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**ANÁLISIS COMPARATIVO Y FUNCIONAL DE CIANOBACTERIAS ASOCIADAS A LA FORMACIÓN DE MICROBIALITAS DE LA LAGUNA BACALAR Y LAGO-CRATER ALCHICHICA**

# TESIS

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## DOCTOR EN CIENCIAS

PRESENTA

**BERNARDO ÁGUILA SALGADO**

**TUTORA PRINCIPAL: DRA. LUISA ISAURA FALCÓN ÁLVAREZ /**

**Instituto de Ecología, UNAM**

**COMITÉ TUTOR: DRA. ROCIO JETZABEL ALCÁNTARA**

**HERNÁNDEZ / Instituto de Geología, UNAM**

**COMITÉ TUTOR: DR. GUSTAVO ALBERTO MONTEJANO ZURITA /**

**Facultad de Ciencias, UNAM**

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Sin otro particular, me es grato enviarle un cordial saludo.

**A T E N T A M E N T E**
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# ÍNDICE

# 1. RESUMEN

En este trabajo se hace un análisis comparativo de las comunidades microbianas que forman microbialitas en el Lago Alchichica (AL), Laguna Bacalar (BL) y el Cenote Azul (CA) en México, con énfasis en el componente de cianobacterias. Para esto se siguió un marco de trabajo polifásico que integró: 1) Métodos microscópicos: incluyendo microscopía óptica, de fluorescencia y confocal láser para análisis morfológicos. 2) Métodos de cultivo: estableciendo las condiciones óptimas de crecimiento para cianobacterias. 3) Métodos moleculares: incluyendo técnicas de genómica dirigida (región hipervariable V4 del gen 16S ARNr) para describir la taxonomía y buscar patrones de estructura filogenética entre sitios, así como técnicas de metagenómica (Shotgun), para buscar patrones y asociaciones en los perfiles funcionales. Los datos morfológicos, taxonómicos, ecológicos y funcionales obtenidos de bacterias y cianobacterias en microbialitas mostraron diferencias a nivel taxonómico, pero similitudes a nivel funcional. Con base en estos datos y una extensa revisión bibliográfica, se hizo una propuesta para englobar a las cianobacterias de microbialitas en grupos funcionales, de acuerdo a su ecología litobióntica y a sus adaptaciones. Esta clasificación facilitará futuros trabajos comparativos de cianobacterias en microbialitas. Finalmente, se remarca la importancia de seguir estudiando cianobacterias de microbialitas en el contexto de ciencias ambientales, atrapamiento de carbono, grupos taxonómicos nuevos y anotación de genes desconocidos.

## ABSTRACT

In this work, a comparative analysis of the microbial communities that form microbialites in Lake Alchichica (AL), Laguna Bacalar (BL) and Cenote Azul (CA) in Mexico is made, with emphasis on the cyanobacterial component. For this, a polyphasic framework was used, which integrated: 1) Microscopic methods: including optical, fluorescence and confocal laser microscopy for morphological analysis. 2) Culture methods: establishing the optimal growth conditions for Cyanobacteria. 3) Molecular methods: including targeted genomic techniques (16S rRNA gen V4 hypervariable region) to describe the taxonomy and phylogenetic structure between sites, as well as metagenomic techniques (Shotgun), to look for patterns and associations in functional profiles. Morphological, taxonomic, ecological and functional data obtained from bacteria and cyanobacteria in microbialites showed differences at the taxonomic level, but similarities at the functional level. Based on these data and an extensive bibliographic review, a proposal to group cyanobacteria of microbialites in functional groups is made according to their lithobiontic ecology and their adaptations. This classification will facilitate future comparative work of cyanobacteria in microbialites. Finally, the importance of continuing to study microbialite cyanobacteria in the context of environmental sciences, carbon sequestration, new taxonomic groups and unknown genes is highlighted.

# 2. INTRODUCCIÓN GENERAL

## 2.1 Definición de microbialitas

Las microbialitas o microbialitos son estructuras bénticas organosedimentarias u organominerales que se forman por actividad microbiana y por el atrapamiento, la acreción y/o la precipitación de minerales (Burne and Moore 1987, Dupraz, Reid et al. 2011). También pueden definirse como tapetes microbianos con la capacidad de litificar (Havemann and Foster 2008, Dupraz, Reid et al. 2011). El término microbialita ha cambiado desde su primera concepción como criptaalgal (Aitken 1967), después estromatolito (Awramik, Margulis et al. 1976) y finalmente microbialita (Burne and Moore 1987). Aunque aún existe discusión sobre si las microbialitas también deben englobar a sistemas organosedimentarios terrestres, como las costras de desierto y a los tapetes microbianos (Riding 1991, Dupraz, Reid et al. 2009). Para esto, se ha propuesto el término "estructura sedimentaria inducida por microbios" o MISS (del inglés *Microbial Induced Sedimentary Structures*), que tiene una clasificación más amplia, y engloba a los sistemas organosedimentarios acuáticos y terrestres (Noffke, Gerdes et al. 2001). Todas las microbialitas se consideran MISS, pero no todas las MISS son microbialitas. Esta tesis se delimita al estudio de microbialitas bentónicas y el papel que tienen las cianobacterias en su formación.

## 2.2 Clasificación morfológica de microbialitas.

Por su morfología estructural interna (microestructura) las microbialitas se clasifican en:
1) Estromatolitos: cuando el carbonato microbiano forma láminas finamente estratificadas dentro del sedimento intersticial; 2) Trombolitos: cuando el carbonato microbiano forma aglomeraciones amorfas dentro del sedimento intersticial; 3) Dendrolitos: cuando el carbonato microbiano forma estructuras dendríticas o ramificadas dentro del sedimento intersticial; 4) Leiolitos: cuando el carbonato microbiano no forma una estructura y el sedimento se compone de grano fino (afanítico) (Riding 2012). Otros trabajos incluyen a los sistemas travertinos y carbonatos microbianos crípticos dentro de las microbialitas. También se mencionan los estromatolitos de tufa, estromatolitos esqueléticos y estromatolitos subaéreos (Riding 1991).

Por su morfología macro estructural, las microbialitas pueden tener diferentes formas, se clasifican en: columnares, ramificados, esponjosos, repisas, digitados, costrosos, domos o montículos, cabezas, conos y oncolitos (**Figura 1**).

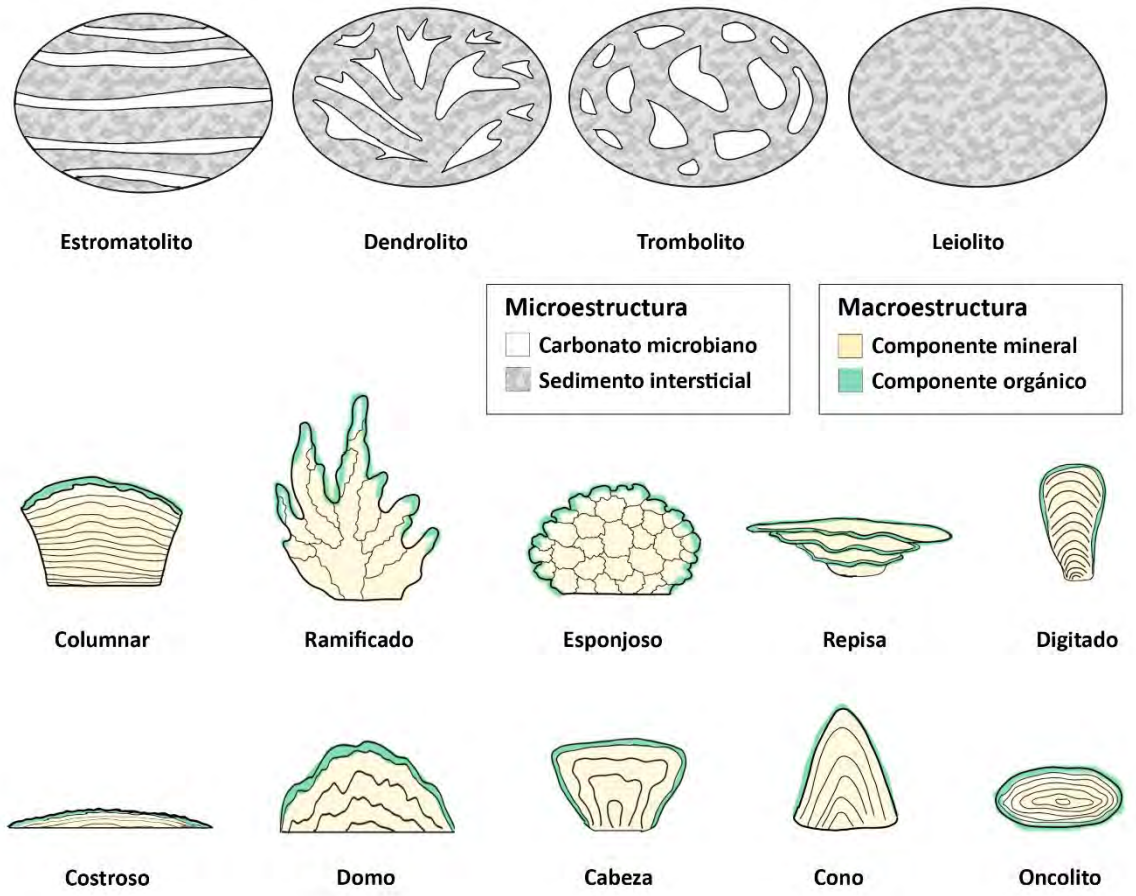


***Figura 1.****Clasificación por morfología interna y externa de las microbialitas, microestructura modificada de (Riding 2011).*

## 2.3 Composición de Microbialitas

Las microbialitas al ser estructuras organominerales, tienen un componente orgánico y otro mineral. El componente orgánico es un tapete microbiano compuesto mayormente por bacterias, donde interaccionan diferentes comunidades de microorganismos con metabolismos acoplados en favor de la precipitación mineral (Laval, Cady et al. 2000). En la zona externa del tapete microbiano se encuentran microorganismos fotótrofos como diatomeas, microalgas y cianobacterias; en la zona intermedia existen heterótrofos aerobios como Proteobacteria, Firmicutes y micro Eucariontes.

También hay fotótrofos anoxigénicos como bacterias púrpuras del azufre, bacterias verdes del azufre, Cloroflexi, Betaproteobacteria, Heliobacteria; en la zona más profunda del tapete hay heterótrofos anaerobios como Bacteroidetes y Planctomycetes, reductores del azufre como bacterias sulfato reductoras y arqueas metanógenas (Arp, Reimer et al. 2003, Krumbein, Brehm et al. 2003, Dupraz 2004, Decho, Visscher et al. 2005, Baumgartner, Reid et al. 2006, Armitage, Gallagher et al. 2012, Beltrán, Centeno et al. 2012, Tarhan, Planavsky et al. 2013, De Anda, Zapata-Peñasco et al. 2018, Roche, Vennin et al. 2019). El componente mineral está conformado por carbonatos, principalmente de calcio y magnesio, aunque menos comúnmente, pueden existir fosfatos (minerales fosfatados), formas de azufre (sulfatos y sulfuros), silicatos, entre otros (Dupraz, Reid et al. 2009) (**Figura 2**).

