacteria together with algae and plants, as well as the scarcity of qualified professionals to identify and classify taxa, have contributed to underestimation of their diversity and a lack of genetic information (Ribeiro et al., 2018). For instance, the number of cyanobacterial genomes amounts to approximately 0.6% of all available prokaryotic genomes (Alvarenga, Fiore, & Varani, 2017). This situation has contributed to a relative lack of attention to other aspects such as the biogeography and phylogeography of cyanobacteria. Studies in this field have recently increased and have provided evidence of geographic isolation for the thermophilic cyanobacterium *Mastigocladus laminosus* Cohn ex Kirchner (Miller, Castenholz, & Pedersen, 2007), allopatric speciation for the filamentous cyanobacterium *Microcoleus vaginatus* Gomont ex Gomont (Dvořák et al., 2012), and niche partitioning for

the marine picocyanobacterium *Prochlorococcus* (Johnson et al., 2006). Since different cyanobacterial species show different distribution patterns (from cosmopolitan to restricted and endemic), a major research topic to be explored is which factors generate these unequal patterns (Ribeiro et al., 2018).

Most phylogeographic studies have examined two species of cosmopolitan freshwater cyanobacteria: *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno (previously named *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju), a filamentous cyanobacterium able to form differentiated cells (akinete and heterocyte); and *Microcystis aeruginosa* (Kützing) Kützing, a cyanobacterium that is not able to form differentiated cells and is often organized into large globular or semi-spherical colonies (Whitton & Potts, 2007). In recent decades, these species have been extensively studied due to their high abundance, widening geographical distributions, and frequent formation of toxic blooms (Cirés et al., 2014; Harke et al., 2016; Janse van Vuuren & Kriel, 2008; Moreira, Vasconcelos, & Antunes, 2013; Sukenik et al., 2012). Increasing evidence suggests that *R. raciborskii* and *M. aeruginosa* originated in Africa and have recently expanded to temperate environments (Moreira, Spillane, Fathalli, Vasconcelos, & Antunes, 2014; Padisák, 1997), or have maintained 'cryptic' populations over time and have only recently proliferated in temperate regions due to climate change (Padisák, Vasas, & Borics, 2016). Previous studies have found phylogeographic patterns, such as lineages clustered by geographical area (Gugger et al., 2005; Haande et al., 2007; Moreira, Fathalli, Vasconcelos, & Antunes, 2011; Moreira et al., 2013; Neilan, Saker, Fastner, Törökné, & Burns, 2003). However, phylogeographic studies on a global scale, comparing latitudinal differences in relation to spatial and climatic variables, and exploring the population dynamics of these species based on genetic data are still lacking.

In this study, we explored and compared the phylogeographic patterns of *R. raciborskii* and *M. aeruginosa*, using sequences of the 16S rRNA gene from populations worldwide. Although both species are cosmopolitan, their different evolutionary histories and morpho-physiological functional traits may lead to different dispersal capabilities and environmental tolerances, reflected in distinct phylogeographic structures. We asked three key questions: (i) Do the genetic diversity patterns of these species vary with latitude? Higher genetic diversity in the tropics than in temperate latitudes would support the hypothesis of a tropical origin and recent dispersal to (or proliferation in) temperate environments; (ii) Do *R. raciborskii* and *M. aeruginosa* show spatially structured genetic diversity related to spatial and climatic variables? If there is dispersal limitation or dispersal occurring at a rate insufficient to homogenize the populations, we would expect a

relationship between genetic divergence and spatial distance. Similarly, if climatic conditions influence the distributions of these species by promoting spatial segregation of ecotypes, we would expect a relationship between genetic divergence and environmental heterogeneity; and (iii) do the demographic histories of *R. raciborskii* and *M. aeruginosa* show signs of recent population expansion? As changes in population size leave traces in genetic information, evidence of a recent population expansion would support the hypothesis of recent dispersal and/or proliferation in temperate environments due to climate change, as is often suggested for these species.

Methods

Data collection

Our study was based on a dataset of 16S rRNA gene sequences from worldwide populations of *R. raciborskii* and *M. aeruginosa*, collected through a search of the public database GenBank (http://www.ncbi.nlm.nih.gov/) undertaken during the year 2018. The 16S rRNA molecular marker, due to its slow rate of mutation, is the most commonly used indicator gene for bacterial biodiversity and has been extensively applied in phylogenies of bacteria (Fox, Wisotzkey, & Jurtshuk Jr, 1992). Nevertheless, although very conservative, some hypervariable regions of this gene may contain high intraspecific variability (Coenye & Vandamme, 2003). Data collection followed specific criteria in order to capture data on the global genetic variability of both species, aiming to obtain as much data as possible (number of sequences and base-pair lengths), and was based on the following steps: 1) the GenBank database was searched using the following terms as keywords: “species name” AND “16S” AND “rRNA”; 2) from all retrieved sequences, we collected only those that came from natural environments and had more than 500 bp (in cases with a large number of sequences from the same sample, as in High-throughput sequencing where hundreds of individuals can be sequenced in the same sample, up to the first 15 sequences were collected and the remainder were not taken into account); 3) after step 2, all retrieved sequences were tabulated along with information available from both GenBank and published papers, such as year and location of sampling; then, all sequences were excluded for which information about the sampling site was incomplete, dubious, or not found; 4) the sequences retained after steps 1, 2 and 3 were aligned through CLUSTALW, using the default parameters (Thompson, Higgins, & Gibson, 1994) implemented in MEGA7 (Tamura, Dudley, Nei, & Kumar, 2007), and carefully improved manually (a few sequences that did not show a part of the sequence were included in the analyses, with the ‘missing information’ symbol added); and 5) since different study approaches and sequencing methods amplify different portions of the 16S rRNA gene, in this final step we

cut off the portion of the gene that had the largest number of matched sequences. Then, we excluded all sequences whose sequenced portion did not match (at least for the most part) with the clipped gene region, resulting in a total of 173 sequences for *R. raciborskii* and 177 sequences for *M. aeruginosa* (Figure 1; Tables S1 and S2).

Using a full-length 16S rRNA gene sequence from *Microcystis aeruginosa* strain NIES-843 (1491 base pairs; GenBank accession: NR_074314) as a template, the regions covered in this study correspond to positions 106–1223 for *Microcystis aeruginosa* and 98–1082 for *R. raciborskii*. Considering the hypervariable regions found in the 16S rRNA gene (Yang, Wang, & Qian, 2016), the sequences used in our study include parts of the V2 region and the V3 and V4 regions. Prior to all analyses, sites with gaps and missing data were excluded, resulting in a total of 782 sites aligned for *R. raciborskii* and 834 sites aligned for *M. aeruginosa*.

Contemporary and past (Last Interglacial – 130–115,000 years ago, Last Glacial Maximum – 20,000 years ago, and Middle Holocene – 6,000 years ago) climatic data used in this study were collected from WorldClim (http://www.worldclim.org/version1), using QGIS software (http://qgis.osgeo.org). The bioclimatic variables were selected considering the cyanobacterial ecology and, after elimination of highly correlated variables, were retained for the analyses: Annual Mean Temperature (Bio1), Mean Diurnal Range (Bio2), Isothermality (Bio3), Temperature Seasonality (Bio4), Temperature Annual Range (Bio7), Annual Precipitation (Bio12) and Precipitation Seasonality (Bio15).

Statistical analyses

First, the global genetic structure of *R. raciborskii* and *M. aeruginosa* was explored through the determination of haplotype and nucleotide diversity, both taking into account all the sequences collected and also separately by latitudinal zones (north temperate zone, tropical zone, and south temperate zone – NTZ, TZ and STZ populations, respectively), by using DnaSP software version 5.10 (Librado & Rozas, 2009). Haplotype networks were designed to evaluate the geographic structure of genetic variation through the Median-joining method, using PopART (Population Analysis with Reticulate Trees) software (Leigh & Bryant, 2015).

To explore the role of spatial and bioclimatic variables in the spatial genetic structure of *R. raciborskii* and *M. aeruginosa*, we performed Mantel tests (Diniz-Filho et al., 2013; Mantel, 1967). The purpose of this analysis was to determine the correlation between the response matrix [biotic matrix (B), which used the genetic divergence calculated as the proportion of mismatched nucleotide sites] and the explanatory matrices [matrices of Euclidean environmental (E) and spatial (S) distances]. Here, as environmental

variables, we used the current and past bioclimatic variables collected from WorldClim described in the 'Data collection' section. The environmental matrices were separated by geological period, resulting in four matrices: the matrix with current environmental data (current matrix), matrix with Middle Holocene data (MH matrix), the matrix with Last Glacial Maximum data (LGM matrix), and finally the matrix with Last Interglacial data (LIG matrix).

The spatial matrix was built from the geographic coordinates of the sampling sites where the sequences were collected. In cases where the precise geographic coordinates were not available in GenBank or in a published paper, we assigned the central coordinates of the locality (for example, the central point of Florida or Lake Balaton). The final spatial matrix contained the geographic distances between the sampling sites, which were log (x+1) transformed before the analyses. Mantel tests were performed for each species, taking into account all the sequences collected, and also separately by latitudinal zones (NTZ, TZ and STZ populations). The significance of correlations between the response matrix and the explanatory matrices was evaluated based on 9999 randomization replicates, using PC-ORD software version 6.22 (Grandin, 2006; McCune & Mefford, 1999).

Tajima's D and Fu's Fs neutrality tests were carried out in order to detect evidence of recent population expansion. These tests were based on the differences between the number of segregating sites and the mean number of nucleotide differences, thus evaluating the hypothesis that all mutations are selectively neutral. Negative values indicate that the population is expanding by a statistically significant amount (Fu & Li, 1993; Tajima, 1989). Tajima's D and Fu's Fs tests were performed with the DnaSP software version 5.10 (Librado & Rozas, 2009). Finally, evidence of expansion was also inferred through estimation of the effective population size (*Ne*) over time directly from the sequence data. For this, the demographic histories of *R. raciborskii* and *M. aeruginosa* were analyzed in a Bayesian framework, using the Bayesian Skyline Plot (BSP) implemented in Beast software (Drummond, Suchard, Xie, & Rambaut, 2012). This approach incorporates uncertainty in the genealogy by using MCMC integration under a coalescent model, in which the timing of dates provides information about *Ne* through time. Regarding the mutation rate, we assumed the same molecular clock for the cyanobacterial 16S rRNA gene used in previous studies (2.0×10^{-11} per site per year) (Bahl et al., 2011; Schirrmeister, de Vos, Antonelli, & Bagheri, 2013). This analysis was run in Beast v1.10.4 for 100 million iterations, using the following parameters: GTR + G + I substitution model without site heterogeneity, relaxed log-normal molecular clock model, and tree prior: coalescent Bayesian skyline, with 10 groups and piecewise-constant skyline model. The results were visualized using Tracer v1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018).

Results

Latitudinal patterns of genetic diversity

Considering the global dataset and excluding sites with gaps and missing data, *R. raciborskii* showed 88 variable (polymorphic) sites and a total of 94 mutations. *M. aeruginosa*, on the other hand, showed 251 variable sites and a total of 278 mutations (see details in Tables S4 and S5). Reflecting these data, *M. aeruginosa* showed higher haplotype and nucleotide genetic diversity (0.941; 0.005, respectively) than *R. raciborskii* (0.800; 0.002) (Table 1). Comparison of these indices between the NTZ and TZ populations separately for each species showed that genetic diversity is higher in the tropical (TZ) than in the north temperate zone (NTZ) populations of both species (Table 1). Since few sequences were collected in the south temperate zone, these data may not provide reliable results and therefore were not considered in these comparisons.

Spatial genetic structure

Mantel tests showed significantly different patterns between the two species (Table 2). For *R. raciborskii*, considering all populations, the analysis showed a significant spatial genetic structure ($r = 0.137$, $p < 0.01$) (Figure 2a). Moreover, we observed a significant correlation between genetic divergence and all environmental distance matrices tested, except for the LGM matrix ($r = 0.055$, $p > 0.05$) (Table 2). When the analyses were performed for each latitudinal zone separately (NTZ, TZ and STZ populations), the genetic divergence of both the NTZ and TZ populations showed significant correlations with environmental and spatial distances. However, TZ populations were influenced only by LIG variables ($r = 0.247$, $p < 0.0001$), while NTZ populations were influenced by all environmental variables except for LGM variables (Table 2). No significant correlations were found for the STZ population (Table 2). In the partial Mantel tests, although different r values were observed, the correlation proportions and the interpretation of the results remained the same (Table S3). In the case of *M. aeruginosa*, considering the global population, the Mantel test showed a significant correlation between genetic divergence and current, MH and LGM environmental variables, but did not indicate any relationship between genetic and geographic distance ($r = –0.080$, $p > 0.05$) (Figure 2b). The analyses of the populations of each latitudinal zone separately found no significant correlations (Table 2). The partial Mantel tests gave the same results (Table S3).

Population growth dynamics

Tajima's D and Fu's Fs neutrality tests supported the evidence of a recent population expansion in both species (Table 1). Both the NTZ and TZ populations of *R.*

raciborskii and *M. aeruginosa* also showed signals of population expansion when considered separately (Table 1). A population expansion of *R. raciborskii* was also apparent in the haplotype network (Figure 3), which showed a 'star-like' pattern together with some terminal branches with accumulated mutations. In the case of *M. aeruginosa*, the haplotype network did not show a clear phylogeographic pattern, but rather many divergent lineages connected by many missing intermediates (Figure 4). Finally, historical population trends inferred by the BSP confirmed the evidence from neutrality tests, showing a population expansion in the last 2,500 years for both *R. raciborskii* and *M. aeruginosa* (Figure 5). Past population dynamics of *R. raciborskii*, specifically, indicated a rapid population growth within the period of 750–250 years ago (Figure 5a), and *M. aeruginosa* showed a relatively constant population expansion from 2,500 years ago to the present, with a faster rate of growth between 1,250–750 years ago (Figure 5b).

Discussion

It is indisputable that both *R. raciborskii* and *M. aeruginosa* are highly successful and invasive species. Several factors may favor their dominance in aquatic ecosystems and their global occurrence, such as anthropogenic nutrient loading, rising temperatures, rising salinity, increased atmospheric levels of CO_2, enhanced vertical stratification, increased water residence time, and more extreme climatic conditions (Mantzouki et al., 2018; Paerl et al., 2019). Specific traits of these two species are also involved in their ecological success, such as buoyancy provided by gas vesicles, which allows them to access optimum light and nutrient conditions in the water column; ability to release toxins; formation of akinetes in *R. raciborskii*; and tolerance to a wide range of light intensities. Also, colony formation in *M. aeruginosa* provides many ecological advantages, including faster floating velocity and protection from chemicals and grazing (Padisák et al., 2016; Rzymski & Poniedziałek, 2014; Xiao, Li, & Reynolds, 2018). Interestingly, *R. raciborskii* has also been implicated in inducing colony formation in *Microcystis* due to the production of allelochemicals (Mello, Soares, Roland, & Lürling, 2012), suggesting a positive correlation between the adaptive advantages of the two species.