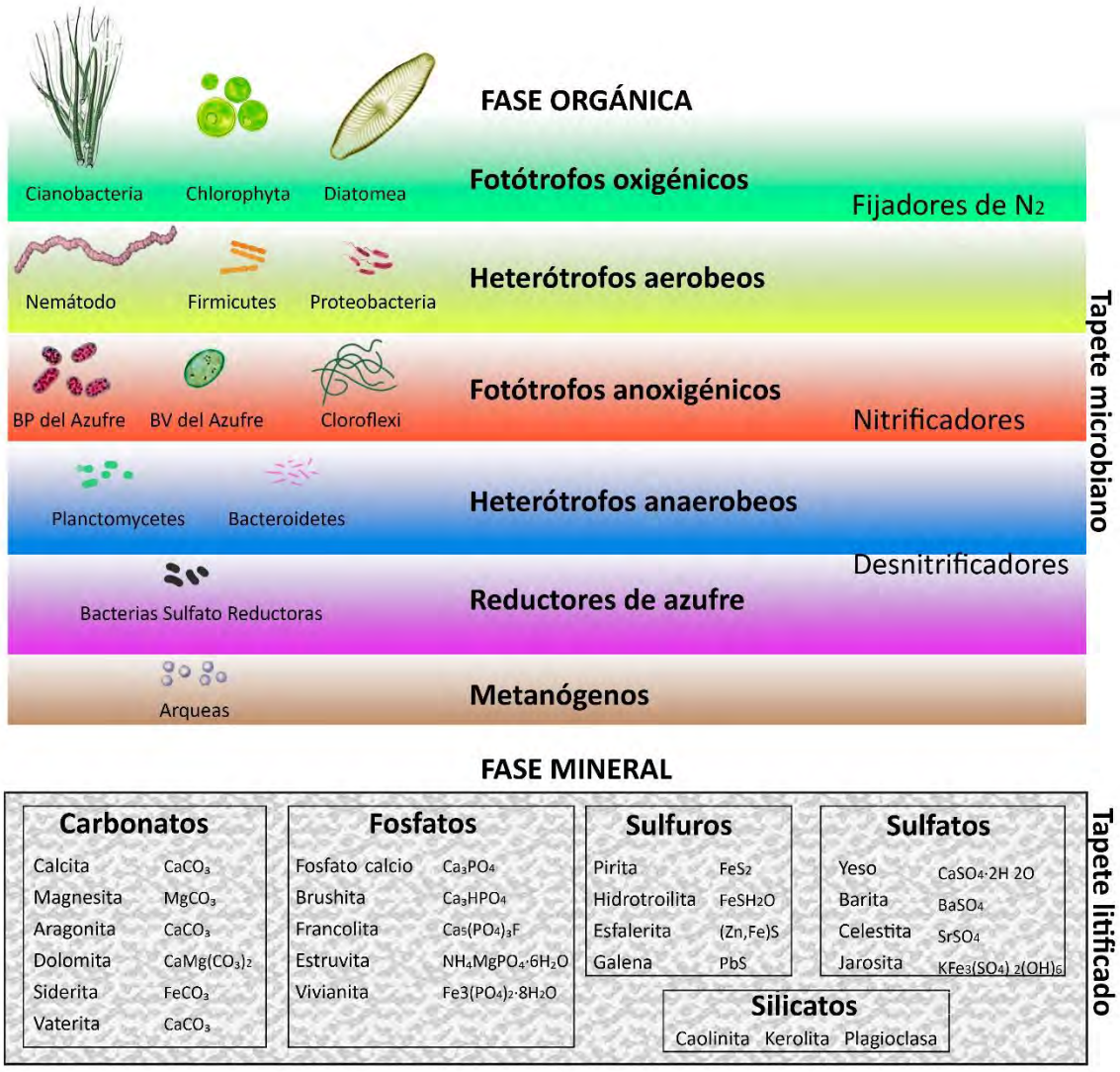


***Figura 2.*** *Composición general de microbialitas modernas incluyendo la fase orgánica compuesto por el tapete microbiano y la fase mineral compuesta por el tapete litificado.*

## 2.4 Ciclo de vida y formación de Microbialitas

En la perspectiva microbiológica, el ciclo de vida de una microbialita puede entenderse como un modelo en donde el componente orgánico (tapete microbiano) litifica con las condiciones abióticas y bióticas favorables, y que con el tiempo el tapete se convierte en componente mineral (tapete litificado). El tapete litificado pasa por procesos de mineralización, cementación y compactación, mientras que paralelamente otro tapete microbiano puede crecer por encima del tapete litificado, repitiendo el ciclo. Con el paso del tiempo, una microbialita puede petrificar y convertirse en una roca sedimentaria o microbialita fósil, pero también puede desmineralizarse o degradarse por factores abióticos o bióticos (Golubic 1991, Riding 2012) (**Figura 3**).


***Figura 3.*** *Ciclo de vida de una microbialita desde su origen como un tapete microbiano, pasando por la litificación hasta su petrificación o desmineralización por condiciones bióticas y abióticas.*

Es importante diferenciar la cronología temporal en la formación de microbialitas, ya que primero pueden llegar los microorganismos a colonizar un sitio y posteriormente, sus metabolismos favorecer la formación de microbialitas (bioformación). Pero también puede existir primero un fenómeno de formación abiótica de la estructura sedimentaria por diagenesis y posteriormente haber una colonización por microorganismos sobre la estructura ya formada (bioturbación). Las revisiones sobre sistemas organosedimentarios postulan que estos dos procesos (bioformación y bioturbación) están alternados o suceden simultáneamente en las microbialitas (Burne and Moore 1987, Garcia-Pichel, Al-Horani et al. 2004, Chagas, Webb et al. 2016, Zhu and Dittrich 2016).

A diferencia de un coral que tiene procesos metabólicos específicos para controlar activamente la mineralización de estructuras (biomineralización), el componente orgánico de las microbialitas no controla directamente la mineralización, pero si la promueve o influencía indirectamente, a esto se le llama organomineralización (Riding 2006, Dupraz, Reid et al. 2009, Zhu and Dittrich 2016) (Dupraz,

Reid et al. 2009). Hay dos mecanismos de organomineralización en microbialitas: 1) Acreción: cuando los microorganismos atrapan activamente materia orgánica, detritos o sedimento (Frantz, Petryshyn et al. 2015); 2) Precipitación, pudiendo ser pasivamente por deposición inorgánica o influenciada por metabolismos microbianos (Dupraz, Reid et al. 2009). Dependiendo el tipo de organomineralización que se lleve a cabo se formará una microestructura diferente (**Figura 4**). Cabe resaltar, que la mayoría de las microbialitas tienen una precipitación híbrida, esto es, ocurre tanto una precipitación inorgánica como orgánica, y se observan estratos alternados de precipitación biótica y abiótica (Kaźmierczak, Fenchel et al. 2015).

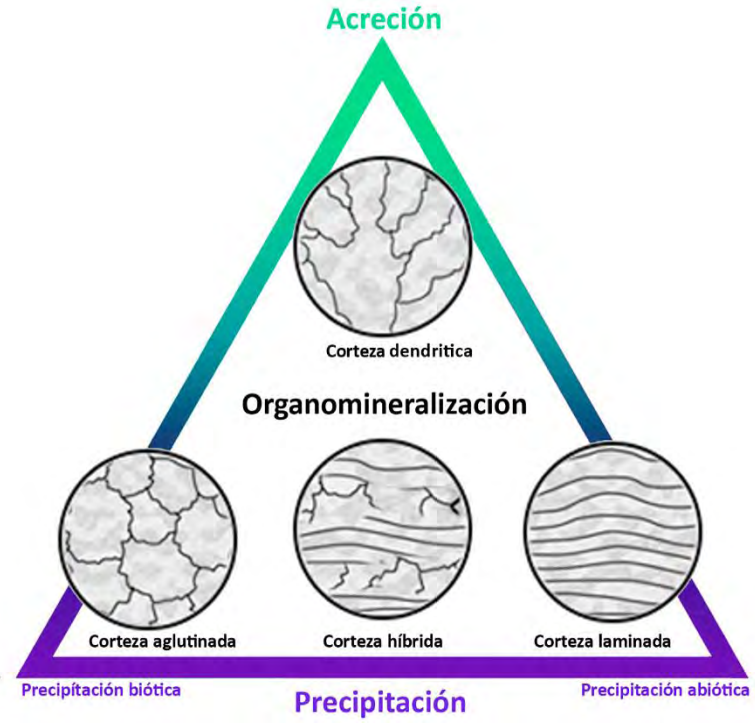


***Figura 4.*** *Las microbialitas pueden formarse por el atrapamiento activo de organismos o por una precipitación ya sea inorgánica o influenciada pasivamente por microorganismos. Dependiendo el tipo de organomineralización, se formará una corteza específica. La mayoría de las microbialitas tienen una corteza híbrida, es decir, una alternancia de capas por precipitación biótica y abiótica.*

## 1.3.1 Factores bióticos que afectan la formación de microbialitas

Para entender el componente orgánico de las microbialitas, se han usado técnicas de microscopía (Kempe, Kazmierczak et al. 1991, Kazmierczak and Kempe 2006, Gischler, Gibson et al. 2008), secuenciación de las regiones del gen 16S ARNr (Schneider, Arp et al. 2013, Chan, Bugler-Lacap et al. 2014, Yanez-Montalvo, Gómez-Acata et al. 2020, Águila, Alcántara-Hernández et al. 2021, Yanez-Montalvo, Águila et al. 2021), técnicas de metagenómica (Breitbart, Hoare et al. 2009, Temperton and Giovannoni 2012, Saghaï, Zivanovic et al. 2016, White III, Chan et al. 2016, Águila, Yanez-Montalvo et al. 2022), y algunos pocos trabajos utilizan técnicas de meta-transcriptómica (Mobberley, Khodadad et al. 2013, Louyakis, Gourlé et al. 2018, Campbell, Coolen et al. 2021). También existen

estudios sobre los mecanismos de precipitación y formación de microbialitas *in vitro,* caracterizando los componentes de las fracciones EPS y tipos de membranas de los microorganismos asociados a las microbialitas (Arp, Reimer et al. 2003, Gautret, Camoin et al. 2004, Parikh and Madamwar 2006, Havemann and Foster 2008, Obst, Dynes et al. 2009, Dittrich and Sibler 2010, Gérard, Ménez et al. 2013, Bundeleva, Shirokova et al. 2014, Han, Yan et al. 2014, Saghaï, Zivanovic et al. 2015, Zhu and Dittrich 2016, Decho and Gutierrez 2017, Payandi-Rolland, Roche et al. 2019). Estos trabajos han descrito diferencias y heterogeneidad en la taxonomía y funcionalidad del componente orgánico. Una generalidad entre microbialitas es que a largo plazo, prevalecen los metabolismos de mineralización sobre metabolismos de degradación y heterotrofía (**Figura 5**), sin embargo estos metabolismos de mineralización y degradación están acoplados y pueden variar en temporadas estacionales y ciclos de día-noche (Dupraz, Reid et al. 2011).

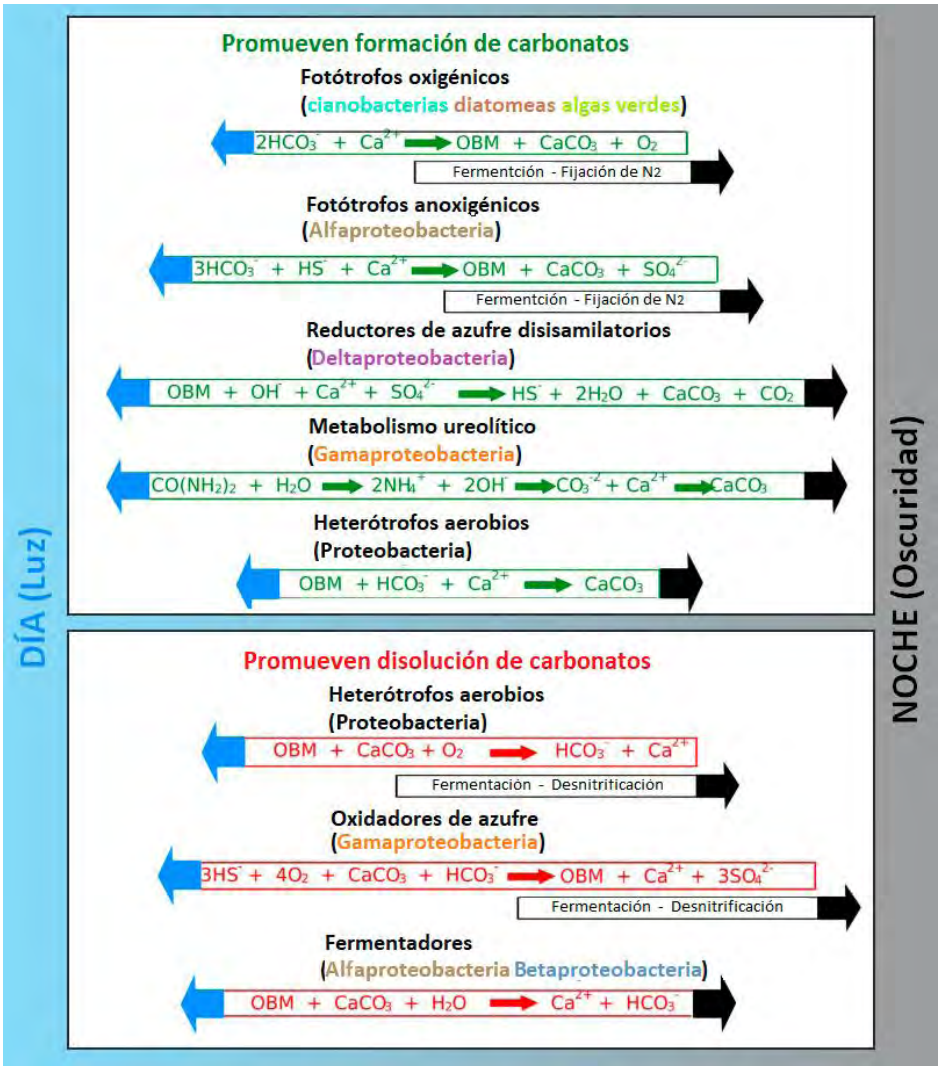


***Figura 5.*** *Principales metabolismos asociados a la formación y degradación de microbialitas y su variación circadiana. Modificado de (Dupraz, Reid et al. 2011).*

La mayoría de los trabajos concuerda que la formación de microbialitas se da por la sinergia y el acoplamiento de metabolismos microbianos, tanto de degradación como de precipitación mineral, siendo la génesis de microbialitas una propiedad emergente de una red de interacción metabólica compleja (Mobberley, Khodadad et al. 2015). Aun así, se ha postulado que existen ciertas especies o grupos calve para la formación de microbialitas, ya que, sin estas especies no podría establecerse ni construirse microbialitas en primer lugar (Chagas, Webb et al. 2016). Estos grupos incluyen a las cianobacterias, las bacterias fotosintéticas anoxigénicas, bacterias heterotróficas degradadoras de EPS, principalmente Alfaproteobacteria junto con bacterias del ciclo del azufre como Deltaproteobacteria (Riding 2012, Mobberley, Khodadad et al. 2013, Chagas, Webb et al. 2016, Kanik, Munro-Ehrlich et al. 2020).