Regarding the global patterns of genetic diversity, we found a low degree of genetic diversity of *R. raciborskii* compared to *M. aeruginosa* (Table 1). The surprisingly high genetic diversity of *M. aeruginosa* was previously observed by Haande et al. (2008) and compared to the marine cyanobacterial genus *Synechococcus* (using the ITS genetic marker) (van Gremberghe et al., 2011). Since geographical isolation favoring diversification in *M. aeruginosa* does not appear to be a plausible explanation (Table 2), local specialization linked to a high mutation rate is a likely cause of the high genetic diversity

found in *M. aeruginosa*. Indeed, Frangeul et al. (2008) showed that the genome of this cyanobacterium is very plastic and displays a high transposon activity. This high genetic diversity may be an important feature of *M. aeruginosa* on which natural selection can act, shaping the population structure through founder effects from a random selection of regional and global strains, whenever new habitat patches are created (Boileau, Hebert, & Schwartz, 1992). The lower degree of genetic diversity of *R. raciborskii* supports the hypothesis that the high phenotypic plasticity of this species has an important role in its recent successful spread (Bonilla et al., 2012). The wide tolerance spectrum of *R. raciborskii* (which may be associated with its ability to form akinetes) is frequently evoked to explain its potential to survive and thrive in novel environments (Rzymski & Poniedziałek, 2014), such as at temperatures as low as 12 °C (Dokulil, 2016).

We also observed that both *R. raciborskii* and *M. aeruginosa* showed higher genetic diversity in the tropical zone than in the north temperate zone (Table 1). This information supports the hypothesis of a tropical origin (or at least that they have existed longest in the tropics) and limited or recent dispersal to temperate areas. Several recent studies have supported the idea of a tropical origin for *R. raciborskii*, indicating Africa as the primary radiation center (Cirés et al., 2014; Haande et al., 2008; Padisák, 1997) and, more recently, South America as a possible radiation center (Panou, Zervou, Kaloudis, Hiskia, & Gkelis, 2018). Further, our findings support the assumption that tropical zones may have served as refuge areas during extreme cold conditions in the past, as suggested in previous studies (e.g. Gugger et al., 2005), leading to higher genetic diversity in the tropics due to a lower extinction rate.

Higher genetic diversity in the tropics may also indicate the existence of a latitudinal gradient of diversity on an intraspecific level. Recently, higher species richness in equatorial zones has been observed for unicellular organisms (Amend et al., 2013; Fuhrman et al., 2008; Sul, Oliver, Ducklow, Amaral-Zettler, & Sogin, 2013), just as it is often found for large organisms. This pattern is frequently attributed to a number of factors that possibly favor speciation processes (Stevens, 1989), and among these, higher primary production (Hawkins, Porter, & Felizola Diniz-Filho, 2003) and intense competition in the tropics (Mittelbach et al., 2007) are directly related to cyanobacteria. Marine picocyanobacteria (*Prochlorococcus* and *Synechococcus)*, for example, are significant primary producers (Li, 1994) with a ubiquitous distribution throughout many ocean regions, in higher abundances in equatorial areas (Flombaum et al., 2013). It is known that the widespread distribution of this group can be attributed to a high degree of genetic and genomic diversity (Fuller, Tarran, Yallop, Orcutt, & Scanlan, 2006). Exploring whether the

genetic diversity distribution fits to the latitudinal gradient of diversity for both this and other cyanobacterial groups deserves attention in future studies.

Significant differences were also observed in the phylogeography of *R. raciborskii* and *M. aeruginosa*. The filamentous cyanobacterium *R. raciborskii* showed significant spatial genetic structure correlated with spatial distance (Table 2; Figure 2a). That is, the populations tended to be genetically closer to each other when they were spatially close. These results indicate that although *R. raciborskii* has a global dispersal capacity, dispersal may occur at a rate insufficient to homogenize the populations, or perhaps has been occurring over a long evolutionary period with mutations accumulating gradually. We also observed a significant correlation between genetic and environmental (current and past) distances (Table 2). Together, these results suggest the existence of ecotypes, as observed in other studies (Chonudomkul et al., 2004; Piccini et al., 2011). Among the possible variables involved, temperature and light are extensively discussed as main driving factors for the growth and maintenance of the various *R. raciborskii* ecotypes (e.g. Briand, Leboulanger, Humbert, Bernard, & Dufour, 2004; Kling, 2009). When these analyses were performed by latitudinal zone separately, the TZ population showed a significant correlation only with climatic variation from the Last Interglacial, whereas the NTZ population was influenced by current and past climatic conditions (Table 2). Here, we speculate that akinete formation may have an important role: not only are akinetes thought to be crucial in the expansion to higher latitudes (Stüken et al., 2006; Wiedner, Rücker, Brüggemann, & Nixdorf, 2007), but also these resistant cells may have formed an 'akinete bank' in the tropics during the cold climatic extremes of the past, leading to greater conservation of the ancient phylogenetic structure of *R. raciborskii*. This result may also indicate that today, other factors such as eutrophication and competition are more important for the TZ population than climatic conditions, since temperature may not be limiting for this species in the tropics. Supporting this idea, Recknagel et al. (2019) have shown that the seasonal population dynamics of *R. raciborskii* are strongly correlated with water temperature in temperate and Mediterranean lakes, but weakly correlated in a tropical lake.

Regarding *M. aeruginosa*, previous studies have shown either the presence (Oberholster, Botha, Muller, & Cloete, 2005) or absence (Haande et al., 2007; Janse van Vuuren & Kriel, 2008; van Gremberghe et al., 2011) of biogeographic structuring at larger geographical scales, depending on the area sampled or the genetic markers used. In our study, the Mantel test did not indicate any relationship between the genetic and geographic distances (Table 2; Figure 2b). The lack of a global phylogeographic structure in *Microcystis* populations was previously described by van Gremberghe et al. (2011), using

the ITS genetic marker. The authors suggested that *M. aeruginosa* represents a young clade that spread globally only recently, through passive dispersal. This hypothesis was also supported by the highly interconnected network and evidence of a recent worldwide population expansion found by the authors, in agreement with our results (Table 1; Figures 4 and 5). Although we found no clear phylogeographic signal, we did find a significant correlation between *M. aeruginosa* genetic divergence and current and past (MH and LGM) environmental variation, for the global population (Table 2). This result indicates the existence of ecotypes, which has been suggested in previous studies. For instance, a link between the ITS sequence type and phenotypic and chemotypic traits has been suggested for *Microcystis* (Janse et al., 2004; Otsuka et al., 1999; Yoshida et al., 2008). Together, these data support the hypothesis that *M. aeruginosa* is a truly cosmopolitan species, with a high dispersal ability influenced mainly by local environmental conditions.

Finally, both the neutrality tests and the BSP analysis supported the hypothesis of recent population expansions for the two species (Table 1; Figure 5). In the case of *R. raciborskii*, a major expansion starting around 750 years before the present (YBP) after a period of relative stability in population size was observed (Figure 5a). For *M. aeruginosa*, on the other hand, the estimates of *Ne* showed a continuous population expansion since 2,500 YBP, with more rapid growth between 1,250 YBP and 750 YBP, followed by relative stability to the present (Figure 5b). Since there have been no extreme climatic changes (such as glaciations) in the last 2,500 years, other factors may be involved in the recent population expansions of *R. raciborskii* and *M. aeruginosa*. For instance, human population growth may have important implications: greater human mobility between continents, facilitating unintentional human transport through shipping; the intensive use and pollution of freshwater ecosystems; and the increase in anthropogenic eutrophication, a widely recognized process that promotes the proliferation of cyanobacteria (O'neil, Davis, Burford, & Gobler, 2012).

Potential caveats

We recognize that using data for a single conserved locus (16S rRNA) may have limited the interpretation of our results. Therefore, we admit that it is necessary to use very high-resolution genetic markers (e.g. protein-encoding genes) in order to confirm the observations found in this and previous studies. In addition, the definition of a cyanobacterial species, because of its prokaryotic nature, is still a matter for discussion, including the two species analyzed here (Aguilera, Gómez, Kaštovský, Echenique, & Salerno, 2018; Otsuka et al., 2001). For instance, a phylogenetic comparison indicated that *Raphidiopsis mediterranea* Skuja may merely represent the non-heterocystous life-cycle

stages of *R. raciborskii*, not a separate species (Moustaka-Gouni, Kormas, Vardaka, Katsiapi, & Gkelis, 2009). Regarding *M. aeruginosa*, traditional taxonomy is still based mainly on the morphology as observed in field populations, and seems to be inconsistent with the results of biochemical or genetic studies for strains that show high phenotypic plasticity of colonies (Otsuka, Suda, Li, Matsumoto, & Watanabe, 2000; Xu et al., 2016). Since phylogeography is closely related to the concept of species, different definitions may result in different patterns, so that further research is needed to address the taxonomy of these species, essential for a better understanding of their phylogeography.

Conclusions

The genetic diversity patterns of *R. raciborskii* and *M. aeruginosa* support the hypothesis that these species originated in tropical regions and have recently been expanding their distributions, or are proliferating more rapidly, in temperate habitats. The phylogeographic structure of *R. raciborskii* suggests that this species may have certain dispersal limitations or has been spreading for a long time. Further, the low genetic diversity of *R. raciborskii* compared to *M. aeruginosa* may be associated with its capacity to form akinetes and with ecological plasticity. *M. aeruginosa*, in contrast, appears to disperse more frequently worldwide and may be more influenced by local environmental conditions. The higher and counterintuitive genetic diversity of this species may be associated with intrinsic characteristics of its genome, together with a high degree of niche specialization. Finally, the rapid population expansion observed in the last 2,500 years indicates that human activities such as intercontinental mobility, pollution and the consequent eutrophication of aquatic ecosystems may be responsible for at least part of the successful spread of these two species.

TABLES

Table 1 Genetic diversity indices and Neutrality tests (Tajima's D and Fu's Fs), considering all populations of each species combined and by latitudinal zone

Raphidiopsis raciborskii	***N***	**Haplotypes**	**Haplotype diversity**	**Nucleotide diversity**	**Tajima's *D***	**Fu's F_s**
NTZ population	95	23	0.762	0.002	–2.216**	–6.888*
TZ population	69	33	0.913	0.003	–2.304**	–4.978*
STZ population	9	7	0.944	0.011	–0.017	–0.311
Global population	173	52	0.800	0.002	–2.611***	–7.840*
Microcystis aeruginosa						
NTZ population	124	58	0.948	0.004	–2.675***	–9.397*
TZ population	44	33	0.979	0.010	–2.613***	–5.205*
STZ population	9	4	0.583	0.001	–0.526	–0.044
Global population	177	82	0.941	0.005	–2.812***	–10.498*

N: number of sequences; NTZ: North Temperate Zone; TZ: Tropical Zone; STZ: South Temperate Zone.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.0001$

Table 2 Correlations (r) between genetic divergence (B) and the spatial (S) and environmental (E) distances, considering all populations of each species combined and by latitudinal zone, determined by standard Mantel tests

Raphidiopsis raciborskii	***N***	**r(BS)**	**r($BE_{current}$)**	**r(BE_{MH})**	**r(BE_{LGM})**	**r(BE_{LIG})**
NTZ population	95	0.251***	0.183***	0.182***	0.037	0.153**
TZ population	69	0.276**	0.058	0.076	0.082	0.247**
STZ population	9	–0.010	–0.167	–0.157	–0.155	–0.057
Global population	173	0.137**	0.103**	0.114**	0.055	0.146**
Microcystis aeruginosa						
NTZ population	124	–0.024	–0.040	–0.041	–0.009	–0.092
TZ population	44	–0.011	0.088	0.016	0.146	0.051
STZ population	9	0.082	0.080	0.082	0.074	0.070
Global population	177	–0.080	0.102*	0.102*	0.089*	0.021

N: number of sequences; $E_{current}$: contemporary climatic conditions; E_{MH}: Middle Holocene climatic conditions; E_{LGM}: Last Glacial Maximum climatic conditions; E_{LIG}: Last Interglacial climatic conditions; NTZ: North Temperate Zone; TZ: Tropical Zone; STZ: South Temperate Zone.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.0001$

List of figure legends

Figure 1 *Raphidiopsis raciborskii* (green) and *Microcystis aeruginosa* (purple) 16S rRNA sequences collected from GenBank used in this study

Figure 2 Genetic divergence of *Raphidiopsis raciborskii* (a) and *Microcystis aeruginosa* (b) sequences plotted against geographical distance between sites

Figure 3 Median-joining network showing the distributions (by latitudinal zone) and frequency (circle sizes) of the 52 haplotypes found across 173 *Raphidiopsis raciborskii* populations. Mutation steps are indicated by hatch marks and missing intermediates by yellow circles

Figure 4 Median-joining network showing the distributions (by latitudinal zone) and frequency (circle sizes) of the 82 haplotypes found across 177 *Microcystis aeruginosa* populations. Mutation steps are indicated by hatch marks and missing intermediates by yellow circles

Figure 5 Bayesian Skyline Plot (BSP) based on *Raphidiopsis raciborskii* (a) and *Microcystis aeruginosa* (b) 16S rRNA sequences. The graph was constructed by merging all populations of each species as a global population. The plot displays changes in the effective population size through time, based on a mutation rate of 2.0×10^{-11} per site per year. Present day is on the left of the x-axis