Las cianobacterias son clave en la formación de microbialitas debido a dos procesos: 1) La fotosíntesis, que conduce al balance de alcalinidad hacia la precipitación de carbonatos, consumiendo bicarbonatos y aumentando el pH localmente (Dupraz, Reid et al. 2009) y 2) La producción de materia orgánica y sustancias poliméricas extracelulares (por sus siglas en inglés, *Exo Polymeric Substances*) que sirven como sustrato a otras bacterias heterotróficas y que atrapa activamente material detrítico y sedimento, mismos que funcionan como centros de nucleación mineral, en un fenómeno conocido como precipitación extracelular. Se han reportado también cianobacterias capaces de precipitar minerales intracelularmente (Moreira, Tavera et al. 2017) y cianobacterias que precipitan extracelularmente sin necesidad de un EPS abundante (Saghaï, Zivanovic et al. 2015) (**Figura 6**). El EPS se ha propuesto como sitio de nucleación de minerales carbonatados asociados a virus (White III, Chan et al. 2016).

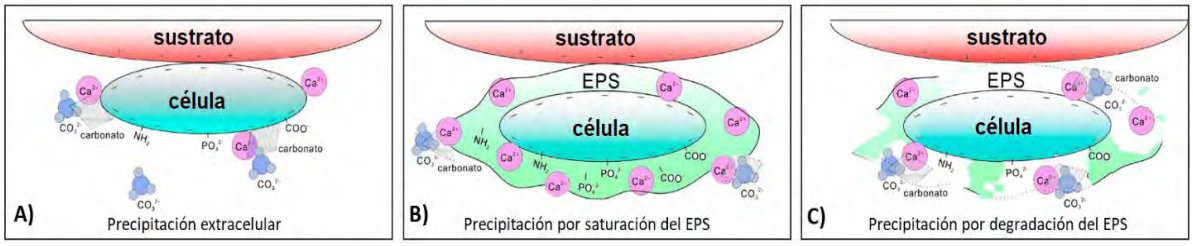


***Figura 6.*** *Mecanismos para la precipitación de carbonatos A) Precipitación directamente en la célula sin formación de un EPS B) Precipitación por saturación de iones en la fracción EPS. C) Precipitación por degradación de EPS que atrapo iones previamente, estos se liberan y precipitan extracelularmente. Modificado de (Zhu and Dittrich 2016).*

Las bacterias reductoras del azufre (SRB) (las *Desulfobacterales, Desulfovibrionales*, y *Syntrophobacterales*) también están reportadas como importantes formadoras de microbialitas ya

que su metabolismo favorece la precipitación de carbonatos por aumento en la alcalinidad, además de que algunas son degradadoras de EPS**,** fenómeno que puede saturar los microambientes con cationes y favorecer la precipitación.

En general las Proteobacterias se han relacionado con una amplia variedad de metabolismos que producen precipitación de carbonatos, incluyendo a las Alfaproteobacteria y en específico, las bacterias verdes no del azufre son relevantes. Algunos estudios remarcan la importancia de Alfaproteobacteria al asociarse con cianobacterias y provocar una precipitación extracelular y crecimiento por laminación (Gérard, De Goeyse et al. 2018). También algunas Gamaproteobacterias (bacterias púrpuras del azufre) que realizan fotosíntesis anoxigénica y que llevan a cabo la ureólisis, pueden elevar el pH localmente y facilitar una precipitación extracelular pasiva. Se han descrito algunos grupos de bacterias que tienen la capacidad de cementar o biocementar, es decir rellenar estructuras porosas de la matriz del sedimento o tapete no consolidado. La biocementación está bien documentada en bacterias como *Bacillus alkalinitrilicus*, una bacteria del suelo resistente a sistemas alcalinos, que al ser mezclada con lactato de calcio mejora la capacidad de autocuración del hormigón. La bacteria consume el oxígeno atrapado en la matriz del hormigón y rellena las microporosidades con carbonatos que precipita (Schwantes-Cezario, Peres et al. 2018).

Otros organismos que suelen estar presentes en microbialitas son Planctomycetes, Verrucomicrobia, Firmicutes, Chlorobi, Bacteriodetes, y en menor proporción Arqueas metanógenas. Además de los procariontes fotosintéticos, es común encontrar clorofitas, algas verdes, diatomeas, y en algunos casos algas rojas, las cuales pueden fungir como productores primarios. Los virus también han sido reportados como importantes factores para la precipitación, ya que provocan la muerte celular de los productores de EPS en los tapetes microbianos y seleccionan a las comunidades de microbialitas (White III, Chan et al. 2016).

Para saber cuál de todos los microorganismos aporta de manera más significativa a la precipitación mineral, se deben conocer los metabolismos presentes y en qué momento se activan, ya que sus productos y metabolitos interactúan con el ambiente y modifican directamente la fisicoquímica del agua. Pocos trabajos han podido realizar meta transcriptómica *in vivo*, la mayoría se ha enfocado en trabajos *ex vivo* (Mobberley, Khodadad et al. 2015, Louyakis, Gourlé et al. 2018). También se han descrito rutas metabólicas que por sí solas promueven la formación de carbonatos y precipitación de carbonatos, estas incluyen la fotosíntesis oxigénica, fotosíntesis anoxigénica, la ureólisis, la

desnitrificación, la amonificación, la reducción de azufre y la oxidación de metano (Zhu and Dittrich 2016).

### 1.3.2 Factores abióticos que afectan la formación de microbialitas

Varios trabajos han tratado de entender la formación de microbialitas comparando las características físicas, químicas y mineralógicas en diferentes regiones (Riding 1991, Reid, Visscher et al. 2000, Camoin and Gautret 2006, Riding 2006, Dupraz, Reid et al. 2009, Woo and Chough 2010, Johnson, Ledesma-Vázquez et al. 2012, Riding 2012, Chagas, Webb et al. 2016, Gérard, De Goeyse et al. 2018, Valdespino-Castillo, Hu et al. 2018, Eymard, Bilmes et al. 2019, Büttner, Isemonger et al. 2021). También se ha encontrado una heterogeneidad en los factores abióticos que influencían la formación de microbialitas. A continuación, se enlistan los más comunes:

1) Alto índice de saturación SI (por sus siglas en inglés: *Saturation Index*) que es la concentración de iones en el medio acuoso. El aumento en el SI puede ocurrir cuando hay fenómenos de surgencia de aguas profundas, mezcla en la columna de agua y florecimientos de fitoplancton asociados a fenómenos de blanqueamiento. Muchas microbialitas se han descrito en condiciones hipersalinas o con alta concentración de carbonatos y sulfatos (Dupraz, Reid et al. 2011, Chagas, Webb et al. 2016). Los altos SI provocan que los minerales y carbonatos precipitados no pueden disolverse y por tanto favorece la mineralización abiótica (Dupraz, Reid et al. 2011).

2) Un pH en un rango de 7 a 10, junto con un alto SI provocan precipitación de minerales en microbialitas carbonatadas (Kempe, Kazmierczak et al. 1991, Last, Last et al. 2010, Couradeau, Benzerara et al. 2011, Chan, Bugler-Lacap et al. 2014). En esta tesis se excluyen a las microbialitas descritas en sistemas ácidos (pH de 2.5 a 4) y sistemas silíceos (Chacon-Baca, Santos et al. 2021) ya que aun se desconoce si sus procesos de formación están más dados por una bioturbación que por una bioformación.

3) Baja disponibilidad de nutrientes como el nitrógeno y fósforo (Souza, Moreno-Letelier et al. 2018, Büttner, Isemonger et al. 2021). Esta baja disponibilidad también se asocia a condiciones de oligotrofia extrema para microorganismos forrajeadores, mismos que inhiben el crecimiento de las microbialitas.

4) Alto flujo de agua subterránea ha sido remarcado en su importancia para la formación de estructuras como trombolitos (Warden, Coshell et al. 2019).

5) Intensidad lumínica, debe ser especifica ya que un exceso de radiación puede destruir a las comunidades fotosintéticas o una falta de luz limita el crecimiento de las mismas (Paterson, Aspden et al. 2008, Russell, Brady et al. 2014, Águila, Yanez-Montalvo et al. 2022).

6) La cementación, que es el proceso de llenado de poros de un sedimento, en donde las partículas precipitadas quedan unidas al sedimento y los poros son rellenados por minerales. Los procesos de cementación abiótica están documentados para depósitos de manantiales termales compuestos típicamente de sílice amorfa (sinterizados) (Berelson, Corsetti et al. 2011), sistemas ricos en azufre y para algunas microbialitas marinas (travertinos) y de cuevas (Planavsky and Ginsburg 2009).

7) La compactación, que es la perdida de porosidad de un sedimento debido a la presión y carga que sufre una capa de sedimento, a medida que más capas de sedimento se depositan sobre un sedimento base, este se va compactando. Es un fenómeno meramente físico de presión y fuerza gravitatoria ejercida sobre el sedimento (Gomez, Anderson et al. 2014).

8) Evaporación y desgasificación, mismos que provocan precipitación inorgánica y forman minerales sin rastro orgánico, por ejemplo la formación de estalactitas y estalagmitas (Bąbel 2004). Algunas microbialitas en cuerpos de agua epicontinentales sufren procesos de desecación y evaporación, lo que cambia su composición mineral (**Figura 7**).


***Figura 7.*** *Principales factores fisicoquímicos que afectan la formación de microbialitas modernas.*

Así mismo, las microbialitas pueden exhibir diferentes grados de porosidad, solidificación o dureza, en función del tiempo de litificación y/o composición mineral (Knoll, Fairchild et al. 1993, Reid, Visscher et al. 2000, Dupraz and Visscher 2005). Por ejemplo, dentro de una misma microbialita coexisten estratos con diferentes grados de dureza, siendo el estrato interno el más duro (el más viejo), mientras que el estrato exterior es el más suave (el más joven), pudiendo ser un tapete en formación, sin consolidar ni mineralizar. Sin embargo, la dureza no siempre es un indicador del tiempo

de litificación, ya que también está dada por el tipo de mineral asociado, siendo más duras o solidas las microbialitas con una composición fosfática, magnesiana o con silicatos en lugar de carbonatos, ya que estas estructuras minerales son menos porosas y mas densas (Valdespino-Castillo, Hu et al. 2018). Contrario a fenómenos abióticos que forman microbialitas, también existen factores abióticos que promueven la degradación o destrucción de las mismas, como son el intemperismo, la desmineralización, asociada a la acidificación del ambiente (Yanez-Montalvo, Águila et al. 2019).

## 1.4 Evolución de microbialitas

Se estima que las microbialitas son una de las primeras evidencias fósiles de vida en la tierra, (Awramik 1971, Dodd, Papineau et al. 2017). Estas estructuras tuvieron un papel importante en la evolución de la atmósfera terrestre, siendo nichos ancestrales en donde surgieron las primeras comunidades microbianas (Riding 2011). Estas comunidades microbianas y sus metabolismos cambiaron paulatinamente los flujos biogeoquímicos de Fe, S, P, carbono orgánico y N (Visscher, Hoeft et al. 2002, Camoin and Gautret 2006). Las microbialitas también cambiaron en morfología y estructura, (estromatolitos, trombolitos y dendrolitos) (Riding 2011). El cambio en la abundancia de las poblaciones de microbialitas se puede ver a través del registro fósil (**Figura 8**). A continuación, se mencionan algunos de los eventos geológicos más relevantes para las microbialitas según su era.

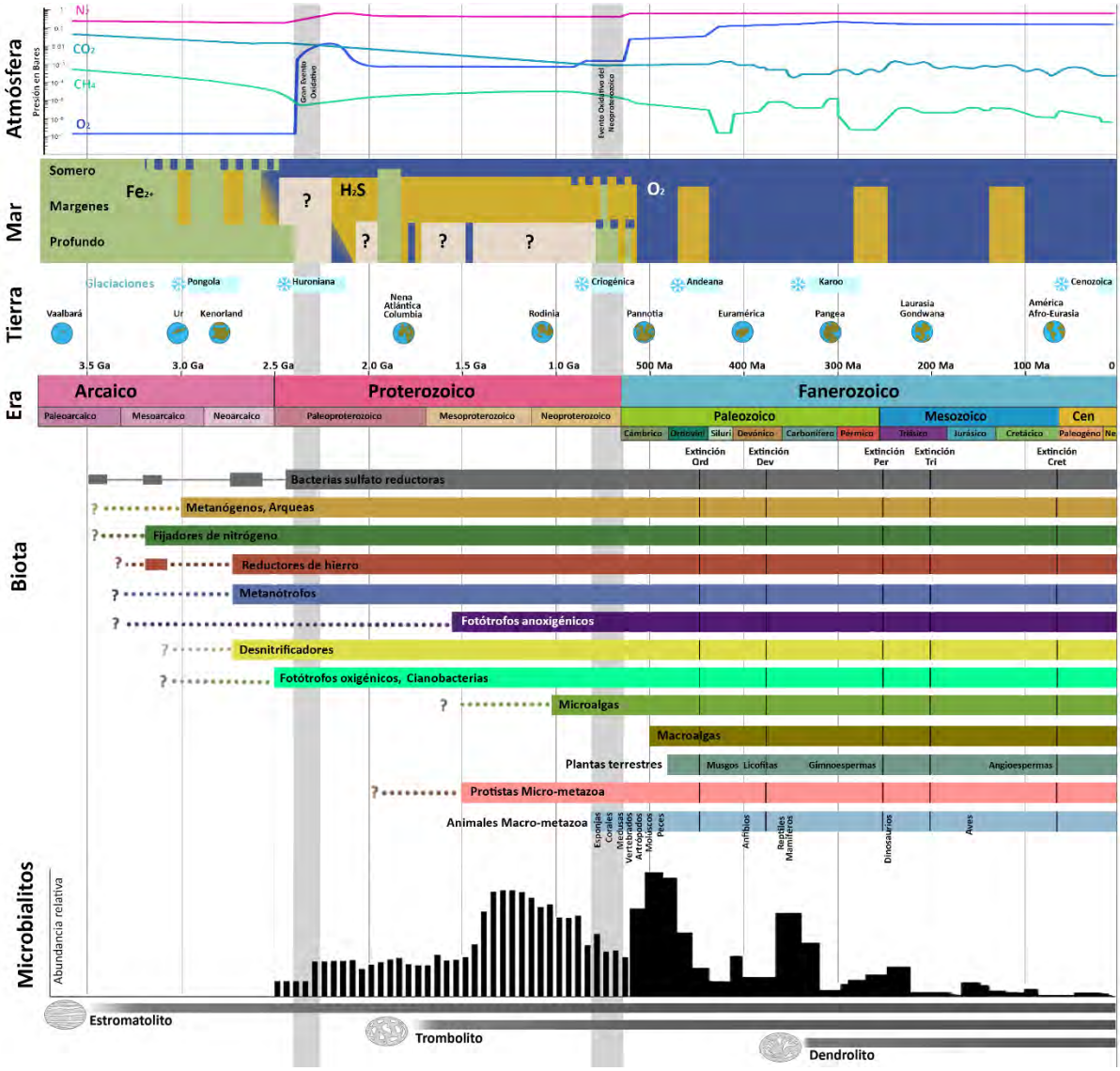


***Figura 8.*** *Abundancias relativas de microbialitas (abajo) comparado contra los cambios atmosféricos, en mares, formaciones continentales, glaciaciones y aparición de metabolismos más importantes en el registro geológico (arriba), modificado de (Riding 2011, Djokic, Van Kranendonk et al. 2017, Catling and Zahnle 2020, Lepot 2020).*

### 1.4.1 Microbialitas del Arcaico

Existe una dificultad inherente en datar y dilucidar si las estructuras de microbialita más tempranas del Arcaico fueron dadas por procesos de diagénesis/bioturbaciones más que por efectos de biogénesis/bioformaciones. Lo mismo para datar el orden cronológico de la aparición de los primeros metabolismos microbianos. Las últimas revisiones (Lepot 2020) sugieren que la evidencia fósil de vida más antigua son microbialitas de pirita con un patrón de isotopos de azufre, indicador indirecto de reducción de azufre microbiana hace 3.48 Ga, en la formación de Dresser, Australia que postula un

modelo de caldera volcánica. Sin embargo, también se sugieren modelos de microbialitas en geiseres y pozas hidrotermales hace 3.5 Ga en la misma formación (Djokic, Van Kranendonk et al. 2017). La metanogénesis microbiana se ha datado por reloj molecular (Wolfe and Fournier 2018) y depleción de $C^{13}$ en rocas sedimentarias kerogénicas entre un rango de 3.5 y 3 Ga (Stüeken and Buick 2018), pero debido a la posible influencia hidrotermal abiótica que estas rocas pudieron tener, no se ha llegado a un acuerdo. La formación de las bandas oxidadas de hierro también han sido un indicador indirecto de la aparición de fotosíntesis anoxigénica y oxigénica datadas entre 3.46 y 3.77 Ga (Sim, Liang et al. 2012, Rego, Busigny et al. 2021). El acuerdo general de las revisiones, es que la aparición de metabolismos microbianos coincide con el incremento de formación de corteza continental en el Neoarcaico, lo que se asocia a un incremento en la diversidad de minerales y compuestos químicos disponibles para los microorganismos (Lepot 2020).

Aunque se estima que existieron pequeños episodios de producción de oxígeno durante el Mesorcaico (oasis oxigénicos), estos no fueron suficientes para modificar químicamente los mares hasta el Proterozoico (Catling and Zahnle 2020). Se especula que las bajas concentraciones de fósforo biodisponible en mares suprimió el crecimiento y la expansión de la fotosíntesis durante mayor parte del Proto arcaico y Meso arcaico (Kipp and Stüeken 2017), hasta el Neoarcaico cuando la actividad volcánica introdujo más compuestos inorgánicos como el $H_2$, $CO_2$, $CH_4$, $SO_2$ y $H_2S$ que promovieron la aparición de nuevos metabolismos (Lepot 2020), como reducción de hierro, hidrogenotrofía, acetotrofía o metanogénesis (Mole, Fiorentini et al. 2014, Planavsky, Asael et al. 2014, Olson and Straub 2016).

Se han documentado apenas 48 ejemplos de estromatolitos, en su mayoría de finales del Arcaico, compuestos por depósitos de carbonatos, láminas de pirita, hematita e hidróxido de hierro, asociados a calderas, ventilas hidrotermales, sinterizados silíceos geiseres y pozas hidrotermales (Schopf 2006, Schopf, Kudryavtsev et al. 2007, Flannery and Walter 2012, Djokic, Van Kranendonk et al. 2017, Dodd, Papineau et al. 2017).

Una dato interesante es que la morfología estromatolitica fue la única macro morfología de microbialitas durante el Arcaico (Riding 2011), lo que podría indicar un mayor papel de los procesos de diagénesis y fenómenos abióticos sobre su bioformación (Dupraz, Reid et al. 2011). Al final del Arcaico, estos estromatolitos estaban construidos principalmente por fotosintetizadores anoxigénicos que usaban $H_2$ y $H_2S$ como donador de electrones (Olson 2006) en las costas del supercontintnete Keroland.

### 1.4.2 Microbialitas del Proterozoico

Para inicios de Proterozoico, el aumento gradual de $O_2$ producido por cianobacterias, conocido como Gran Evento Oxidativo (GEO) (Gumsley, Chamberlain et al. 2017) comenzó a ocurrir entre aproximadamente 2-2.4 Ga. El GEO llevó a la formación de la capa de ozono, la disminución del metano en la atmósfera y el consecuente enfriamiento de la tierra, lo que causó una glaciación global en el Huroniano, un evento que cubrió gran parte de la tierra en una capa de hielo de 2.5 Ga hasta hace 2.2 Ga (Kopp, Kirschvink et al. 2005) y frenó temporalmente el crecimiento de microorganismos.

Tras el deshielo en el Mesoproterozoico por actividad volcánica, se empezaban a formar los continentes Atlantica Columbia y Nena, lo que facilitó la formación de microbialitas en sus márgenes, (Sarkar, Eriksson et al. 2004), sus abundancias dependían del nivel del mar, nutrientes disueltos e intensidad lumínica (Stüeken, Catling et al. 2012). Las microbialitas ya eran productoras eficientes de materia orgánica, y estaban formadas por carbonatos, láminas de calcita y dolomita. También ya podían distinguirse distintas morfologías como estromatolitos columnares, en conos y domos. La formación de la capa de ozono permitió a microorganismos y tapetes microbianos comenzar a colonizar regiones continentales (Cockell and Raven 2007). También en el Mesoproterozoico aparecen las primeras microbialitas con morfologías trombolíticas, hace 1.9 Ga (Riding 2011). Para entonces los trombolitos y estromatolitos del Proterozoico estaban construidos esencialmente por cianobacterias (Riding 2006). Las microbialitas tuvieron su máximo apogeo a finales del Mesoproterozoico hace 1.3 Ga, cuando el continente Columbia se separó formando Rodinia y varios sistemas insulares, creando un área costera mayor y donde grandes cantidades de nutrientes ingresaron los océanos (Wang, Bolhar et al. 2018). Se especula que este gran florecimiento y distribución de nutrientes en el mar, también favoreció la evolución de vida libre- planctónica y de organismos con metabolismos aerobios y nitrificantes (Chen and Strous 2013, Michiels, Darchambeau et al. 2017). Debido la competencia fótica entre los organismos fotosintéticos planctónicos y bentónicos, aunado a la aparición de Eucariontes fotótrofos, las poblaciones de microbialitas volvieron a declinar drásticamente durante el evento oxidativo del Neo Proterozoico (0.85-0.54 Ga ) y durante la glaciación del Criogénico (Rose, Maloof et al. 2013, van Smeerdijk Hood and Wallace 2015).

### 1.4.3 Microbialitas del Fanerozoico

La vida multicelular y eucarionte radió al terminar la glaciación del Criogénico a inicios del Fanerozoico, teniendo su máxima expresión durante la explosión del Cámbrico (Marshall 2006). Existe una recuperación en las comunidades de microbialitas durante el Fanerozoico (Riding 2012). Sin embargo, en el Cámbrico, de nuevo hay un decrecimiento de microbialitas, el cual se especula fue debido a la aparición de organismos heterotróficos forrajeadores, como foraminíferos y protozoos (Awramik 1971, Pratt 1982, Riding 2012). También se sugiere que aumentó la competencia por espacio lumínico en el bentos, ya que aparecen los primeros animales fotosintéticos bentónicos con mayor tasa de acreción y crecimiento por biomineralización, como esponjas y corales (Zamagni, Košir et al. 2009). A finales del Fanerozoico las poblaciones de microbialitas tuvieron pequeños picos de recuperación asociadas a las extinciones masivas de eucariontes. Por esto, las microbialitas del Fanerozoico medio y tardío se consideran proxis importantes al funcionar como "formas de desastre" ya que describen con precisión las divisiones geológicas (Mata and Bottjer 2012). Las microbialitas del Fanerozoico son las estructuras más diversas morfológicamente, se observan fábricas fenestrales (estructuras porosas), estructuras finamente laminadas, estructuras híbridas y son los primeros con estructura granular gruesa y dendrolitica. La dificultad de estudiar sistemas sedimentarios como microbialitas fósiles, radica en identificar el componente orgánico destruido por fenómenos de diagénesis. En algunos fósiles aún se discute si las formaciones sedimentarias fueron consecuencia de fenómenos meramente abióticos, que después sufrieron bioturbación (Lepland, van Zuilen et al. 2005).