Figure 1

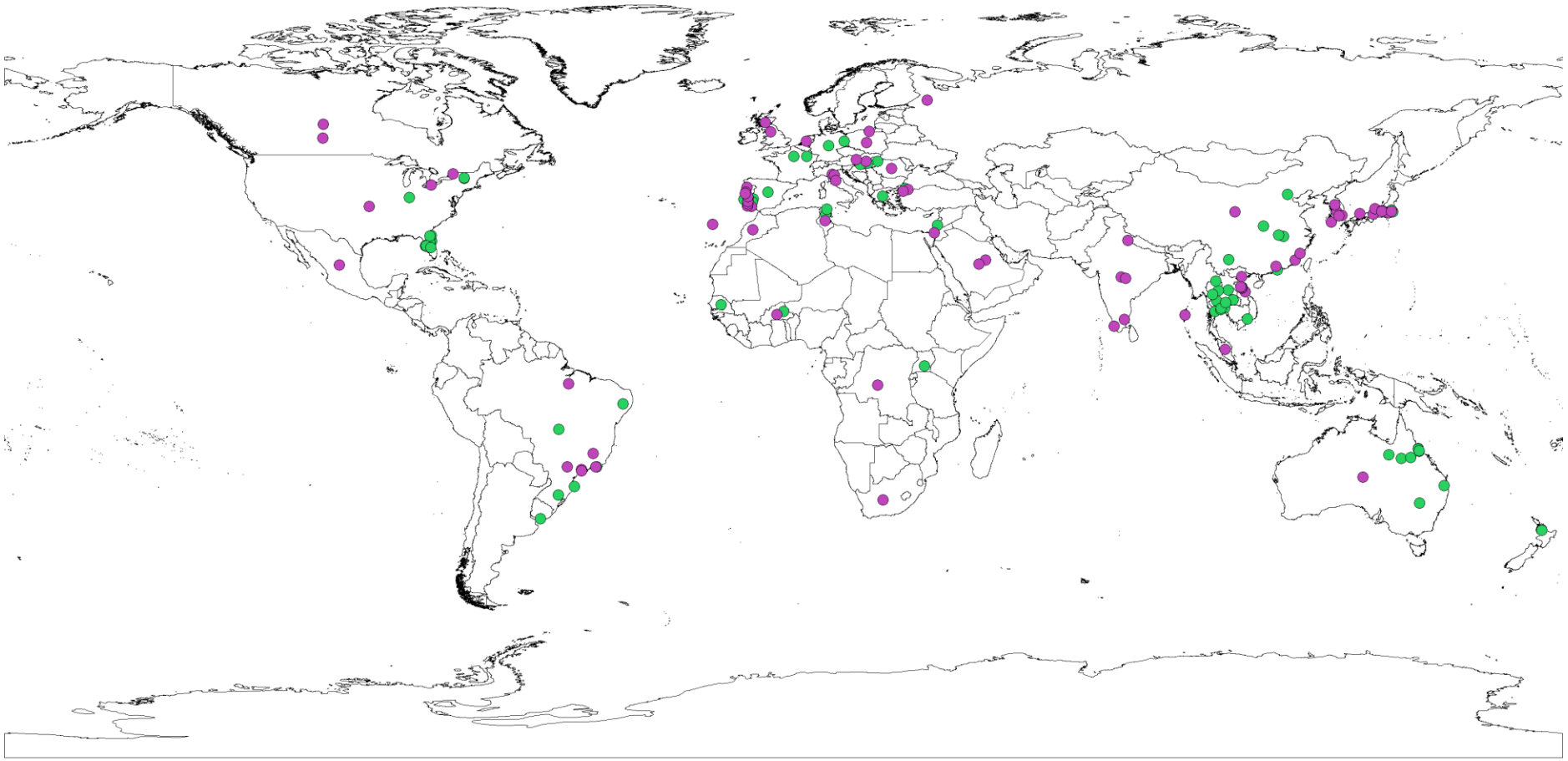
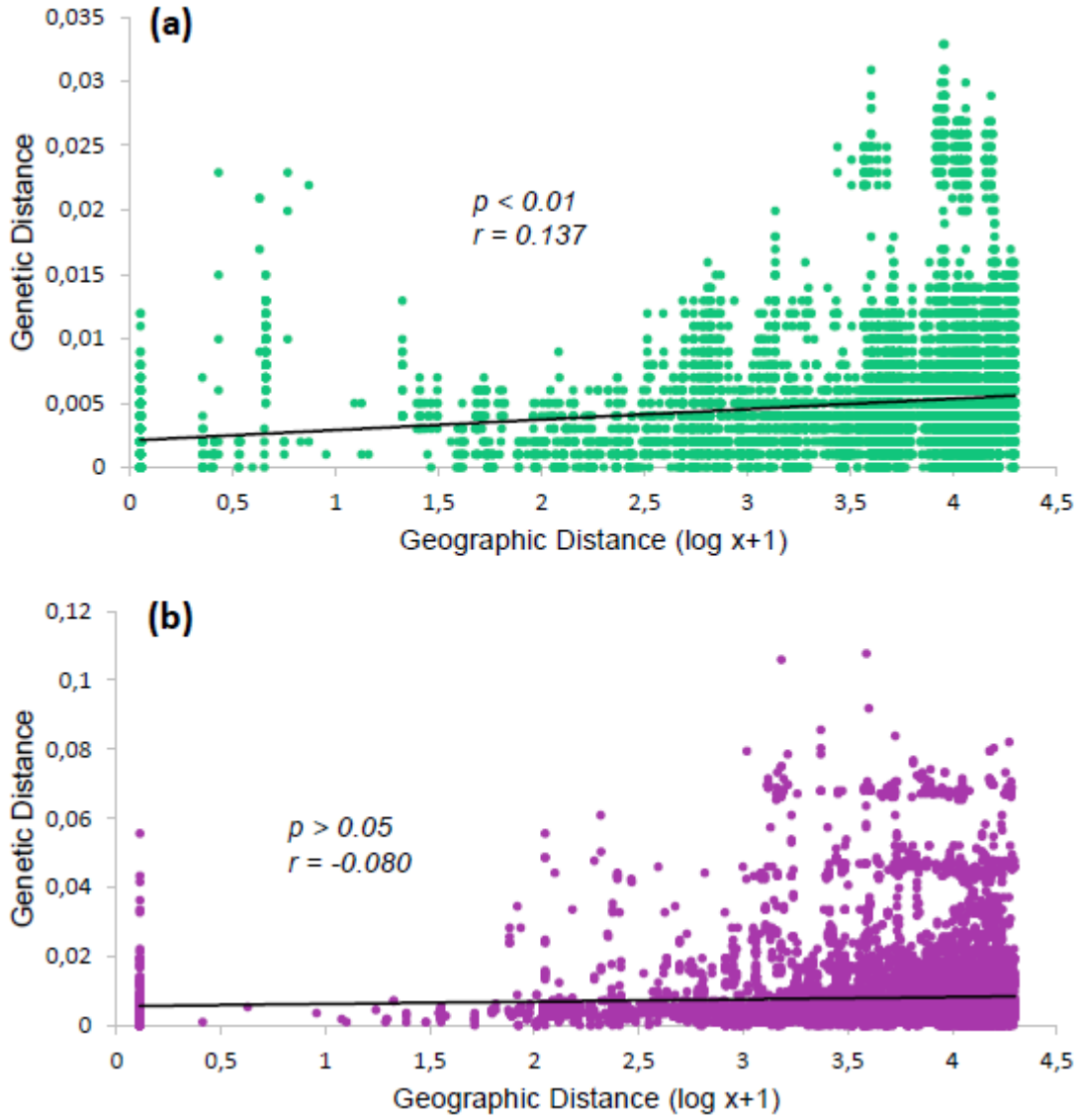


Figure 2


(a)
Genetic Distance
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0,03
0,025
0,02
0,015
0,01
0,005
0
p < 0.01
r = 0.137
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
Geographic Distance (log x+1)
(b)
Genetic Distance
0,12
0,1
0,08
0,06
0,04
0,02
0
p > 0.05
r = -0.080
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3,5
4
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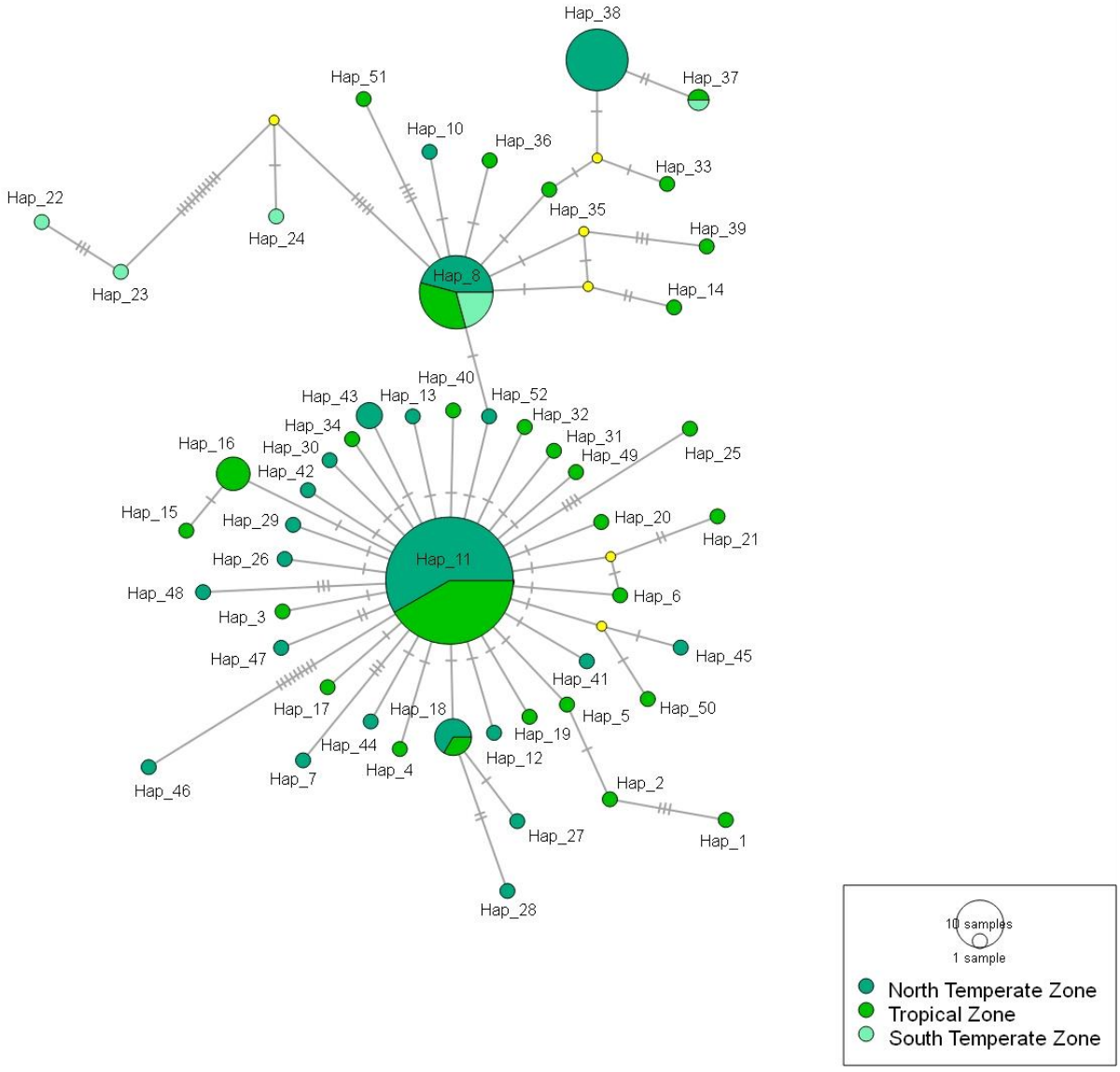
Geographic Distance (log x+1)

Figure 3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Hap_14
Hap_40
Hap_43
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Hap_16
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Hap_50
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Hap_19
Hap_44
Hap_12
Hap_4
Hap_7
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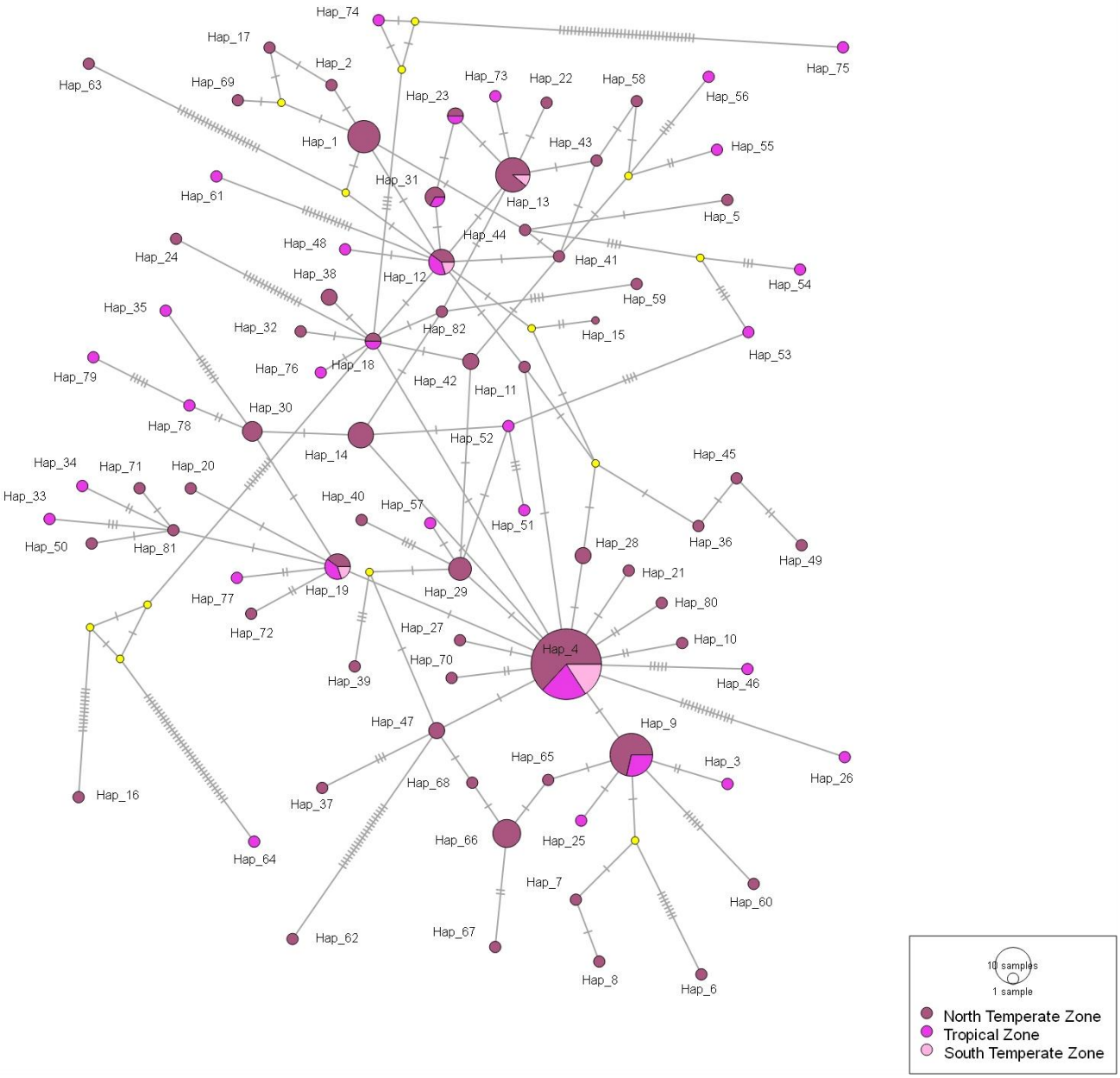
10 samples
1 sample
North Temperate Zone
Tropical Zone
South Temperate Zone

Figure 4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Hap_25
Hap_64
Hap_7
Hap_60
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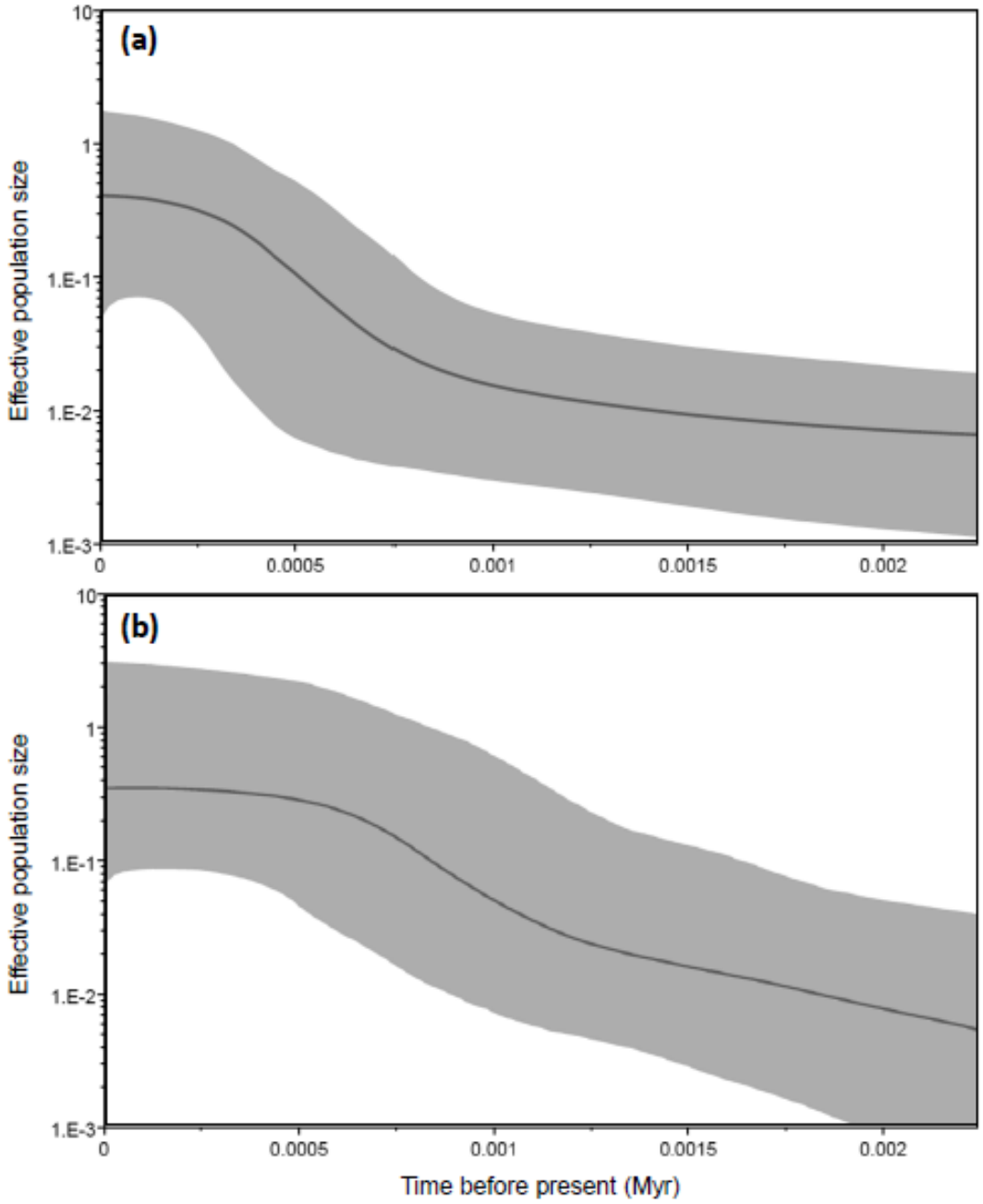
Hap_62
Hap_8
Hap_6
10 samples
1 sample
North Temperate Zone
Tropical Zone
South Temperate Zone

Figure 5


(a)
(b)
Effective population size
Effective population size
10
1
1.E-1
1.E-2
1.E-3
0
0.0005
0.001
0.0015
0.002
Time before present (Myr)

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DATA AVAILABILITY STATEMENT

Genetic distance matrices and climatic data are archived in Dryad Digital Repository (https://doi.org/10.5061/dryad.dbrv15dx6).

Biosketch

Karine Felix Ribeiro is broadly interested in biogeography and evolutionary biology of prokaryotes, with a special focus on cyanobacteria. This paper constitutes one of the goals of her PhD thesis at the Universidade Federal do Rio Grande do Sul. The other authors collaborate on different aspects of the evolutionary ecology, phylogeography and ecology of cyanobacteria. **Author contributions:** K.F.R. designed the research, performed the analyses and wrote the manuscript, with active assistance and input from L.D., A.C.T. and L.O.C. A.P.F. collected the data and helped in the analyses. All authors helped to improve the final version.

CAPÍTULO III

Alfa and beta diversity of planktonic and periphytic cyanobacteria based on 16S rRNA gene in a subtropical lake system: How similar are they?*

*Este capítulo está nas normas da revista **Environmental Microbiology**, para onde será submetido futuramente.

Alfa and beta diversity of planktonic and periphytic cyanobacteria based on 16S rRNA gene in a subtropical lake system: How similar are they?

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Running title
Distribution of planktonic and periphytic cyanobacteria

Originality-Significance Statement
This work compared alfa and beta diversity patterns of cyanobacteria from open waters and macrophyte-attached freshwater habitats. Previous studies in this context incorporate cyanobacteria into the total algal community using less sensitive methods of quantifying diversity (e.g. microscopy). The present study specifically focused on the diversity of cyanobacteria at the level of the 16S rRNA gene, a still pioneering and valuable approach to access the distribution of diversity in this bacterial group from two distinct habitats.

Summary

Cyanobacteria are an abundant component of both free-living (phytoplankton) and surface-attached (periphyton) freshwater communities. Despite this, it remains unknown whether planktonic and periphytic cyanobacteria are structured by the same ecological processes. Here, we explored the distribution of planktonic and periphytic cyanobacteria diversity, based on 16S rRNA gene, in a subtropical lake system. Variation partitioning analysis was employed to determine the influence of environmental (water conditions) and biotic (abundance of heterotrophic bacteria) variables on the cyanobacteria variation. We found that periphytic assemblages showed a richness about three times higher than the planktonic ones, highlighting the importance of the periphytic community to the overall diversity in subtropical lakes. The picocyanobacteria *Synechococcus* was the dominant component of the plankton, a taxon often underestimated using classical methods of quantification (e.g. optical microscopy). Species replacement was the major component explaining the distribution of diversity in both planktonic and periphytic assemblages. However, while planktonic cyanobacteria was primarily influenced by water conditions, periphytic cyanobacteria was influenced only by the abundance of heterotrophic bacteria. Together, our findings showed that assemblages of planktonic and periphytic cyanobacteria are distinct, suggesting a high habitat specialization, and the importance of environmental filtering and biotic interactions in structuring planktonic and periphytic cyanobacteria distribution, respectively.

Introduction

Earth is home to nearly one trillion (10^{12}) microbial species that have evolved over ~4 billion years (Locey and Lennon, 2016). This microscopic world is quite intriguing, since it represents the largest proportion of biomass and genetic diversity of the biosphere (Whitman *et al*., 1998), yet its ecological patterns remain mysterious. The importance of environmental filtering for bacteria was established by Bass-Becking (Baas-Becking, 1934) and highlights the relevance of trade-offs in ecological traits among distinct taxa, thus evoking only the role of local environment on bacterial distribution. Since then, the study of bacterial distribution has become increasingly significant due to the advent of powerful molecular techniques to describe diversity at the genotype level, which has uncovered a previously unrevealed rare biosphere (Ramette and Tiedje, 2007) and facilitated the detection of patterns previously unknown to bacteria, such as geographical isolation (e.g. Papke *et al*., 2003). However, although the existence of distribution patterns in prokaryotes is known, relatively little remain understood regarding the processes underlying these patterns (Hanson *et al*., 2012). While recent global surveys have indicated that most bacteria are restricted to broad habitat types, since there is little overlap among bacterial taxa found in soils, sediments, freshwater, and seawater (Nemergut *et al*., 2011), how environmental, biotic, and historical factors affect the distribution of bacterial diversity at regional and local scales remain relevant questions (Ramette and Tiedje, 2007).

Among the prokaryotes, cyanobacteria are distinguished by their ability to perform oxygenic photosynthesis, which is responsible for the largest proportion of global primary productivity (Li, 1994). Throughout its evolution, cyanobacteria became one of the most diverse and widely distributed prokaryotes, occupying many niches within terrestrial, planktonic, and benthic habitats (Whitton and Potts, 2007). These oxygenic phototrophs present high ecological plasticity, serve key roles in biogeochemical and metabolic processes and exhibit remarkable variability in morphology and ultrastructure (from unicellular to filamentous forms with differentiated cells) (Whitton and Potts, 2007). Cyanobacteria are particularly abundant and important organisms at the base of aquatic food webs in freshwater ecosystems (Christoffersen, 1996; Padisák *et al*., 2016). Some freshwater species are also frequently cited regarding their potential invasiveness as well as their ability to release toxins and form blooms (Sukenik *et al*., 2012; Moreira *et al*., 2013; Cirés *et al*., 2014; Wilk-Woźniak *et al*., 2016). These characteristics may have severe effects on freshwater systems worldwide, such as oxygen depletion, decreased water quality, eutrophication, and the possible rise of harmful cyanobacterial blooms associated with climate change (Paerl and Huisman, 2008; O'neil *et al*., 2012; Paerl *et al*., 2019).

Due to concerns related to public health and the conservation of freshwater ecosystems, less attention has been paid to the diversity of cyanobacteria (Nabout *et al*., 2013) and other ecological aspects such as their biogeography (Ribeiro, Duarte, *et al*., 2018). Despite this context, an increasing number of studies on the distribution of cyanobacteria diversity is found in recent literature. Most of these studies have focused on the influence of niche-related factors such as hydrological characteristics (e.g. water body depth), water chemistry (e.g. concentration of nutrients), and biotic interactions on the distribution of cyanobacteria diversity (Lu *et al*., 2013; Chamberlain *et al*., 2014; Zhang *et al*., 2016). More recently, some studies have also sought to explore the influence of historical (dispersal and evolutionary processes) and neutral (stochastic demographic dynamics) factors on cyanobacteria distribution in freshwater systems (Drakare and Liess, 2010; Östman *et al*., 2012; Marmen *et al*., 2016; Ribeiro, da Rocha, *et al*., 2018). Although these factors seem ineffective in explaining distribution patterns in lakes dominated by cyanobacteria (Östman *et al*., 2012), dispersal limitation and geographical isolation have already been recorded for some cyanobacterial taxa on continental and global scales (Papke *et al*., 2003; Miller *et al*., 2007; Bahl *et al*., 2011; Moreira *et al*., 2013). To date, these findings suggest that, at regional and local spatial scales, environmental filtering represents the major mechanism structuring freshwater cyanobacteria assemblages. Since cyanobacterial assemblages in freshwater systems frequently contain bloom-forming species, it seems plausive that the distribution of cyanobacteria best fit the environmental filtering paradigm, since these blooms are typically triggered by specific environmental conditions (Drakare & Liess, 2010).

Although an increasing number of studies seek to understand the factors affecting cyanobacteria distribution, generalizations cannot be made since distinct patterns were reported based on spatial scale, biogeographic area, and habitat type (Ribeiro, Duarte, *et al*., 2018). Notably, most studies on freshwater cyanobacteria distribution accessed species composition by microscopy, relying only on morphology data (e.g. Lu *et al*., 2013; Ribeiro, da Rocha, *et al*., 2018). It is known that, in cyanobacteria, studying morphology alone often lacks resolution at the species level, while completely ignoring cryptic species and underestimating the diversity of picocyanobacteria (Dvořák *et al*., 2015). Moreover, most studies in freshwater systems have explored the planktonic cyanobacteria, without considering other microhabitats in these environments. Besides being a component of the phytoplankton floating along water currents, cyanobacteria may also be associated with microbial biofilms attached to some type of substrate, such as rocks and macrophytes (periphyton). Previous studies have demonstrated that microbial communities from open water and macrophyte-associated habitats are highly distinct, in terms of both the total

bacterial community (bacterioplankton versus bacterial biofilm) (Crump *et al*., 1999; Souffreau *et al*., 2018) and the total microalgae community (phytoplankton versus periphyton) (Crossetti *et al*., 2013; Dunck *et al*., 2013, 2018; Santana and Ferragut, 2016; de Souza Cardoso *et al*., 2019). These differences are primarily attributed to habitat specialization: while planktonic microrganisms are directly dependent on the physical and chemical water parameters for their metabolism (Souffreau *et al*., 2015), as they are suspended in open waters, periphytic microrganisms are also dependent on the type and quality of the substrate (Rodrigues *et al*., 2005) and mutualistic relationships between the autotrophic and heterotrophic components of the periphyton (Wetzel, 1990, 1993), which enable the effective cycling of nutrients within the biofilm. Despite these findings and to the best of our knowledge, however, comparison of distribution patterns of planktonic and periphytic cyanobacteria within freshwater ecosystems, mainly based on more sensitive methods (e.g. high-throughput sequencing), are still lacking. These analyses are valuable since they could reveal whether different types of cyanobacterial assemblages are shaped by the same ecological factors, and how related these assemblages are to one another.

In this study, we explored the alpha (diversity within sites) and beta diversity (compositional variation among sites) patterns of planktonic and periphytic (associated to *Scirpus californicus* (C.A. Mey) Steud) cyanobacteria in five lakes with high environmental heterogeneity, and the influence of environmental (physical and chemical water conditions) and biotic (abundance of heterotrophic bacteria) factors on cyanobacteria variation. The assemblages were sampled in subtropical shallow lakes from a river basin located in Southern Brazil, which remains poorly studied despite the frequent occurrence of blooms and high richness of cyanobacteria (Bohnenberger *et al*., 2018). The sampled assemblages were characterized using partial 16S rRNA gene amplicon sequencing. We tested the following hypotheses: (1) considering the total dataset, due to habitat specialization, cyanobacteria is firstly structured regarding the habitat type (open water and macrophyte-attached); (2) due to environmental filtering and considering the environmental heterogeneity between the lakes, species replacement (turnover component of the beta diversity) is the major component explaining the beta diversity of both planktonic and periphytic cyanobacteria; (3) while environmental factor is the main predictor of planktonic cyanobacteria beta diversity due to their high dependence on water conditions, biotic factor (representing biological interactions) is the main predictor of periphytic cyanobacteria beta diversity since cyanobacteria and heterotrophic bacteria are mutually related through biological interactions that are essential to the biofilm functioning.