### 1.4.4 Microbialitas modernas

Las microbialitas modernas son metabólicamente activas, se formaron en el Cenozoico tardío durante el Holoceno y Pleistoceno (Kempe, Kazmierczak et al. 1991, Gischler, Gibson et al. 2008, Chagas, Webb et al. 2016, Newell, Jensen et al. 2017, Eymard, Bilmes et al. 2019). La complejidad de definir a las microbialitas modernas es la misma que a las fósiles, respecto a que exhiben una composición mineral y de microorganismos heterogénea. Mas aún, existen microbialitas modernas coexistiendo geográficamente con microbialitas fósiles, ya que sus formaciones se han dado sobre cratones o roca madre del Precámbrico (Proterozoico y Arcaico). Este es el caso para microbialitas de Australia, sur de África, Norte América, la cuenca de Brasil, parte de China y el norte de Europa. El tipo de cratón o roca madre también ayuda a diferenciar a las microbialitas por su tipo de cuenca o provincia geológica asociada (**Figura 9**).

Aunque las convergencias estructurales entre microbialitas modernas y fósiles son evidentes a simple vista, funcionalmente no existen análogos modernos a las estructuras de microbialitas del Arcaico y Proterozoico temprano, ya que los metabolismos aeróbicos y taxones recientes (evolutivamente), no existían en microbialitas antiguas (Myshrall, Dupraz et al. 2014). Se estima que los homólogos modernos más parecidos a las microbialitas fósiles, podrían ser aquellos asociados a ventilas hidrotermales para el Proterozoico y lagos hipersalinos alcalinos para las del Fanerozoico (Laval, Cady et al. 2000, Dupraz, Reid et al. 2011). También, se ha postulado que las microbialitas han funcionado como centros de origen de diversidad (hotspots), y refugios de microorganismos durante eventos de catástrofe ambiental, haciendo de las microbialitas modernas una de las estructuras más complejas a nivel metabólico y funcional que se conocen en la actualidad (White III, Chan et al. 2016). Se han reportado que sus metabolismos pueden estar haciendo sintrofía y sus comunidades microbianas tienen efectos de mutualismo obligado y efectos de rey rojo o de reina negra, complementándose metabólicamente para llevar acabo sus funciones (Souza, Moreno-Letelier et al. 2018).

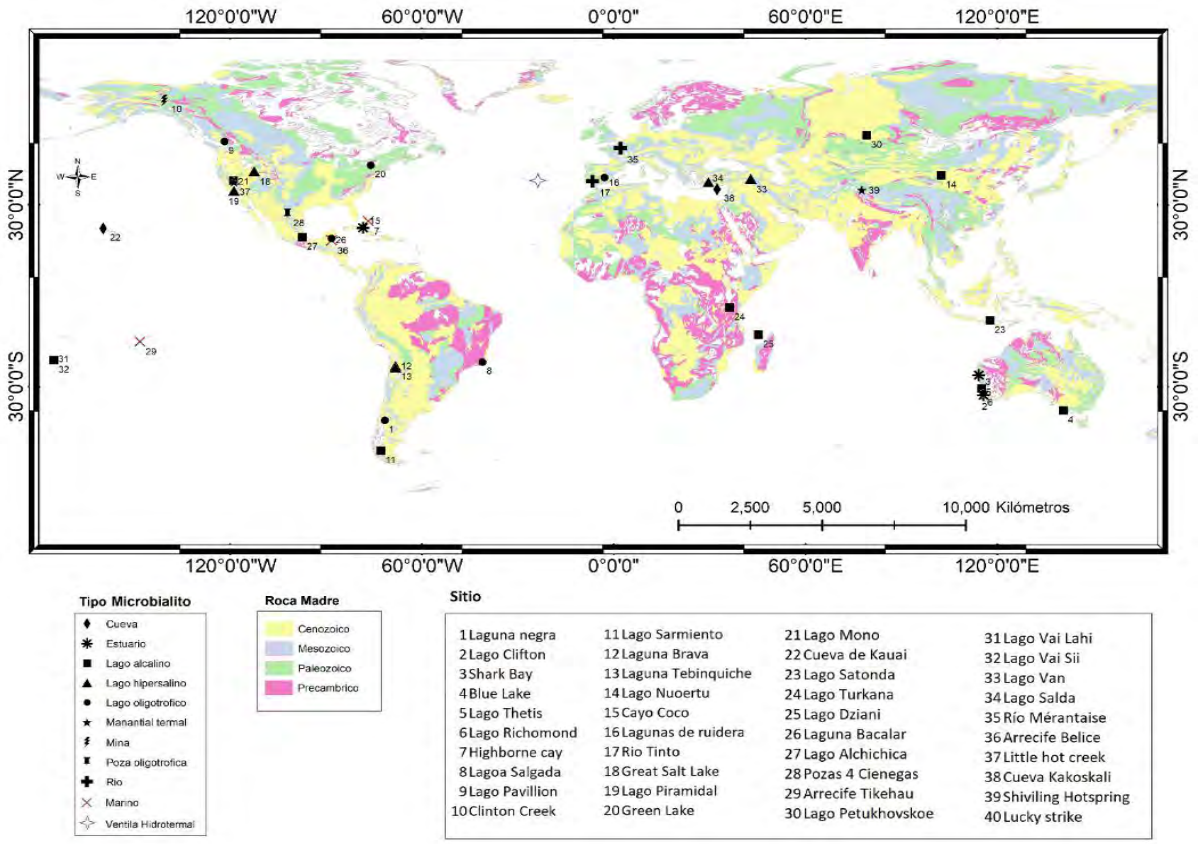


***Figura 9.*** *Mapa de distribución de microbialitas modernas asociados a su provincia geológica (Roca madre).*

## 1.5 Distribución de las microbialitas modernas

Las microbialitas modernas se encuentran confinadas en zonas donde la actividad heterotrófica es baja, generalmente en microambientes con condiciones de hipersalinidad, oligotrofía extrema (en especial N y P), altas o bajas temperaturas, altos o bajos niveles de pH (de 7 a 10 o de 2 a 5), o con condiciones extremas para microorganismos eucariontes forrajeadores (Valdespino-Castillo, Hu et al. 2018). Diversos trabajos han descrito microbialitas modernas en diferentes sistemas del mundo (**Tabla 1**). Por sus ubicación geográfica y características ambientales se dividen en tres grandes tipos: microbialitas profundas (asociados a ventilas hidrotermales), microbialitas marinas (asociados a sistemas costeros y con intrusión salina), y microbialitas de aguas continentales. Las microbialitas de aguas continentales se subdividen en estuarios, lagos hipersalinos, lagos alcalinos, lagos oligotróficos, manantiales/pozos termales y pozas oligotróficas (Foster et al., 2019). Se han descrito en menor medida, en ríos, cuevas, minas ácidas, sistemas de sinterizados silíceos (geiserita), sistemas ricos en sulfato de calcio (yeso) y sistemas con fosfatos de calcio.

**Tabla 1** Principales localidades donde se han reportado microbialitas modernas (datadas máximo en 12 mil años). En negritas sistemas que se estudiaron en este trabajo.

| País | Localidad | Sistema | Referencia |
|---|---|---|---|
| Argentina | Laguna negra | Laguna dulce | (Boidi, Mlewski et al. 2020) |
| Australia | Lake Clifton | Estuario | (Burne, Moore et al. 2014) |
| Australia | Shark Bay | Estuario | (Burns, Goh et al. 2004) |
| Australia | Lake Thetis | Lago alcalino | (Reitner, Paul et al. 1996) |
| Australia | Lake Walyungup | Lago costero | (Coshell, Rosen et al. 1998) |
| Bahamas | Highborne cay | Marino | (Reid, Macintyre et al. 1999) |
| Brasil | Laguna Vermelha | Laguna hipersalina | (Keim, dos Santos et al. 2020) |
| Brasil | Lagoa Salgada | Laguna | (da Silva, Mansur et al. 2019) |
| Canadá | Lago Pavillion | Lago agua dulce | (Russell, Brady et al. 2014) |
| Canadá | Clinton Creek | Mina abandonada | (White III 2014) |
| Canada | Manito Lake | Lago alcalino | (Last, Last et al. 2010) |
| Chile | Lago Sarmiento | Lago alcalino | (Graham, Knack et al. 2014) |
| Chile | Laguna Brava | Laguna hipersalina | (Rasuk, Visscher et al. 2020) |
| Chile | Laguna Tebenquiche | Laguna hipersalina | (Farias, Contreras et al. 2014) |
| China | Lake Nuoertu | Lago alcalino | (Arp, Hofmann et al. 1998) |
| Cuba | Cayo Coco | Lago costero | (Bouton, Vennin et al. 2016) |
| España | Lagunas de ruidera | Laguna dulce | (Santos, Pena et al. 2010) |
| España | Rio Tinto | Mina abandonada | (Chacon-Baca, Santos et al. 2021) |
| Estados Unidos | Great Salt Lake | Lago salino | (Lindsay, Dunham et al. 2020) |
| Estados Unidos | Lago Piramidal | Lago hipersalino | (Arp, Thiel et al. 1999) |
| Estados Unidos | Fayetteville Green Lake | Lago agua dulce | (Uveges, Teece et al. 2018) |
| Estados Unidos | Mono Lake | Lago alcalino | (Souza-Egipsy, Wierzchos et al. 2005) |
| Hawái | Cueva isla kauai | Cueva basáltica | (Léveillé, Fyfe et al. 2000) |
| Indonesia | Lago Satonda | Lago cráter | (Arp, Reimer et al. 2003) |
| Kenia | Lago Bogoria | Lago alcalino | (McCall 2010) |
| Mayotte | Lago Dziani | Lago cráter | (Gérard, De Goeyse et al. 2018) |
| **México** | **Laguna Bacalar** | **Laguna dulce** | **(Gischler, Gibson et al. 2008)** |
| **México** | **Lago Alchichica** | **Lago cráter** | **(Kazmierczak, Kempe et al. 2008)** |
| **México** | **Cenote Azul** | **Poza oligotrófica** | **(Yanez-Montalvo, Águila et al. 2021)** |
| México | Cuatro Ciénegas | Pozas alcalinas | (Souza, Siefert et al. 2012) |
| Polinesias fanc | Arrecife Tikehau | Marino | (Abed, Golubic et al. 2003) |
| Rusia | Lago Petukhovskoe | Lago hipersalino | (Samylina and Zaytseva 2019) |
| Tonga | Lago Vai Lahi y Vai Sii | Lago cráter | (Kremer, Kazmierczak et al. 2012) |
| Turquía | Lago Van | Lago salino | (Kempe, Kazmierczak et al. 1991) |
| Turquía | Lago Salda | Lago cráter | (Russell, Ingham et al. 1999) |

## 1.6 Sitios de estudio

### 1.6.1 Lago cráter Alchichica

El lago cráter de Alchichica (AL) es un lago salino alcalino que contiene microbialitas, se encuentra en el Eje Neovolcánico Transversal, en la Cuenca Oriental de Puebla (19° 24' N, 97° 24' O, 2300 m s.n.m) en México (**Figura 10**). La columna de agua es alcalina (pH 8.7-9.2) y salina (conductividad eléctrica 13 dS $m^{-1}$). Los aniones dominantes son los cloruros y bicarbonatos; mientras que los cationes dominantes son el sodio, magnesio, potasio y calcio (Alcocer and Lugo 2003, Armienta, Vilaclara et al. 2008, Mancilla Villa, Bautista Olivas et al. 2014). El lago es monomíctico presentando un periodo de mezcla en invierno y estratificación la mayor parte del año. Las microbialitas se han descrito en toda la periferia del lago hasta 30 m de profundidad (Águila, Alcántara-Hernández et al. 2021). Este sistema lacustre ha sido objeto de estudios hidrológicos, geológicos y de ecología microbiana (Tavera and Komárek 1996, Falcón, Escobar-Briones et al. 2002, Couradeau, Benzerara et al. 2011, Kaźmierczak, Kempe et al. 2011, Beltrán, Centeno et al. 2012, Centeno, Mejía et al. 2016, Alcántara-Hernández, Valdespino-Castillo et al. 2017, Valdespino-Castillo, Águila et al. 2022).