Results

Differences in cyanobacterial composition between open water and macrophyte-attached microhabitats (hypothesis 1)

The complete dataset of thirty libraries consisted of 1.068.076 sets of paired-end raw reads. After trimming, filtering, denoising and chimera removal steps, 315.955 effective sequences were obtained. A summary of the preprocessing steps is provided in Table S1 (Supporting Information). A total of 1.262 amplicon sequence variants (ASVs) were obtained among all samples, in which 227 ASVs were attributed to the Cyanobacteria phylum, and the remainder to other bacterial taxa. The 227 cyanobacterial ASVs were distributed into 20 identified genera, which 15 occurred only in the macrophyte-attached habitat, two only in the open water habitat, and three were shared among the two habitat types. Considering the ASVs, 193 occurred only in the macrophyte-attached habitat, 28 only in the open water habitat, and six were shared among the two habitat types. Periphytic assemblages had on average (n = 15) an ASV richness (20.7; standard deviation, 16.8) approximately three times higher than the planktonic assemblages (7.5; standard deviation, 2.5).

Considering the total dataset of cyanobacteria, the two most abundant genera were *Synechococcus* (51%) and *Leptolyngbya* (16%). For planktonic assemblages, the two most abundant genera were *Synechococcus* (88%), which was dominant in all samples except the Lake Marcelino, and *Microcystis* (6%), which was dominant in Lake Marcelino (Figure 2). For periphytic assemblages, *Leptolyngbya* and *Calothrix* were the two most abundant genera (disregarding the unidentified genera), representing 35% and 17% of the total cyanobacterial abundance, respectively (Figure 2). Moreover, three taxa were differentially abundant between the lakes (ANCOM analysis): *Planktothrix* sp., *Microcystis aeruginosa* (Kützing) Kützing and *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno, which were only detected in Lake Marcelino. Finally, Bray-Curtis MDS ordination showed a separate clustering of all planktonic assemblages sampled in Lake Marcelino, a second clustering of all periphytic assemblages, and a third clustering of the remaining planktonic assemblages (Figure 3). These compositional differences were confirmed by a significant result in the PERMANOVA test both among lakes ($p_{PERMANOVA}$ = 0.001) as well as between the two assemblage types ($p_{PERMANOVA}$ = 0.003).

Alpha and beta diversity patterns and the contribution of turnover and nestedness components (hypothesis 2)

In relation to alpha diversity patterns, we found that Lake Cidreira presented a significant higher diversity (Shannon index) than Lake Marcelino ($p_{\text{Wilcoxon signed-rank}}$ = 0.04) and Lake Passo ($p_{\text{Wilcoxon signed-rank}}$ = 0.02) (Figure 4a). However, when taking into account both richness and evenness (Simpson index), Lake Cidreira showed an opposite pattern. That is, although the Lake Cidreira had a greater diversity, the abundance of distinct cyanobacterial taxa is not evenly distributed (some taxa are highly abundant while others are rare) (Figure 4b). On the other hand, when comparing the two assemblage types, Shannon and Simpson diversity indices were not significantly different between planktonic and periphytic cyanobacteria ($p_{\text{Wilcoxon signed-rank}} > 0.05$) (Figure 5a and b). Regarding beta diversity, estimated overall beta diversity was very similar when considering the total dataset (β_{BRAY} = 0.98), as well as for planktonic (β_{BRAY} = 0.91) and periphytic (β_{BRAY} = 0.97) cyanobacteria separately (Table 2). When this overall beta diversity was partitioned into its turnover and nestedness components, turnover was clearly the responsible for most of the beta diversity in both planktonic (β_{SIM} = 0.87) and periphytic (β_{SIM} = 0.96) assemblages, as well as when considering the total dataset (β_{SIM} = 0.87) (Table 2).

Relative importance of environmental and biotic variables on cyanobacteria beta diversity (hypothesis 3)

Considering the total dataset, both the environment (E) and heterotrophic bacteria (Bac) fractions had a significant influence on the variation of cyanobacteria assemblages, in which most of the variation explained was by the biotic fraction (R^2_{adj} = 0.304; $p < 0.001$) (Table 3). When comparing the influence of theses fractions on the planktonic and periphytic cyanobacteria assemblages separately, strong differences were found. The environmental fraction (E) had a major importance in explaining the variation in planktonic assemblages (R^2_{adj} = 0.347; $p < 0.001$), while it was not significant for the variation in periphytic assemblages (R^2_{adj} = 0.025; $p > 0.05$). The biotic fraction (Bac) had a significant influence in variation of both planktonic (R^2_{adj} = 0.186; $p < 0.001$) and periphytic (R^2_{adj} = 0.125; $p < 0.05$) assemblages (Table 3). There was, although of minor importance, a shared influence of the environmental and biotic fractions (E + Bac) to all datasets (Table 3). Besides that, the planktonic dataset had a higher amount of variance explained (total variation explained, including pure and shared fractions = 69.5%; unexplained variance = 30.5%) when compared to the periphytic dataset (total variation explained, including pure and shared fractions = 24.8%; unexplained variance = 75.2%) (Table 3). The

environmental and biotic variables retained for each dataset by forward selection are also given in Table 3.

Discussion

Overall, our results demonstrate that planktonic and periphytic assemblages of cyanobacteria are highly distinct and different ecological factors are acting on the biotic structure of these two habitats. Simultaneous assessment of planktonic and periphytic cyanobacteria are extremely important for understanding the structure and dynamics of these primary producers and their relationships with environmental factors in distinct freshwater habitats. Differences in microalgae richness and composition, including cyanobacteria, in open water and macrophyte-attached habitats have already been observed in freshwater ecosystems through microscopic quantification methods (Dunck *et al*., 2013, 2018; Santana and Ferragut, 2016). Although we have found no difference regarding alpha diversity (Shannon and Simpson indices) between planktonic and periphytic assemblages, we found a higher average richness (based on ASV number) in the periphytic assemblages. This finding is congruent with previous observations of higher microbial richness in biofilms when compared to open water in freshwater ecosystems, both in studies on the total microalgae community (Dunck *et al*., 2013; Santana and Ferragut, 2016) and on the total bacterial community (Souffreau *et al*., 2018). Between lakes, alpha diversity indices were significantly distinct in Lake Cidreira when compared to Lake Marcelino and Lake Passo, which had the highest cyanobacterial ASVs richness and diversity (Shannon index). Interestingly, ASV richness and diversity (Shannon index) showed a decrease from southwestern to northeastern lakes (Table 1; Figure 4a), contrary to wind direction which blows steadily from the northeast to southwest during summer (Bohnenberger *et al*., 2018). Notably, Lima *et al*. (2020) studied 25 lakes from TRS and also discovered that bacterial richness (estimated at the OTU level using the ARISA method) decreased from southwestern to northeastern lakes. Thus, a possible explanation is that, especially in wind-dominated landscape as is the TRS, wind direction may be critically associated with the higher transportation rates of bacterial propagules from northern to southern regions.

Regarding cyanobacterial composition in open water and surface-attached habitats, previous studies have already recorded differences between these two habitat types in freshwater ecosystems (Crossetti *et al*., 2013; Dunck *et al*., 2013; Santana and Ferragut, 2016; de Souza Cardoso *et al*., 2019). Supporting these data and our first hypothesis, the largest compositional difference in our dataset were between the planktonic and periphytic assemblages (Figures 2 and 3). These differences were mostly attributed to the expressive

dominance of *Synechococcus* in the plankton (except in Lake Marcelino), and the dominance of *Leptolyngbya*, *Planktothrix* and *Calothrix* in the periphyton. In addition, only 6 of the 227 cyanobacterial ASVs recorded in our study were shared between the open water and macrophyte-attached habitats, suggesting a high specificity of habitat. Similarly, through a wide bibliographic synthesis on the occurrence and distribution area of planktonic and periphytic microalgae in a Brazilian floodplain, of the 938 species of algae (562 periphytic, 482 planktonic), only 103 co-occurred in the two habitats (Dunck *et al*., 2018). Regarding adaptive characteristics to the type of habitat, it is known that filamentous species typically dominate the cyanobacterial component of submerged attached communities (Komárek and Johansen, 2015), and several studies have recorded this pattern (Cavati and de Oliveira Fernandes, 2008; França *et al*., 2011; Santana and Ferragut, 2016; Cordeiro *et al*., 2017). *Phormidium* and *Calothrix*, for instance, are two genera that are commonly found attached to the surface of submerged rocks and macrophytes in lentic or lotic systems (Komárek and Johansen, 2015) and, in agreement with these data, both genera were recorded only in the periphyton in our study. *Leptolyngbya* —one of the most abundant genera in the periphytic assemblages in our study— is also very common in the periphyton and metaphyton of freshwater systems (Fonseca and Rodrigues, 2007; Dunck *et al*., 2018). It is worth noting, however, that most of these studies analyzed the total algal community, in which cyanobacteria were included, using optic microscopic quantification methods. It is known that purely optical microscopic quantification methods in cyanobacteria are problematic since they ignore cryptic species and underestimate the contribution of picocyanobacteria (Dvořák *et al*., 2015). In Santanna & Ferragut (2016), for instance, although cyanobacteria were the second most abundant taxon in the dataset, they were represented by a few species. Furthermore, considering the total algal community, picocyanobacteria were not the most abundant group of phytoplankton in their study, probably due to the underestimation of its richness by the usual microscopic method used.

The dominance of the picocyanobacteria *Synechococcus* in open water habitats, as observed in this study thanks to the molecular method used, is a very common pattern described in the literature in both marine and freshwater ecosystems (Li, 1998; Camacho *et al*., 2003; Flombaum *et al*., 2013; Cabello-Yeves *et al*., 2017), and is often related to (1) its free-living cosmopolitan characteristic with a high passive dispersal capacity through the air and (2) the occurrence of several recognized genetically distinct populations adapted to a wide range of environmental conditions (ecotypes), which favor their dominance in a set of spatially close habitats even with a high environmental heterogeneity. Here, the only exception was Lake Marcelino, whose cyanobacterial composition in planktonic

assemblages was markedly different from the other studied lakes (Figures 2 and 3). Lake Marcelino is highly impacted by anthropogenic activities and commonly dominated by cyanoblooms (Mello and Castro, 2013; Bohnenberger *et al*., 2018), which were observed during the sampling collection in the present study. This observation was confirmed by the fact that, contrary to the other lakes, the planktonic assemblages in Lake Marcelino were dominated by the genera *Microcystis*, *Raphidiopsis*, and *Planktothrix*, all of which are known for their ability to form massive blooms in freshwater lakes and reservoirs (Oliver and Ganf, 2000; Paerl *et al*., 2001). Thus, the compositional difference of planktonic cyanobacteria in this lake highlights the well-known pattern triggered by bloom formation in which rare species of cyanobacteria on a regional scale may become very locally abundant (Drakare and Liess, 2010). Interestingly, the typically planktonic genus *Planktothrix* (Komárek and Johansen, 2015) was recorded in high abundance in both planktonic and periphytic assemblages in Lake Marcelino. It is known that cyanoblooms can affect the structure of the periphyton (De Oliveria *et al*., 2010; Borduqui and Ferragut, 2012), since cyanoblooms are an indirect factor determining the availability of resources (mainly light and nutrients) and, hence, periphyton community dynamics (Borduqui and Ferragut, 2012). In this context, therefore, it is possible that the eutrophic condition of Lake Marcelino is influencing the composition of the periphyton, due to the high abundance of *Planktothrix* in the plankton, especially by decreasing light availability, and by providing inoculum of species able to establish in this habitat, as it is the case of some planktonic shade-adapted *Planktothrix* species, commonly found in benthic and periphytic samples (Fonseca and Rodrigues, 2007; Borduqui and Ferragut, 2012; Martins *et al*., 2012).

Estimated overall beta diversity was very similar when considering the total dataset as well as for planktonic and periphytic cyanobacteria separately, which was mostly explained by the turnover component (Table 2), supporting our second hypothesis. The predominance of turnover has commonly been observed in studies evaluating beta diversity across several taxa (including bacteria) and ecosystems (Soininen *et al*., 2018). Species replacement across spatial gradients has largely been attributed to environmental heterogeneity linked to habitat specialization, although spatial and historical constraints related to dispersal limitation may also be related (Qian *et al*., 2005; Baselga, 2010). In the present study, we assumed unrestricted dispersal due to the high wind dispersal capacity of cyanobacteria and the region of our study system being constantly exposed to ocean winds (Bohnenberger *et al*., 2018). Thus, the predominance of turnover due to environmental filtering was expected since high turnover can even be generated in an environmentally heterogeneous ecosystem— such as our study system —if efficient dispersal exists among sites (Baselga, 2010). In agreement with our findings, consistent

beta diversity patterns of freshwater bacteria and microalgae related to environmental gradients have increased in the literature, which is mainly due to variation in chemical and physical water conditions (Lima *et al*.; Van der Gucht *et al*., 2007; Lindström and Langenheder, 2012; Souffreau *et al*., 2015, 2018; Bohnenberger *et al*., 2018). However, although dispersal limitation seems to be less important than environmental conditions in the spatial structuring of aquatic microbial communities (Beisner *et al*., 2006), it is worth noting that it should not be completely discarded. Patterns related to dispersal limitation, such as distance-decay of community similarity and taxa-area relationships, have also been observed in aquatic microbes at both fine (Crump *et al*., 2007) and large spatial scales (Papke *et al*., 2003). While we did not measure dispersal rates directly in the present study, this is an approach that deserves particular attention in future studies.

Despite the prevalence of turnover to beta diversity in both planktonic and periphytic cyanobacteria, different ecological factors were found to act on the cyanobacteria variation in these two assemblage types (Table 3). Our third hypothesis predicted that the variation in planktonic cyanobacteria would be predominantly explained by the water conditions (e.g. nutrients) since they depend directly on these conditions for metabolism. In support of this assumption, when comparing the relative importance of the environment (using physical and chemical water conditions data) and heterotrophic bacteria (using bacterial abundance data at ASV level) driving the variation in planktonic cyanobacteria, variation partitioning showed that the environment explained a higher fraction of the total variation than the biotic fraction. Indeed, several studies on planktonic cyanobacteria and their structuring variables have shown that water conditions such as nutrients, light, and temperature are the most important predictors of compositional variation (Rejmánková *et al*., 2004; Johnson *et al*., 2006; Bonilla *et al*., 2012; Lu *et al*., 2013; Chamberlain *et al*., 2014; Ribeiro, Duarte, *et al*., 2018). Although to a lesser extent, the biotic fraction also had a significant influence on planktonic cyanobacteria variation. Moreover, the shared fraction (E + Bac) explained an amount of variation similar to the pure biotic fraction (Table 3). These findings may indicate an effect either of species interactions or other bacteria sharing the same driving abiotic variables with planktonic cyanobacteria. Interactions between cyanobacteria and other bacteria in pelagic environments have already been reported in previous studies. For instance, it is known that phytoplankton, including cyanobacteria, may exudate different types of dissolved organic matter (Baines and Pace, 1991), such as amino acids, carbohydrates and carboxylic acids, generating high species specificity in the bacterial use of algal organic matter (Sarmento *et al*., 2013; Tada and Suzuki, 2016), which may result in strong spatial coupling between cyanobacteria and heterotrophic bacteria in lakes (Lima *et al*., 2016; Li *et al*., 2017). Moreover, cyanobacterial blooms occurring in response to

eutrophic conditions may be followed by associations with heterotrophic bacteria that may be capable of enhancing cyanobacterial growth (Berg *et al*., 2009). On the other hand, a strong negative association between cyanobacteria and *Chloroflexi* (non-sulfur green bacteria) also suggests possible competitive interactions for habitat and/or limiting resources between these two bacterial taxa (Miller *et al*., 2009).

Contrary to planktonic cyanobacteria, periphytic cyanobacteria were significantly influenced by only the biotic fraction (Table 3), which is also in agreement with our third hypothesis. This hypothesis derived from the fact that (1) although periphyton can assimilate and retain nutrients of water column (Vadeboncoeur and Steinman, 2002), their constituents are surrounded by a polysaccharide matrix forming a biofilm, which may inhibit nutrient flow from the surrounding water due to diffusion difficulties (Riber and Wetzel, 1987); (2) to maintain a high primary production within the periphyton, mutualistic relationships between autotrophic (cyanobacteria) and heterotrophic bacteria are crucial to enable the effective internal nutrient cycling (Wetzel, 1990, 1993). Such a mutualistic relationship suggests a strong correlation between cyanobacteria and other bacterial taxa in periphytic assemblages, which has been reported by several authors (Haack and McFeters, 1982; Neely and Wetzel, 1995). Moreover, a higher fraction of the total variation in assemblage composition could be explained for planktonic cyanobacteria (69.5%) when compared to periphytic cyanobacteria (24.8%). This suggests a lower explanatory power of the environmental variables measured in our study on the variation of periphytic cyanobacteria. Previous studies have demonstrated that the structure and spatial-temporal dynamics of periphytic algal assemblages are also associated with hydrological regime (dry and rainy periods) (França *et al*., 2011), long-lasting effects of stochasticity during initial biofilm formation (Jackson *et al*., 2001), as well as the type and quality of the substrate (Rodrigues *et al*., 2005; Cordeiro *et al*., 2017), since periphytic cyanobacteria may also utilize nutrients from the host plant (Vadeboncoeur *et al*., 2006). Notably, we did not quantify any of these factors for the studied periphytic assemblages, which may be related to the relatively high unexplained variation in periphytic cyanobacteria within our study system.

In conclusion, our results indicate that cyanobacteria assemblages from open water and macrophyte-attached habitats are distinct and structured by different ecological factors. Periphyton had an average three times higher richness of cyanobacteria than the plankton, evidencing the importance of the periphytic community to the overall diversity of the subtropical studied lakes. Compositional variation in both planktonic and periphytic assemblages was determined by species replacement among the sites, however, while planktonic cyanobacteria was primarily influenced by water conditions, periphytic

cyanobacteria was related only to the abundance of heterotrophic bacteria. These results emphasize the importance of environmental filtering and biotic interactions in structuring planktonic and periphytic cyanobacteria assemblages, respectively, in subtropical shallow lakes. Additionally, the relatively large proportion of unexplained variation in periphytic cyanobacteria also indicates that further attention is needed on alternative potential assemblage-shaping factors for periphyton, which may include priority effects and host plant conditions. Moreover, the molecular method used in this study to characterize the assemblages, in contrast to the traditional microscopy, made it possible to perceive the high contribution of the picocyanobacterial component, specifically *Synechococcus*, to plankton. Finally, particular differences were observed in the eutrophic Lake Marcelino. Lake Marcelino presented the lowest richness of cyanobacteria and the dominance of three harmful bloom-forming genera in the plankton, which is associated with its condition highly impacted by anthropogenic activities.

Experimental Procedures

Study site and field sampling

Our study system comprised five coastal shallow lakes located within the Tramandaí River System (TRS), in southern Brazil (Figure 1). The TRS consists of 41 shallow lakes that have different degrees of connectivity and a single connection to the Atlantic Ocean through the Tramandaí Estuary. This system is located on a Holocene coastal plain (ca. 5000 BP) formed after a series of marine regressions and transgressions, mainly influenced by northeast and southwest winds with a humid subtropical climate and hot summers (Schwarzbold and Schäfer, 1984; Tomazelli *et al*., 2000; Mello and Castro, 2013; Bohnenberger *et al*., 2018). The TRS lakes are subjected to various anthropogenic uses, such as fishing and sewage disposal. In the summer, due to the intensification of tourism, the demand for the use of these lakes increases significantly, intensifying the impacts of human activities (Mello and Castro, 2013). The five lakes studied here differ strongly in ecological characteristics: some lakes in a turbid, cyanobacterial-bloom dominated state and subjected to impacts of human activities (e.g. Lake Marcelino), and others in a clear-water state with abundant submerged vegetation (e.g. Lake Custódia) (Table 1) (see Mello and Castro, 2013; Guimarães *et al*., 2014; Bohnenberger *et al*., 2018 for details on the TRS).

The sampling was conducted in the five lakes, at six sites within each lake (totalizing 30 sampling sites), which was performed once at the end of the warm season of the year 2018. For collecting planktonic cyanobacteria, water samples were taken at three sites, approximately 10 m apart, in the central region of the pelagic zone. These samples

were collected through 500 mL sterile flasks on the subsurface of the water column, which were subsequently filtered to a total volume of 200 mL. For collecting periphytic cyanobacteria, we sampled at three sites, also approximately 10 m apart, in emergent macrophyte beds (formed by *Scirpus californicus*) located on the banks. At each sampling site, 10 to 15 plant stems of approximately 10 cm long were cut off at 10 cm depth. Then, the stems were subsequently scraped with a sterile brush and rinsed with sterile milliQ in a total volume of 250 mL stored in sterile flasks, and from this solution, 50 mL was subsequently filtered. Both planktonic and periphytic samples (final volumes) were filtered in the laboratory immediately after collection over hydrophilic polyvinylidene difluoride (PVDF) sterile membranes (pore size 0.22 µm, diameter 47 mm; EMD Millipore, Billerica, MA, USA) and stored at -20°C.

Environmental variables data were obtained by collecting three additional samples at each of the 30 sampling sites: 500 mL of subsurface water for chemical analysis, which were immediately frozen after collection; 500 mL of subsurface water stored in frosted white flasks for determination of chlorophyll a concentration (Chl-a), which were filtered through GF/F filters (Whatman) and the pigments were measured by spectrophotometry after extraction with ethanol (Jespersen and Christoffersen, 1987); and 300 mL of subsurface water using glass BOD bottles for determination of dissolved oxygen concentration (DO) following the Winkler method, which was performed immediately after collection. *In situ*, lake depth and water transparency (transp), pH and water temperature (temp), and conductivity (cond) were measured using Secchi disk, portable pH meter and portable conductivity meter, respectively. Geographical coordinates were determined with a GPSMAPVR 62s (Garmin Ltd., Southampton, UK). In the laboratory, suspended solids were estimated through filtration on glass fiber filters, which were dried (total suspended solids, TSS) and ignited (fixed suspended solids, FSS) at 350°C > 3h. Volatile suspended solids (VSS) were calculated as the difference between TSS and FSS (Apha, 2012). Total carbon (TOC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and particulate organic carbon (POC) were evaluated using TOC V equipment (VCPH 5000; Shimadzu). Nitrate (NO_3^-), nitrite (NO_2^-), ammoniacal nitrogen ($N\text{-}NH_3^-$), soluble reactive phosphorus (SRP), soluble reactive silica (SRSi), total phosphorus (TP), total nitrogen (TN) were measured following the standard methods by Apha (2012).

DNA extraction, PCR amplification and sequencing

Cyanobacteria assemblages were characterized through high throughput sequencing. DNA was extracted using the DNeasy PowerSoil Kit (MO BIO, Qiagen Sciences Inc., Germantown, MD, USA) following the manufacturer's instructions and using

the PVDF membranes as starting material. DNA concentration of each sample was measured spectrophotometrically (NanoDrop® ND-1000, Thermo Fisher Scientific Inc., DE) and subsequently standardized over samples (2ng/µl). The libraries were constructed from the amplification of the V4 hypervariable region of the 16S rRNA gene using the eubacterial universal primers F515 and R806 with the Illumina adapters (Caporaso *et al*., 2012). PCR was performed in a final reaction volume of 25 µl containing 1 µl of DNA, 200 µM of dNTP, 0.2 µM of forward and reverse primer oligonucleotides, 1.5 mM of $MgCl_2$, 1x PCR Buffer and 2U of Platinum Taq DNA Polymerase (Invitrogen). Then, PCR was carried out on the thermal cycler (BioRad) using the following conditions: initial denaturation of 95°C for 3 min, 25 cycles of denaturation of 95°C for 30 s, annealing of 55°C for 30 s, extension of 72°C for 30 s and final extension of 72°C for 5 min. The amplicons were visualized on a 1% agarose gel. The libraries were purified using Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN) according to the manufacturer's protocol and the sequencing was performed on the Illumina® MiSeq platform using the Illumina v2 reagent kit.

Bioinformatic analyses

Bioinformatics analysis of 16S rRNA amplicons were performed using QIIME 2 version 2019.7 (Bolyen *et al*., 2018). Raw sequence data were quality filtered, denoised and chimera filtered using the q2-dada2 plugin with DADA2 pipeline Callahan (Callahan *et al*., 2016). The 5' end 20 nucleotide bases were trimmed from forward and reverse read sequences due to low quality. Reads with a number of expected errors higher than 2 (following Callahan *et al*., 2016) were discarded. Read length filtering was applied and the reads were trimmed at the first instance of a quality score less than or equal to 11. The resulting reads with nucleotide overlap between the forward and reverse reads below 20 and shorter than 230 bp length were discarded. Chimera removal was performed using the consensus method. The amplicon sequence variants (ASVs) obtained by DADA2 pipeline were merged into a single feature table using the q2-feature-table plugin. Taxonomy was assigned to ASVs using the q2-feature-classifier (Bokulich *et al*., 2018) classify-sklearn naïve Bayes taxonomy classifier. The classifier was trained using extracted Greengenes 13.8 reference sequences with 99% similarity truncated at 250 bp length from the V4 hypervariable region of the 16S rRNA gene.

Before further analyses, the ASVs that were observed in less than two samples and/or with a frequency less than 10 were removed from the feature table. Moreover, the total dataset (ASVs compositional matrix) was subdivided into two matrices: one containing only the ASVs assigned to the Cyanobacteria phylum (used as the response matrix in the following analyses), and the other containing the remaining ASVs (used as the explanatory

biotic matrix in the following analyses). In these matrices, the frequency of each ASV (number of sequences per sample) was used as a measure of abundance. Differential abundance in compositional data of cyanobacteria were performed with ANCOM (Analysis of Composition of Microbiomes) using q2-composition plugin, with mean difference as fold difference in ASVs abundances across groups and centered log-ratio (clr) as transform-function for volcano plot. ANCOM can be applied to identify features (ASVs at a certain taxonomic level) that are present in different abundances across sample groups. ANCOM is done by calculating pairwise log ratios between all features and performing a significance test to determine if there is a significant difference in feature ratios with respect to the variable of interest (Mandal *et al*., 2015). For ANCOM analysis, we grouped the ASVs at species level using q2-taxa plugin. Lastly, the ASVs attributed to Cyanobacteria were aligned with MAFFT (via q2-alignment) (Katoh *et al*., 2002) and used to construct a phylogeny (Jukes-Cantor + CAT model) with fasttree2 (via q2-phylogeny) (see details in Price *et al*., 2010). The resulting compositional matrices, rooted tree from reconstructed phylogeny, and taxonomy classification of all ASVs were imported from QIIME 2 to R environment v3.6.1 (R Core Team, 2018) for further data analysis.

Statistical analyses

To visualize the largest variation in the cyanobacteria composition data, Multidimensional Scaling (MDS) using the Bray-Curtis dissimilarity index was performed using plot ordination function of the R-package *Phyloseq* (McMurdie and Holmes, 2013). Alpha (using Shannon and Simpson indices) and beta (using Bray-Curtis dissimilarity index) diversity patterns were estimated on cyanobacteria dataset using the R-packages *Microbiome* (Lahti *et al*., 2017) and *Phyloseq* (McMurdie and Holmes, 2013). Non-parametric statistics using the Wilcoxon signed-rank (Wilcoxon, 1992) and Permutational multivariate analysis of variance (PERMANOVA) tests were performed to assess significant differences in alpha and beta diversity between the sampling sites, respectively. Non-parametric statistics were carried out through adonis function of the R-package *vegan* (Oksanen *et al*., 2010) considering a *p*-value significance of 0.05 with 9999 permutations. The disentangling of the contribution of species replacement (turnover component) and species loss (nestedness component) to total beta diversity was calculated according to Baselga (2010) considering the total dataset and separately for planktonic and periphytic assemblages, using the multiple-site Bray-Curtis dissimilarity index (β_{BRAY}) applied to abundance data (Baselga, 2013). The total beta diversity (β_{BRAY}) was then partitioned into turnover component (β_{SIM}) by using the Simpson dissimilarity index, and nestedness component (β_{NES}) following the equation: $\beta_{NES} = \beta_{BRAY} - \beta_{SIM}$. Partition of beta diversity

analyses were performed through beta.multi.abund function of the R-package *betapart* (Baselga and Orme, 2012).

To explore the relative importance of environmental (physical and chemical water conditions) and biotic (abundance of heterotrophic bacteria) factors in explaining the variation in cyanobacteria assemblages, we assessed the contribution of the environment (E), heterotrophic bacteria (Bac) and their combined effect (E + Bac) on the cyanobacteria variation (based on the abundance of the cyanobacterial ASVs) through partial redundancy analyses (pRDA) in association with variation partitioning procedures (Borcard *et al*., 1992; Legendre and Legendre, 2012). These procedures were performed considering the total dataset (total cyanobacteria: 30 samples, 227 ASVs) and subdividing the total dataset into two subsets based on the type of assemblage (planktonic cyanobacteria: 15 samples, 34 ASVs; periphytic cyanobacteria: 15 samples, 199 ASVs). Variation partitioning was performed on Hellinger-transformed abundance data of both cyanobacteria and heterotrophic bacteria. For each of the three datasets (total cyanobacteria, planktonic cyanobacteria and periphytic cyanobacteria), unique environmental and biotic models were constructed *a priori* by selecting sets of environmental and biotic variables, respectively, that significantly explain (part of the) variation in cyanobacteria composition.

To construct the environmental (E) and biotic (Bac) models, the forward selection method (Blanchet *et al*., 2008) was applied to the z-score transformed environmental variables and the Hellinger-transformed biotic variables using the function forward.sel from the R-package *packfor* (Dray *et al*., 2009) with a threshold *p*-value of 0.05. Variables significantly contributing to the model were retained for variation partitioning. Before forward selection, we explored the presence of collinearities among the non-transformed environmental variables by computing variance inflation factors (VIFs) using the function vif.cca from the R-package *vegan* (Oksanen *et al*., 2010). Then, only the environmental variables with VIFs < 3 were maintained in the analyses. Variation partitioning was performed using the function varpart of the R-package *vegan* (Oksanen *et al*., 2010) and the conditional effects of the environmental (pure environment without biotic effects) and biotic (pure bacteria without environmental effects) fractions were calculated as adjusted canonical R^2 values by pRDA using the function rda, followed by a ANOVA-like permutation test using the function anova.cca of the same R-package.