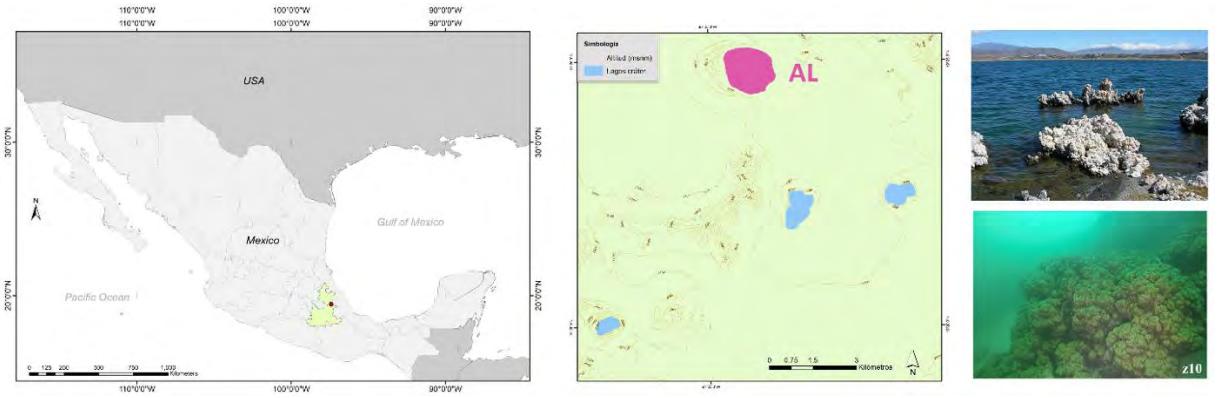


***Figura 10.*** *El lago cráter Alchichica tiene 1 km de diámetro y alcanza los 60m de profundidad, donde se han encontrado microbialitas modernas hasta los 30 m de profundidad, datadas entre los 1200 y 2000 mil años. Alchichica es parte de un sistema de seis lagos cráter en el estado de Puebla, México.*

**Tabla 2.** Valores fisicoquímicos para el lago cráter Alchichica

| Sistema | pH | Conductividad | $Cl^-$ | $SO_4^{2-}$ | NOx | P(soluble) | Mg | Ca | Na | K | $NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alchichica | 9.55 | 13 | 3094.785 | 803.5419 | 6.04 | 1.6 | 432.7 | 14.72 | 2311.5 | 207.48 | 2.23 |

## 1.6.2 Laguna Bacalar y Cenote Azul

La laguna Bacalar (BL) es una laguna de agua dulce rica en sulfatos ubicada en Quintana Roo, México (18°40’27’’N 88°22’57’’W) (**Figura 11**). Formada por un sistema de fallas geológicas cretácicas, con 40km de longitud, y profundidad promedio de 20 metros, es parte del sistema kárstico de la península de Yucatán. Su columna de agua es alcalina (3.9 meq/L). A lo largo de la laguna se encuentran el arrecife de microbialitas de agua dulce más extenso del mundo, los cuales son trombolitos ricos en carbonato de calcio. Existen algunos trabajos descriptivos (Gischler, Gibson et al. 2008, Johnson, Beddows et al. 2018, Valdespino-Castillo, Hu et al. 2018). Se estima que su formación se debe principalmente a cianobacterias filamentosas, bacterias reductoras del azufre y diatomeas. A 100 metros de la laguna se encuentra el Cenote Azul (CA) un cenote con más de 70 m de profundidad donde también se han reportado microbialitas de 5 a 30 m de profundidad (Yanez-Montalvo, Aguila et al. 2022). En su lado oeste, las condiciones fisicoquímicas e hidrológicas son similares a Laguna Bacalar con la excepción de las condiciones lumínicas, menor fósforo soluble y tener una edad de formación anterior a la laguna. CA se formó antes del cuaternario mientras que BL está cubierto por material eyectado por el cráter Chicxulub (Perry, Velazquez-Oliman et al. 2002, Perry, Paytan et al. 2009).

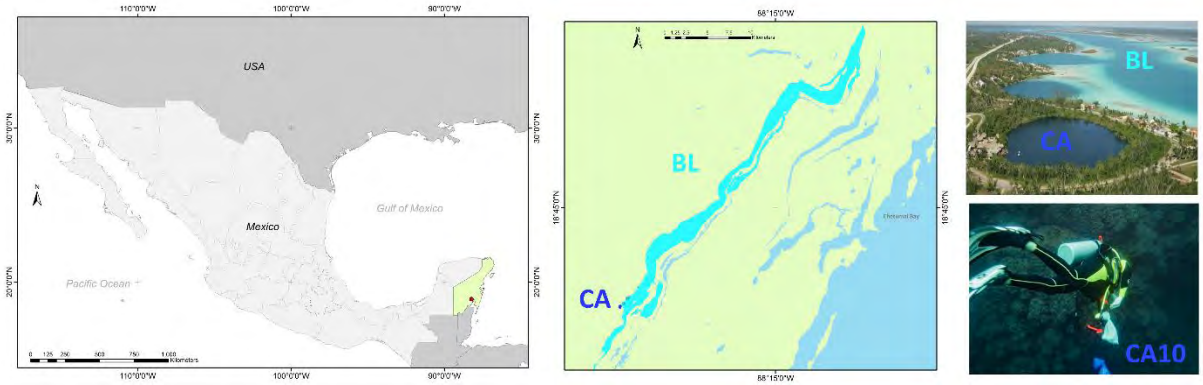


***Figura 11.*** *La laguna de Bacalar tiene un flujo de agua subterránea del norte al centro y de sur al centro saliendo su corriente con dirección a la costa de Chetumal, sus microbialitas están datadas entre 2 y 9.5 mil años. CA está al sur del poblado Bacalar, tiene microbialitas hasta los 30 m de profundidad, aún no están datados pero su origen geológico se calcula anterior a los de BL.*

**Tabla 3.** Valores fisicoquímicos para laguna Bacalar y el Cenote Azul.

| Sistema | pH | Conductividad | $Cl^-$ | $SO_4^{2-}$ | NOx | P(soluble) | Mg | Ca | Na | K | $NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Laguna Bacalar | 7.2 | 2.786 | 43.6 | 1139.3 | 7.03 | 0.0857 | 88.8 | 310 | 108.38 | 7.63 | 3.626 |
| Cenote Azul | 8 | 2.31 | 62.125 | 1234.62 | 7.0325 | 0.001 | 86.2 | 424.075 | 33.25 | 3.8 | 0.001 |

# 2. OBJETIVOS

## Objetivo general

Analizar y comparar la composición taxonómica y el perfil funcional de cianobacterias asociadas a microbialitas del lago cráter Alchichica y del sistema Laguna Bacalar (incluyendo al Cenote Azul).

## Objetivos específicos:

1) Caracterizar y comparar mediante microscopía, las comunidades de cianobacterias asociadas a microbialitas en Alchichica, Bacalar y Cenote Azul, para clasificarlas con métodos morfométricos a nivel de género.

2) Caracterizar y comparar mediante técnicas de genómica dirigida (región hipervariable V4 del gen 16S ARNr) la composición y estructura de microbialitas en Alchichica, Bacalar y Cenote Azul, para definir si existen biorregiones y buscar patrones de estructura comunitaria.

3) Caracterizar y comparar mediante técnicas de metagenómica (Shotgun), los perfiles funcionales de cianobacterias en microbialitas de Alchichica, Bacalar y Cenote Azul, para buscar patrones y asociaciones funcionales dentro de los sistemas.

4) Establecer las condiciones óptimas de crecimiento para cianobacterias de microbialitas en Alchichica, Bacalar y Cenote Azul, para aislar y resguardar los cultivos en la colección del Laboratorio de Ficología y el Herbario del Instituto de Biología (UNAM) para futuros estudios bioquímicos y fisiológicos.

## 3. HIPÓTESIS

Ho: Las cianobacterias asociadas a microbialitas de los sistemas Alchichica, Bacalar y Cenote Azul, tendrán diferencias en su taxonomía y en su perfil funcional, debido a las diferencias fisicoquímicas y geológicas.

Ha: Las cianobacterias asociadas a microbialitas de los sistemas Alchichica, Bacalar y Cenote Azul, no tendrán diferencias en su taxonomía y en su perfil funcional, debido a las similitudes en mecanismos de formación e historia biogeográfica.

# 4. MÉTODOS Y TÉCNICAS

**1) Métodos de microscopía.**

Se identificaron mediante microscopía óptica, microscopía de fluorescencia y microscopía confocal láser, a las comunidades microbianas presentes en las muestras de microbialitas de los sistemas Alchichica (AL), Laguna Bacalar (BL) y Cenote Azul (CA) haciendo énfasis en las cianobacterias. (Águila, Alcántara-Hernández et al. 2021, Águila, Yanez-Montalvo et al. 2022).

**2) Métodos de cultivo.**

Se sembraron muestras frescas de microbialitas con agua pre filtrada del sistema correspondiente (AL, BL, CA) y medio BG-11(0) (Rippka, Deruelles et al. 1979). Paralelamente se probaron diferentes condiciones de luz temperatura y nutrientes. Los cultivos obtenidos fueron puestos a disposición del cepario del laboratorio de Ficología de la Facultad de Ciencias UNAM, México. Muestras semipermanentes de cultivos fueron agregados a la colección (PA-FCMA) del Herbario Nacional del Instituto de Biología UNAM.

**3) Métodos moleculares.**

- **Extracción de ácidos ribonucleicos (ADN):** Las extracciones de ADN se realizaron por quintuplicado por submuestra, juntando de tres a cinco submuestras (fragmentos de 0.25 g) por individuo (microbialita), por punto de muestreo, en cada sistema de estudio. Las extracciones se hicieron con el kit Power Soil (QIAGEN Inc.) y conforme a protocolos establecidos ((Centeno, Legendre et al. 2012, Yanez-Montalvo, Gómez-Acata et al. 2020, Águila, Yanez-Montalvo et al. 2022).
- **Amplificación del gen 16S ARNr:**

  Región V4 del gen 16S ARNr de bacterias y arqueas conforme (Carrillo-Araujo, Taş et al. 2015).

  Región V2-V5 del gen 16S ARNr de cianobacterias con los cebadores (Nübel, Garcia-Pichel et al. 1997).

  Fragmento de aprox. 1450 pb, del gen 16S ARNr de cianobacteria aisladas con los cebadores (Weisburg, Barns et al. 1991).
- **Secuenciación y análisis del gen 16S ARNr.**

  Las regiones V4 se secuenciaron mediante la plataforma Ilumina MiSeq y se analizaron las secuencias conforme (Caporaso, Lauber et al. 2012). Las regiones V2-V5 y fragmentos casi

completos del gen 16S ARNr de cianobacterias se clonaron y secuenciaron mediante técnica Sanger. Los análisis filogenéticos se hicieron en ARBSilva (Pruesse, Peplies et al. 2012). Los análisis estadísticos se llevaron a cabo en R Studio 3.6.2 conforme (Yanez-Montalvo, Gómez-Acata et al. 2020).

- **Secuenciación y análisis de metagenomas.** El DNA genómico sin amplificar se fragmentó en ~400 pb. para hacer una biblioteca genómica NEXTERA y se secuenció mediante la plataforma Ilumina NovaSeq 2x150. Las secuencias resultantes se ensamblaron en MEGAHIT v1.2.9 conforme los protocolos de (Águila, Yanez-Montalvo et al. 2022). Paralelamente se subieron las secuencias a MG-RAST para anotación en otras bases de datos (Meyer, Bagchi et al. 2019). Se usó STAMP v2.1.3. para la visualización de datos funcionales.

# 5. RESULTADOS

Como parte de los resultados de esta tesis, se publicaron: un artículo de divulgación, cinco artículos científicos indexados y dos capítulos de libro.