Tables

Table 1. Average values of environmental conditions and biotic characteristics of the five lakes studied (n = 6 per lake: 3 in the pelagic zone and 3 in the emergent macrophyte beds on the banks). Only the environmental variables that significantly correlated with cyanobacterial composition variation (Redundancy Analysis test) are shown, except by Secchi depth and TP values.

	Ramalhete	Passo	Marcelino	Custódia	Cidreira
Geographical coordinates	S 29°45.566 W 50°08.570	S 29°51.969 W 50°06.315	S 29°53.218 W 50°15.160	S 30°00.951 W 50°11.337	S 30°13.956 W 50°15.959
Maximum lake depth (cm)	190	150	60	150	90
Secchi depth (cm)	40	70	10	75	50
TP ($mg.L^{-1}$)	0.02	0.08	0.80	0.16	0.12
SRSi ($mg.L^{-1}$)	9.57	7.37	12.73	0.88	5.35
$N\text{-}NH_3^-$ ($mg.L^{-1}$)	0.13	0.11	0.05	0.32	0.22
SRP ($mg.L^{-1}$)	0.01	0.07	0.22	0.13	0.07
DIC ($mg.L^{-1}$)	2.67	4.24	30.81	9.20	2.22
VSS ($mg.L^{-1}$)	8.83	5.83	18.67	36.83	7.67
DO ($mg.L^{-1}$)	7.45	8.73	7.00	8.40	7.35
Water temperature (°C)	23.13	25.60	25.47	25.35	27.05
Total cyanobacterial ASVs	49	28	16	52	104
Total other bacterial ASVs	117	134	99	309	461

Table 2. Decomposition of the beta diversity of cyanobacteria assemblages into species replacement (turnover) and species loss (nestedness), considering the total dataset and separately for the planktonic and periphytic assemblages.

	Total Cyanobacteria (n=30)	Planktonic Cyanobacteria (n=15)	Periphytic Cyanobacteria (n=15)
Overall beta diversity (β_{BRAY})	0.98	0.91	0.97
Turnover component (β_{SIM})	0.96	0.87	0.94
Nestedness component (β_{NES})	0.02	0.04	0.03

n: number of samples

Table 3. Relative importance of the pure environment (E), pure bacteria (Bac), and the shared (E + Bac) fractions on the variation of cyanobacteria assemblages, expressed as the adjusted canonical R^2 values, considering the total dataset and separately for the planktonic and periphytic assemblages. Variables were selected by forward selection (see Methods). Significant fractions are indicated with asterisks.

	Total Cyanobacteria (n=30)	Planktonic Cyanobacteria (n=15)	Periphytic Cyanobacteria (n=15)
Pure Environment (E)	0.054**	0.347***	0.025
Pure Heterotrophic bacteria (Bac)	0.304***	0.186***	0.125**
E + Bac	0.098	0.162	0.098
Unexplained	0.544	0.305	0.752
Environment selected	depth, SRSi, $N\text{-}NH_3^-$, SRP	DIC, VSS, DO, temp	depth, DIC
Bacteria selected (at class level)	Actinobacteria, Spartobacteria, Gammaproteobacteria, Saprospirae, Betaproteobacteria, Alphaproteobacteria, Cytophagia	Spartobacteria, Betaproteobacteria, Saprospirae, Actinobacteria, Chloroflexi	Saprospirae, Gammaproteobacteria, Actinobacteria, Cytophagia

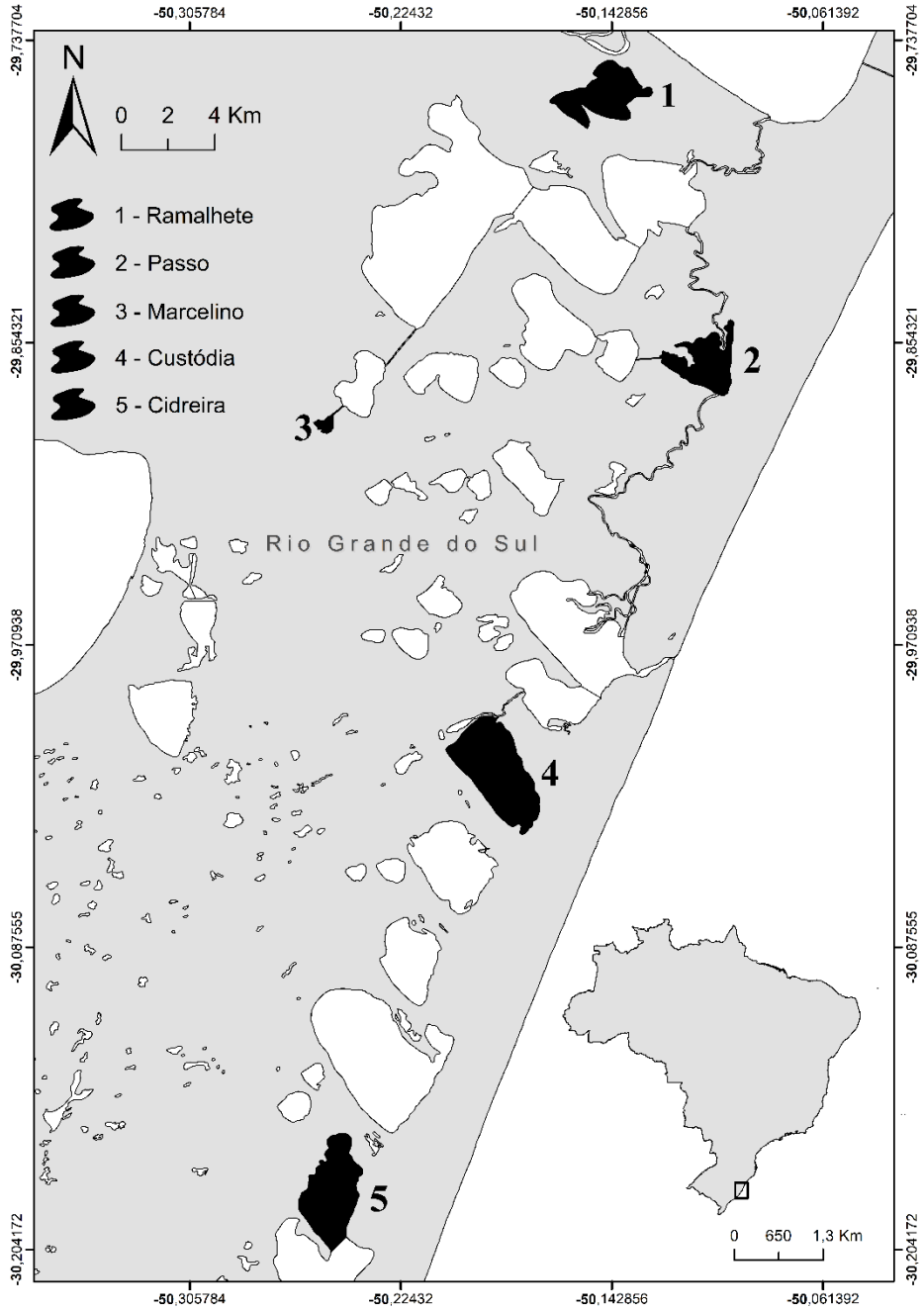
SRSi: silica; $N\text{-}NH_3$: ammoniacal nitrogen; SRP: soluble reactive phosphorus; DIC: dissolved inorganic carbon; VSS: volatile suspended solids; DO: dissolved oxygen; temp: water temperature

n: number of samples

**$p < 0.005$, ***$p < 0.001$

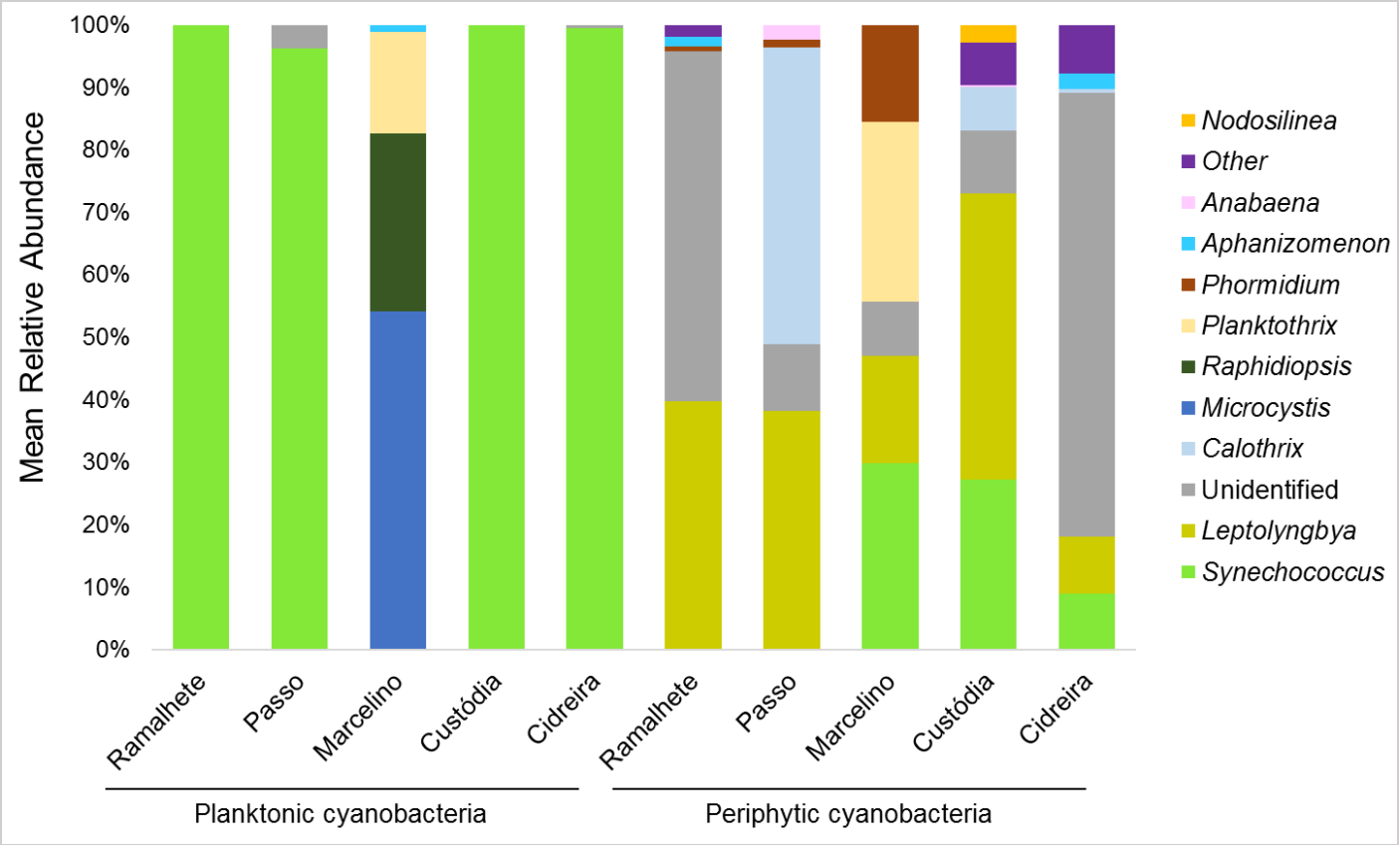
Figures

Figure 1



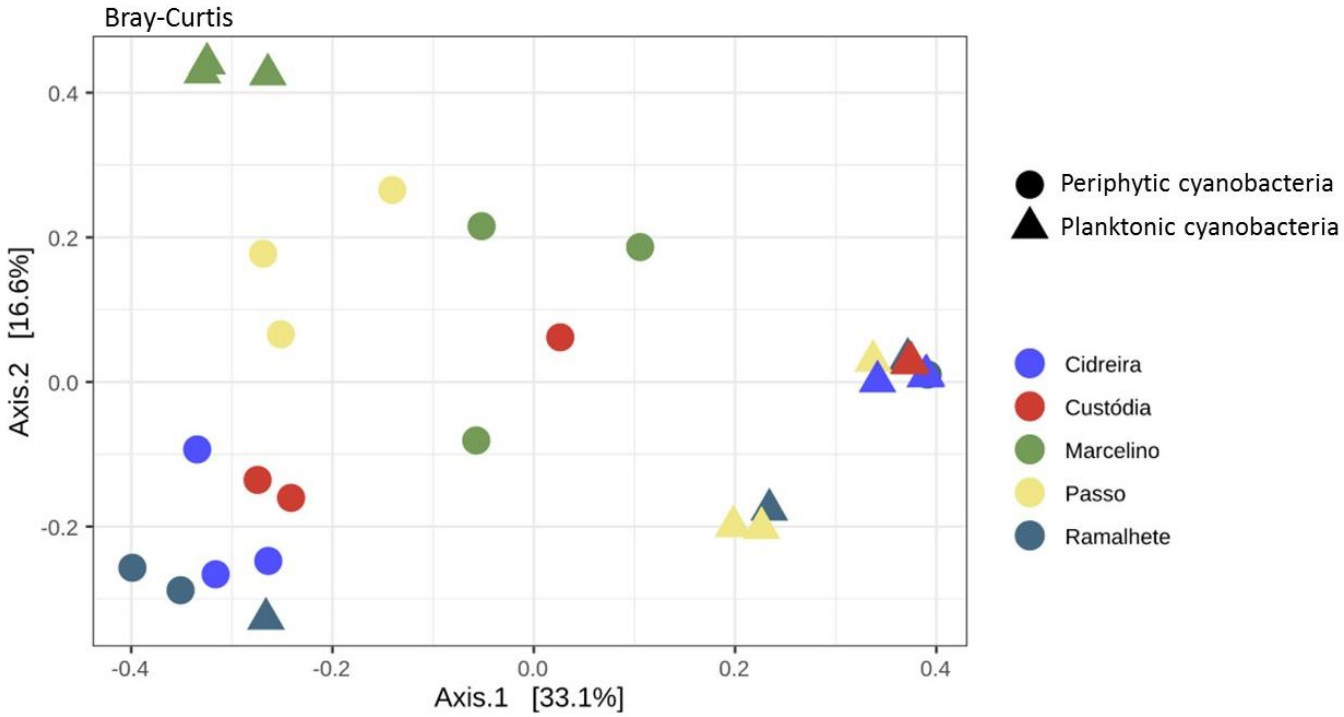
Legend: Map of Rio Grande do Sul, southern Brazil, showing the locations of the five coastal lakes sampled in this study.