Artículos científicos indexados como primer autor:

1. **Águila B**, Alcántara-Hernández RJ, Montejano Zurita GA, López Martínez RA, Falcón LI, Absalón I. (2021). Cyanobacteria in microbialites of Alchichica crater lake: a polyphasic characterization. Europ. J. Phycology. https://doi.org/10.1080/09670262.2020.1853815
2. **Águila B**, Yanez-Montalvo A, Mercado-Juárez R. A, Montejano G.A, Becerra-Absalón I, Falcón L.I. (2022). Microbialites show distinct cyanobacterial phylogenetic structure and functional redundancy in Bacalar lagoon and Cenote Azul sinkhole, Yucatan peninsula, Mexico FEMS Microbiology Ecology. https://doi.org/10.1093/femsec/fiac039
3. **Águila B.**, Becerra-Absalón I., Montejano-Zurita G, Falcón L.I. (2022). Cyanobacteria in microbialites: A lithobiontic perspective. MDPI Microorganisms (aceptado).

## 5.3 Artículos científicos indexados

### 5.3.1 Cyanobacteria in microbialites of Alchichica Crater Lake: A polyphasic approach.

**Águila, B.**, Alcántara-Hernández, R. J., Montejano, G., López-Martínez, R., Falcón, L. I., & Becerra-Absalón, I. (2021). Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization. European Journal of Phycology, 56(4), 428-443.

https://doi.org/10.1080/09670262.2020.1853815

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# Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization

Bernardo Águila[a], Rocío J. Alcántara-Hernández[b], Gustavo Montejano[c], Rafael López-Martínez[b], Luisa I. Falcón[a] and Itzel Becerra-Absalón[c]

[a]Laboratorio de Ecología Bacteriana, Instituto de Ecología, Universidad Nacional Autónoma de México, Parque Científico y Tecnológico de Yucatán, 97302, México; [b]Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México; [c]Laboratorio de Ficología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México

**ABSTRACT**

We analysed and characterized the cyanobacteria in microbialites of Alchichica crater lake over a depth gradient, from 3 to 30 m. A polyphasic approach was followed using morphometry, optical and epifluorescence microscopy, and molecular methods. Regions V3–V4 of the 16S rRNA conserved for cyanobacteria were amplified and used for clone library construction. Independently, a NGS library was constructed using the V4 region of the 16S rRNA. To complement the analysis, eight monocultures of cyanobacteria were isolated from microbialites, which were also characterized by microscopy and 16S rRNA sequencing. In total, we described 18 genera of cyanobacteria isolated from microbialites of Alchichica crater lake. The distribution pattern suggests that on the surface there were mostly Nostocales and filamentous Synechococcales, while at depth we found single-celled Synechococcales. Pleurocapsales and Chroococcales were found at all depths, increasing in abundance at 20 and 30 m.

**ARTICLE HISTORY** Received 7 May 2020; Revised 10 November 2020; Accepted 14 November 2020

**KEYWORDS** Clone libraries; depth gradient; microscopy; NGS library; oxyphotobacteria; stromatolites; taxonomy

## Introduction

Microbialites are organo-sedimentary structures, formed by accumulation, precipitation or binding of minerals, mediated by the interaction of a diverse microbial community (Burne & Moore, 1987; Riding, 2011). Microbialites are considered to be the modern analogues of fossil stromatolites dated to ~3.7 billion years before present (ybp) (Flannery & Walter, 2012; Nutman *et al.*, 2016). Stromatolites have played an important role in the early stages of terrestrial biogeochemistry (Krumbein *et al.*, 2003) but their composition and microbial metabolisms have changed throughout geological time (Awramik, 1971).

Modern microbialites are rare and are mainly confined to environments with low nutrient concentrations and extreme conditions for prokaryotic grazers to grow. These include soda, hypersaline, freshwater and crater lakes, oligotrophic pits or pools, brackish waters, and a few marine environments (Russell, 1996; Laval *et al.*, 2000; Arp *et al.*, 2003; López-García *et al.*, 2005; Falcón *et al.*, 2007, 2020; Dupraz *et al.*, 2009; Foster *et al.*, 2009; Berelson *et al.*, 2011; Power *et al.*, 2011; Centeno *et al.*, 2012; Kempe & Kaźmierczak, 2012; Souza *et al.*, 2012; Valdespino-Castillo *et al.*, 2014; Bouton *et al.*, 2016; Gérard *et al.*, 2018; Johnson *et al.*, 2018; Yanez-Montalvo *et al.*, 2020).

Although microbialite formation is not completely understood to date, cyanobacteria are postulated to play an important role for microbialite genesis related to their photosynthetic metabolism, which can passively influence extracellular precipitation (Merz, 1992; Chagas *et al.*, 2016; Zhu & Dittrich, 2016). Cyanobacteria can trap cations (e.g. $Mg^{2+}$ or $Ca^{2+}$) with extracellular polymeric substances (EPS), which can form carbonates with the later degradation of the EPS fraction (Obst *et al.*, 2009; Pereira *et al.*, 2011; Kamennaya *et al.*, 2012). Some works have also evaluated the intracellular precipitation potential of cyanobacteria (Ragon *et al.*, 2014; Cam *et al.*, 2018).

Alchichica Lake is a maar-type crater located in central Mexico. The lake is alkaline, has a depth of 60 m, and exhibits a monomictic circulation pattern being oligotrophic most of the year (Alcocer & Lugo, 2003; Mancilla Villa *et al.*, 2014). Within the lake, two distinct morphological generations of microbialites have been described: (1) columnar–dome-like structures, rich in aragonite, forming near the shoreline, dated to ~1100 ybp; and (2) spongy, cauliflower-like thrombolytic structures that dominate inside the lake, which are mainly composed of hydromagnesite, huntite and calcite, dated to ~2800 ybp. It is hypothesized that these distinct morphologies and mineral compositions were the result of different periods of drought and flood affecting groundwater in the lake (Kaźmierczak *et al.*, 2008).

One of the first microbial characterizations of Alchichica (Tavera & Komárek, 1996) highlighted the

**CONTACT** Itzel Becerra-Absalón iba@ciencias.unam.mx


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uniqueness and rarity of the cyanobacterial species found within the lake. This work described new cyanobacterial species such as *Xenococcus candelariae, Heteroleibleinia profunda*, *Entophysalys litophyla, E. atrata* and *Chamaesiphon halophilus*. The authors suggested that some Chroococcales within the Entophysalidaceae family could be involved in microbialite formation. The study also stated the difficulty involved in the typification of cyanobacteria, because of their endolithic ecology, which hindered microscopic observations with carbonates and detrital particles.

The first sequencing study in Alchichica found that microbial diversity in microbialites changed along a 14 m depth gradient (Couradeau *et al.*, 2011). This study found a high abundance of Alphaproteobacteria and Betaproteobacteria along with Verrucomicrobia and Cyanobacteria, of which Oscillatoriales were abundant at 0.5 and 4 m depth, Gloeobacterales were also detected at these depths, and Pleurocapsales were detected at 0.5 and 4 m and were very abundant at 14 m. Nostocales were only detected at 4 m, while Chroococcales, Acaryochlorales and Prochlorales were only detected at 14 m depth. Another molecular analysis (Kaźmierczak *et al.*, 2011) amplified longer cyanobacterial sequences from 3–4, 6 and 8 m microbialites, and found *Rivularia* and *Calothrix* phylotypes at 3–4 and 6 m, while *Leptolyngbya* and *Halomicronema* phylotypes were found abundantly at 6 m. *Pleurocapsa* phylotypes were found abundantly at 3–4 and 8 m, while *Acaryochloris* phylotypes, Chroococcales and Prochlorales were only found in 8 m samples.

Finally, a recent work that focused on cyanobacteria from Alchichica (Saghaï *et al.*, 2015) analysed microbialite samples from 1, 5, 10 and 15 m depth and found that Pleurocapsales and Chroococcales increased in abundance with depth, while Nostocales and Oscillatoriales decreased. Prochlorales were found at 1, 5 and 10 m. 10–20% of cyanobacterial sequences were unclassified. The same depth gradient of this study was used for a later comparative metagenomic analysis (Saghaï *et al.*, 2016).

Although several studies have already addressed microbial composition in microbialites in Alchichica, a more detailed and updated description is needed for the cyanobacterial component. Historically, cyanobacteria have been a very difficult group to work with taxonomically, yet current advances in molecular information (Garcia-Pichel *et al.*, 2020) have led to a reclassification of species, which at the same time has caused an accumulation of misclassified taxa in the molecular databases (Soo *et al.*, 2017). Moreover, the different codes, either prokaryotic or botanical, used to name cyanobacteria at the genus level, are still under intense discussion (Oren & Ventura, 2017). To avoid the accumulation of confusing cyanobacterial classifications, a polyphasic classification system has been proposed, where information from ecology, morphometry, physiology and molecular approaches are combined to establish a robust characterization (Komárek *et al.*, 2014).

In this study we applied a polyphasic approach to study the cyanobacterial component of microbialites of Alchichica crater lake, recovering and discussing valuable information from previous studies. We also expand on previous studies by including microbialites from deeper than 15 m. This work aims to characterize in detail the cyanobacteria present in the microbialites of Alchichica crater lake along a depth gradient.

## Materials and methods

### *Study site*

Alchichica crater lake is located in the Trans-Mexican Volcanic Belt (19°24'N, 97°24'W, 2300 m asl) in the state of Puebla, Mexico. It has a maximum depth of 60 m, and the water is saline-alkaline, with an electric conductivity of 13 dS $m^{-1}$ and a pH of 8.7–9.2. The dominant anions are chlorides and bicarbonates, while the dominant cations are sodium and magnesium (Armienta *et al.*, 2008). A map of the sampling site and footage from the diving zones are shown in Supplementary fig. S1.

### *Sampling*

Microbialites were collected by SCUBA diving in the northern part of the lake (19°25′13.43″N, 97°24′27.83″W) where ~200 g of microbialite fragments were sampled at four different depths: 3 m (Z3), 10 m (Z10), 20 m (Z20) and 30 m (Z30); subsamples were separated into: (1) 100 g stored in blotting paper for microscopic analysis, (2) 10 g stored in sterile-sealed culture containers and (3) 5 g immediately frozen in liquid nitrogen for DNA extraction. Sampling was carried out in October 2016 and March 2017, when the two main hydrodynamic stages of the lake were present (stratification and mixing, respectively).

### *Microscopic methods*

Semi-permanent preparations, with phenol-glycerin (20/20 g $l^{-1}$) of dry microbialites treated with 5% acetic acid for demineralization of carbonates, were observed in a DIC Olympus BXX51 microscope and a BX51 microscope coupled to a Hg lamp, equipped with a U-MNG2 Green filter (excitation 530/50 nm; emission 590 nm) for autofluorescence of phycoerythrin to discriminate cyanobacteria from chlorophytes. Sigma Scan Pro V5 software (Systat Software Inc., San Jose, California, USA) was used to take morphometric data of cyanobacterial cells from fresh microbialite preparations and monocultures. Characterizations were made according to the

uniqueness and rarity of the cyanobacterial species found within the lake. This work described new cyanobacterial species such as *Xenococcus candelariae, Heteroleibleinia profunda*, *Entophysalys litophyla, E. atrata* and *Chamaesiphon halophilus*. The authors suggested that some Chroococcales within the Entophysalidaceae family could be involved in microbialite formation. The study also stated the difficulty involved in the typification of cyanobacteria, because of their endolithic ecology, which hindered microscopic observations with carbonates and detrital particles.

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with the QIAquick® gel extraction kit (QIAGEN Inc.). Purified amplicons were sequenced in the UNAM Institute of Biotechnology (IBT-UNAM, Mexico). Sequences quality were manually checked with PhyDE® (Phylogenetic Data Editor) software, then uploaded to GenBank: MK713349–MK713355 (420 bp) and MN822189–MN822192 (1400 bp). Primers used in this study are shown in Supplementary table S1.

### *Sequence analysis*

A Neighbour joining (NJ) phylogenetic tree was built in ARBSilva (Pruesse *et al.*, 2012); the parameters used were: FastTree GTR Model, Gamma rate for likelihood, Denovo construction with user sequences only, 10 neighbours per query sequence and 0.95 of minimum identity with query sequence. Fixed Positional variation was set at highly and moderately variable positions (1–9). SILVA, RDP, GTDB, LTP and EMBL-EBI/ENA databases were used for the 16S phylogeny. Sequences submitted to ARBSilva consisted of: (1) clone libraries (420 bp), (2) MiSeq libraries (250 bp), (3) sequences from monocultures (420–1400 bp), (4) Alchichica clones from previous work (400–800 bp), and curated 16S rDNA cyanobacterial sequences from databases (1400 bp).