Figure 2



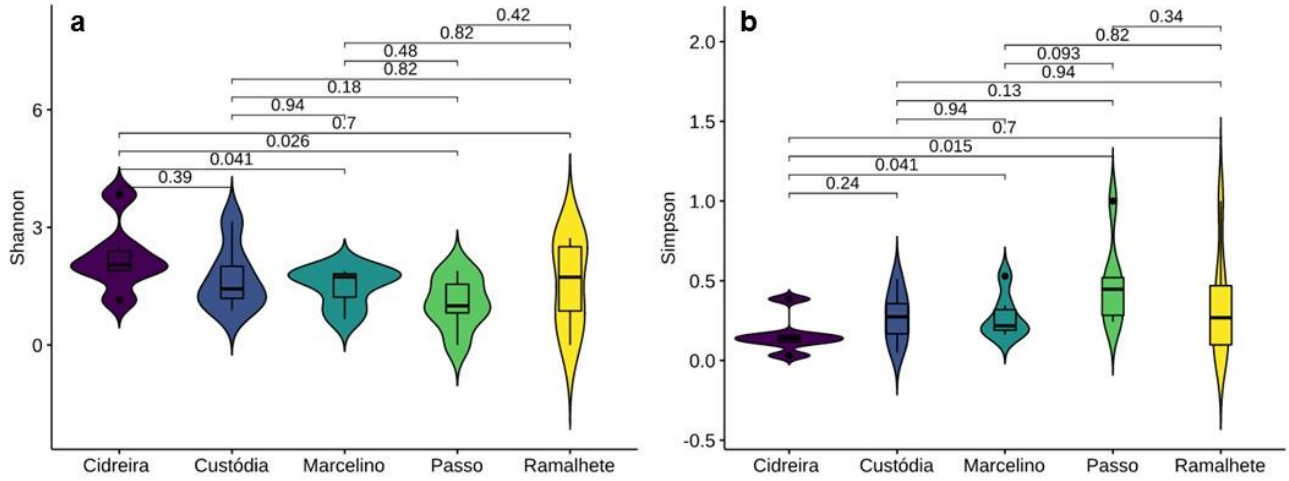
Legend: Mean relative abundance at genus level in planktonic and periphytic cyanobacteria assemblages per lake.

Figure 3



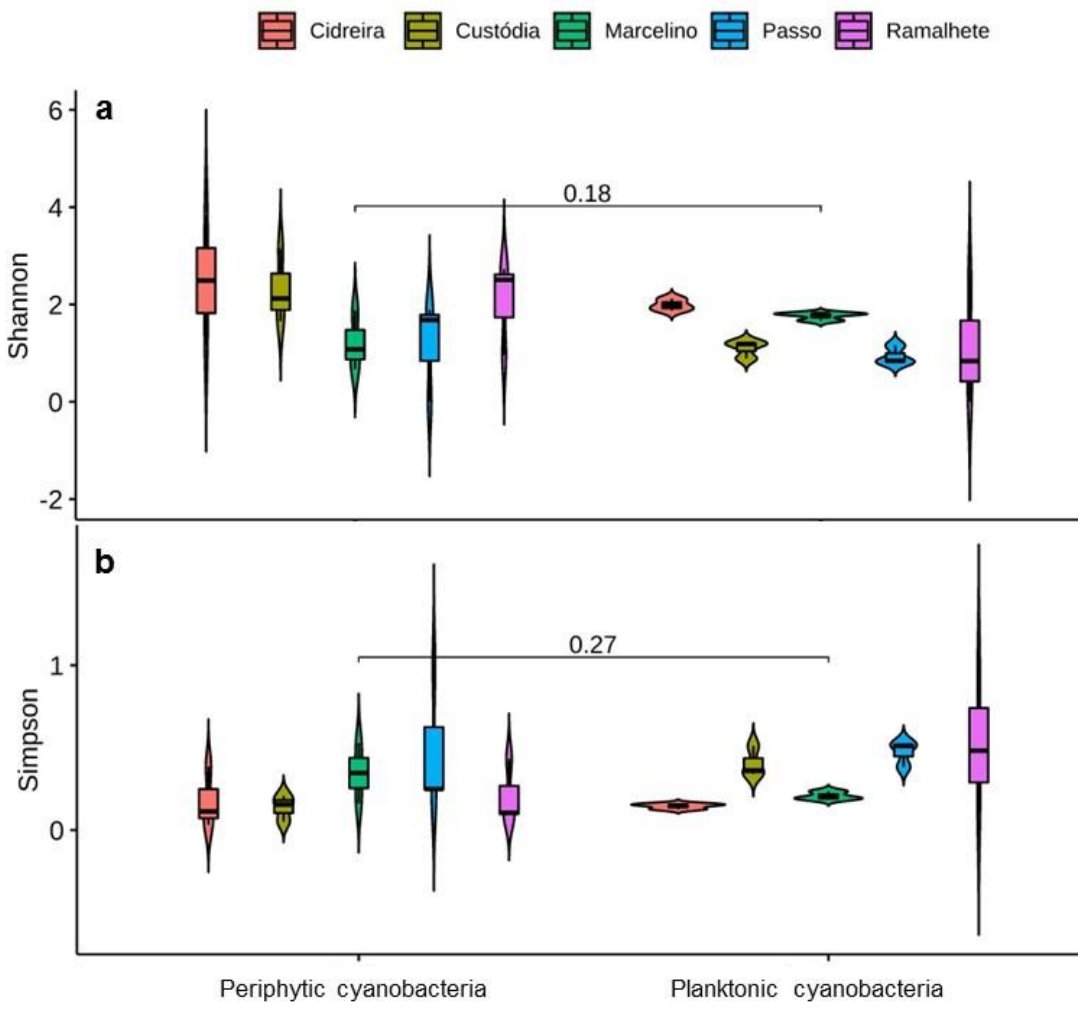
Legend: Multidimensional scaling (MDS) plot based on Bray-Curtis dissimilarities for the 30 samples of the two assemblage types (circle = periphytic cyanobacteria; triangle = planktonic cyanobacteria) sampled in the five lakes (represented by colours).

Figure 4



Legend: Boxplot of the (a) Shannon and (b) Simpson alpha diversity indices calculated for each lake (n=6). The lower part of the boxes represents the lower quartile, the upper part of the box represents the upper quartile, the bar inside the box marks the median and outliers are shown as small circles. The numbers represent the *p*-values obtained from the Wilcoxon signed-rank test (among lakes).

Figure 5



Legend: Boxplot of the (a) Shannon and (b) Simpson alpha diversity indices calculated for periphytic and planktonic cyanobacteria (n=15). The lower part of the boxes represents the lower quartile, the upper part of the box represents the upper quartile and the bar inside the box marks the median. The numbers represent the *p*-values obtained from the Wilcoxon signed-rank test (among assemblage type).

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CONSIDERAÇÕES FINAIS

Entre os procariotos, as cianobactérias destacam-se pela enorme diversidade morfológica e fisiológica (de unicelulares a multicelulares), e a existência de táxons com diferentes padrões de distribuição (de endêmicos a cosmopolitas) Estas características únicas viabilizam abordagens interessantes para estudos na área de distribuição microbiana que ainda são pouco exploradas. Em ecologia microbiana, estudos com foco em cianobactérias, especialmente nas áreas de biogeografia e filogeografia e com uso de dados moleculares, ainda são recentes e concentrados a determinadas regiões e táxons específicos. Inúmeras questões neste campo precisam ser melhor exploradas, como por exemplo se espécies consideradas cosmopolitas na verdade representam ecótipos com distribuições restritas, ou se espécies consideradas endêmicas podem na verdade ser o resultado de uma subestimativa de ocorrência devido ao esforço insuficiente de amostragem. Assim, empreender esforços para conhecer e mapear a real diversidade de cianobactérias nos mais diversos ecossistemas é o primeiro passo para alavancar o conhecimento sobre padrões de distribuição desse grupo.

A partir do conjunto de dados obtido na revisão bibliográfica (Capítulo I), ficou evidente a importância de fatores ecológicos sobre padrões de distribuição biogeográfica de cianobactérias em uma variedade de táxons, ecossistemas e escalas espaciais. Condições ambientais locais, sobretudo relacionadas à concentração de nutrientes e radiação solar, são frequentemente mencionadas como principais preditoras da distribuição de cianobactérias. Evidências mais recentes, entretanto, demonstram a importância também de fatores puramente espaciais, históricos e estocásticos, como o limite de dispersão, isolamento geográfico e especiação alopátrica, sobre a distribuição de cianobactérias. Ao mesmo tempo, ficou claro que a relação entre padrões de distribuição biogeográfica e variáveis preditoras varia dependendo do nível de diversidade explorada (e.g. taxonômica, genética), do conceito de espécie adotado, do tipo de ecossistema, da escala espacial, e dos vieses das metodologias aplicadas nos estudos. Portanto, esforços de amostragem contínuos, geração de dados genômicos para resolver questões filogenéticas, e teste de hipóteses no contexto da biogeografia e filogeografia com dados já existentes, como dados de ocorrência e abundância, são necessários para um melhor entendimento da biogeografia e da ecologia de cianobactérias.

Na área da filogeografia, estudos com foco em cianobactérias ainda são raros e concentrados em espécies potencialmente problemáticas, principalmente formadoras de florações tóxicas. A partir da comparação da filogeografia (baseada na diversidade genética do gene 16S rRNA) de duas espécies cosmopolitas de cianobactérias (Capítulo II), foi corroborada a hipótese de que essas espécies se originaram em regiões tropicais (em função da maior diversidade genética encontrada nos trópicos) e recentemente expandiram suas distribuições para habitats temperados. Além disso, a estrutura filogeográfica de *R. raciborskii* sugere que esta espécie possa ter alguma limitação de dispersão ou esteja se dispersando ao longo do globo por um maior período de tempo. *M. aeruginosa*, ao contrário, parece ter uma maior taxa de dispersão intercontinental, em concordância com estudos anteriores que denominam esta espécie como verdadeiramente cosmopolita. Finalmente, a evidência de rápida expansão populacional nos últimos 2.500 anos, através das análises de dinâmica populacional, sugere que atividades humanas recentes, como a maior mobilidade intercontinental e uso de ecossistemas aquáticos, podem ser responsáveis por pelo menos parte da propagação global bem-sucedida dessas duas espécies.

O fato de haver espécies de cianobactérias verdadeiramente cosmopolitas em escala global, no entanto, não implica que cianobactérias sejam amplamente difundidas em escalas regionais e locais. Além disso, pouco se sabe sobre como as cianobactérias se distribuem em diferentes microhabitats (e.g. zonas pelágica, bentônica e litorânea) dentro de um mesmo ecossistema. A partir de uma abordagem comparativa entre assembleias de cianobactérias planctônicas e perifíticas (Capítulo III), caracterizadas através do sequenciamento parcial do gene 16S rRNA, ficou claro que os dois tipos de assembleias são distintos, em termos de composição de cianobactérias, e estruturados por diferentes fatores ecológicos. Estes achados corroboram a ideia de que diferentes táxons de cianobactérias são adaptados à vida livre, como componente do plâncton, e outros à vida séssil, como em biofilmes aderidos a superfícies. Ao particionar a diversidade beta das cianobactérias planctônicas e perifíticas, ficou evidente que o *turnover* (substituição de espécies entre as unidades amostrais) é o principal componente da variação de cianobactérias no sistema de estudo. Entretanto, enquanto a variação das cianobactérias planctônicas foi influenciada principalmente pelas condições da água, a variação das cianobactérias perifíticas esteve relacionada apenas à abundância de outras bactérias presentes nos biofilmes. Em resumo, estes achados enfatizam a importância do filtro ambiental e das interações bióticas na estruturação de assembleias de cianobactérias planctônicas e perifíticas, respectivamente.

Por fim, em função das diversas possibilidades ainda inexploradas no estudo da distribuição da diversidade de cianobactérias, especialmente biogeografia e filogeografia, germinam perspectivas instigantes em estudos futuros. Com ferramentas de pesquisa cada vez mais poderosas no campo da biologia molecular e bioinformática, experimentos criativos, grandes conjuntos de dados espaciais temporais, e novos modelos teóricos, as perspectivas na área de ecologia de cianobactérias prometem transformar nossa compreensão dos processos que moldam a sua distribuição, essencial para um grupo bacteriano que está intimamente relacionado à conservação de um dos recursos mais importantes para a população humana, a água. Além disso, abordagens ainda pouco exploradas, como a análise de distribuição de atributos funcionais e análises baseadas em experimentos controlados, por exemplo, serão necessárias para complementar o entendimento da ecologia de cianobactérias como um todo.

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MATERIAL SUPLEMENTAR

Capítulo II

O Material Suplementar pode ser acessado em https://doi.org/10.1111/jbi.13785, na sessão ‘Supporting Information’.

Capítulo III

Table S1. Total sequences obtained after trimming, filtering, denoising and chimera removal steps

Sample ID	Lake	Assemblage type	Input	Filtered	Denoised	Merged	Non-chimeric
1	Ramalhete	Planktonic	28368	24155	20865	8549	7716
2	Ramalhete	Planktonic	29336	26466	21121	7756	6465
3	Ramalhete	Planktonic	24767	22309	19747	8211	7276
4	Ramalhete	Periphytic	38260	36613	30178	16941	12080
5	Ramalhete	Periphytic	40959	40176	35291	16816	14939
6	Ramalhete	Periphytic	40972	38961	32828	16375	12769
13	Passo	Planktonic	41685	41034	34756	15923	13879
14	Passo	Planktonic	52646	51937	45303	19797	18263
15	Passo	Planktonic	42900	41789	35977	15222	14249
16	Passo	Periphytic	59397	56837	51399	26439	17548
17	Passo	Periphytic	46290	44707	39725	12570	8984
18	Passo	Periphytic	65482	64452	53951	28038	19537
25	Marcelino	Planktonic	38384	37730	34286	10177	8295
26	Marcelino	Planktonic	23418	15774	13336	3621	2927
27	Marcelino	Planktonic	18817	16421	15313	3792	3436
28	Marcelino	Periphytic	25239	23570	20445	8394	6899
29	Marcelino	Periphytic	33247	32493	28475	10594	9057
30	Marcelino	Periphytic	23843	19899	16065	4766	4040
49	Cidreira	Planktonic	46964	46501	41109	15042	13881
50	Cidreira	Planktonic	32511	31867	26649	11831	11473
51	Cidreira	Planktonic	40835	40226	35184	14392	13344
52	Cidreira	Periphytic	55116	53573	42566	17904	14049
53	Cidreira	Periphytic	48635	47762	43587	32129	19212
54	Cidreira	Periphytic	40128	39365	30971	9685	8112
55	Custódia	Planktonic	13925	13267	11549	5688	5688
56	Custódia	Planktonic	13878	12864	11052	7598	7598
57	Custódia	Planktonic	11963	11082	9738	5714	5576
58	Custódia	Periphytic	16711	16177	14161	7975	6803
59	Custódia	Periphytic	17027	16678	13603	5874	5675
60	Custódia	Periphytic	51250	50559	48285	39750	16185