### *Relative abundance analysis*

For comparing different molecular methods (clones and MiSeq) relative abundances of OTUs from the different methods were calculated separately and then averaged. Taxa tables and biome files from MiSeq were curated to match the cyanobacterial classification proposed by Komárek *et al.* (2014).

Relative abundances were plotted with Phyloseq and ggplot in RStudio 3.6.2 (Wickham, 2016). The OTUs abundance table and manually classified taxa were submitted to a GitHub repository: https://github.com/Burn121212/Alchichica-Reltive-Abundance/blob/main/Table%20calssification.xlsx

## Results

### *Microscopic analysis*

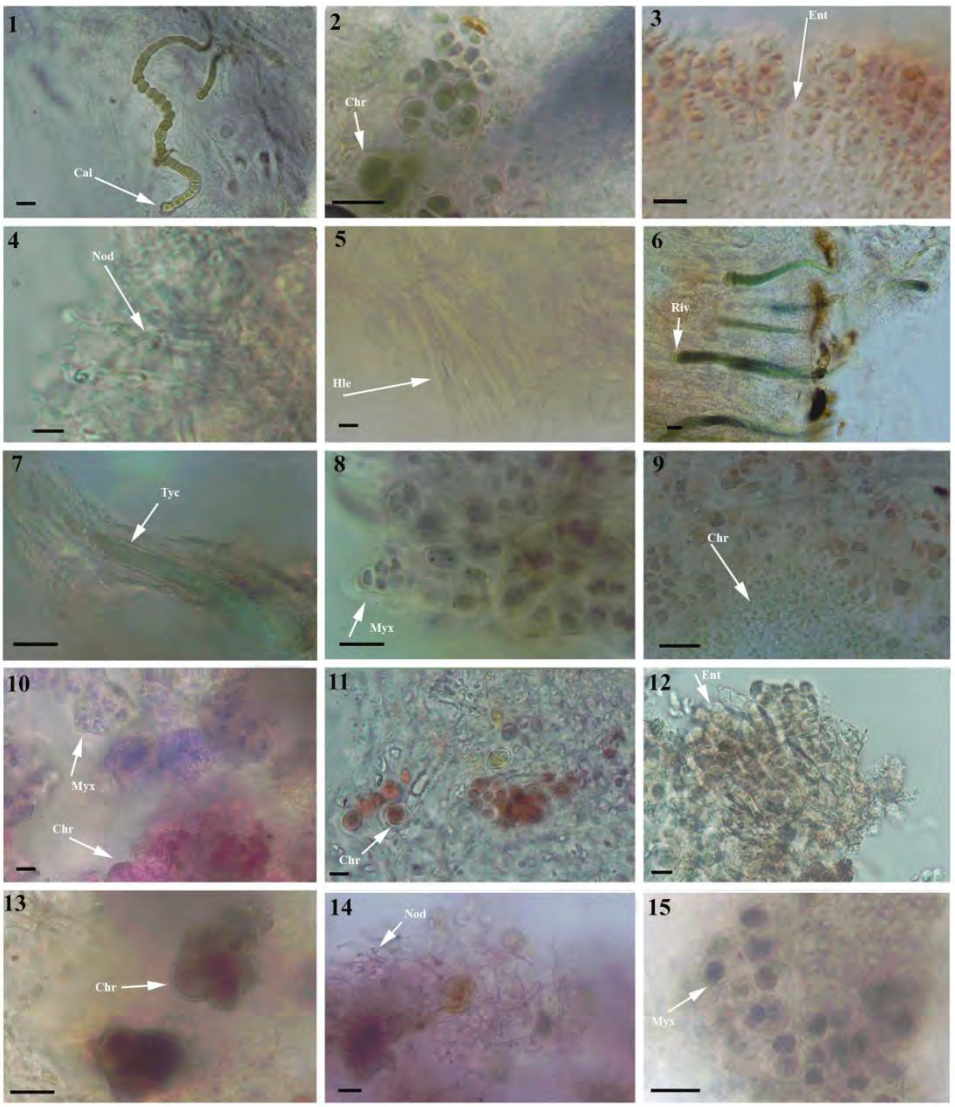
A total of 20 semi-permanent preparations in triplicate for four different depths (Z3, Z10, Z20 and Z30) and two sampling dates were examined (n = 120). Optical microscopy images of the cyanobacteria from microbialites collected are shown in Figs 1–15 and Figs 16–30. Autofluorescence of cyanobacteria is presented in Supplementary fig. S3.

### *Cyanobacterial observation for each sampling depth*

*3 m*: The most abundant cyanobacteria observed by microscopy were filamentous Synechococcales, mostly *Nodosilinea* sp. and *Haloleptolyngbya* sp. These cyanobacteria formed a consistent mat in the outer part of microbialites giving the microbialite a blue-green colouration. The small filaments were hard to identify by optical microscopy, but autofluorescence microscopy revealed nodule formations with similar morphometry to the *Nodosilinea* sp. cultures. Radial colonies of *Rivularia* sp. with heteropolar growth were abundant in the black portions of the microbialites, with basal heterocysts embedded into the substrate. Very few filaments of *Calothrix* sp. were found endolithic. Filaments of *Nodularia* sp. were observed in the samples from March 2017. No strict Oscillatoriales were observed microscopically except for a possible *Tychonema* sp. filament. In the Chroococcales, abundant colonies of *Entophysalis* sp. were observed in the brown and red patches of the microbialite, exhibiting pseudo filaments growing parallel to the substrate, sometimes forming radial colonies similar to *Myxosarcina* sp. but with no baeocytes. In Pleurocapsales, blue-green colonies of *Chroococcidium gelatinosum* with transparent sheets were attached to the base of a filamentous chlorophyte, probably *Cladophora*, as previously reported by Tavera & Komárek (1996).

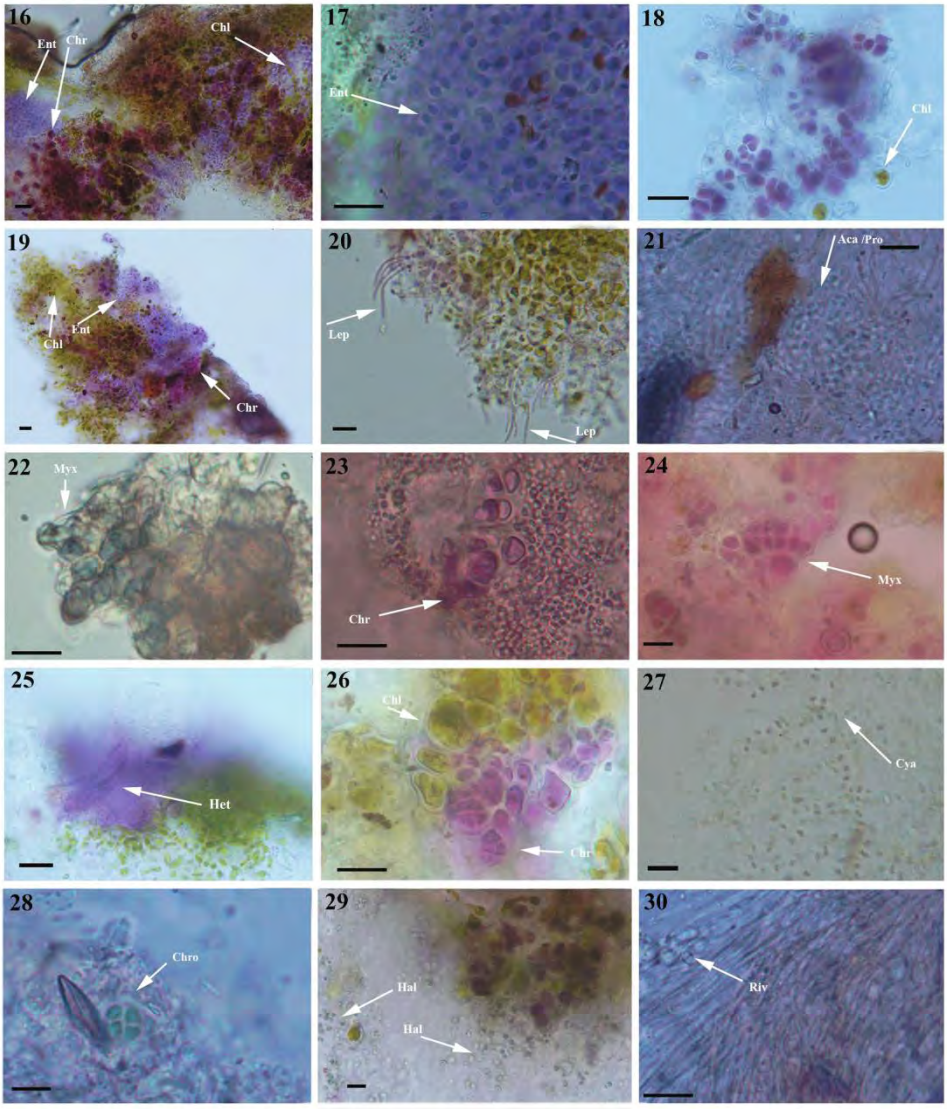
*10 m*: Microbialites showed a notable increase in abundance of Pleurocapsales, including *Myxorascina* sp., and *Chroococcidium gelatinosum*. The Chroococcales observed included *Entophysalis* sp. which were common in the black-brown parts of microbialite fragments. Few solitary cells of *Chroococcus* sp. were also found. Filaments of *Calothrix* sp. were scarce and only detected by fluorescence microscopy, but colonies of *Haloleptolyngbya* sp. and *Nodosilinea* sp. were observed in the green portion of the microbialites. Dead cells and empty sheaths of filamentous cyanobacteria were observed in the red portions of the microbialites. Picocyanobacteria < 2 µm were very abundant at this depth but could not be completely characterized by standard microscopic methods; typical bacillus forms similar to *Cyanobium* sp. were identified.

*20 m*: *Entophysalis* sp. and *Chroococcidium gelatinosum* were the most abundant cyanobacteria observed at 20 m depth, forming a ubiquitous consortium with chlorophyte cells. A few endolithic and isolated *Myxosarcina* sp. colonies were also present. No filamentous cyanobacteria were observed at this depth, but picocyanobacteria were still common, as well as filamentous Chlorophyta where *Heteroleibleina* sp. cells were growing epiphytically. Empty dead sheaths of Pleurocapsales and filamentous cells were also present. At this depth all Pleurocapsales exhibited high concentrations of phycoerythrin giving them a purple and a dark pink colour.




Figs 1–15. Optical microscopy of microbialites. **Fig. 1**. *Calothrix* sp. filaments are scarcely found in Z3 and Z10 with a characteristic olive-green colour. **Fig. 2**. Reproductive phases of a *Chroococcidium* sp. in Z3; at this depth this cyanobacterium exhibited green colouration, and exhibited dark pink colourations in Z10, Z20 and Z30. **Fig. 3**. *Entophysalis* sp. colonies incrusted in carbonates in Z3. **Fig. 4**. Probable filament of *Nodosilinea* sp. **Fig. 5**. Probable filaments of *Haloleptolyngbya* sp. at Z3. **Fig. 6**. Colonies of *Rivularia* sp. in Z3. **Fig. 7**. The only observation that could belong to *Tychonema* sp. **Fig. 8**. Colonies of *Myxosarcina* sp. with pseudo-filaments at Z10. **Fig. 9**. Green baeocytes of *Chroococcidium* sp. at Z3. **Fig. 10**. Purple colonies of *Myxosarcina* sp. and dark pink colonies of *Chroococcidium* sp. at Z10. **Fig. 11**. Cells of *Chroococcidium* sp. with spherical morphology. **Fig. 12**. Colonies of *Entophysalis* sp. with brown colourations in Z10. **Fig. 13**. Thick cell wall from *Chroococcidium* sp. **Fig. 14**. Nodules formations of *Nodosilinea* sp. **Fig. 15**. Colonies of *Myxosarcina* in brown. Scale bar represents 10 µm.

*30 m: Chroococcidium gelatinosum* and *Myxosarcina* sp. were the only identifiable cyanobacteria. These cyanobacteria were present in the olive-green patches of all microbialites. *Chroococcidium* presented blue green colouration at 3 and 10 m, but it was more often dark pink at 20 and 30 m. It was sometimes wrapped in a




Figs 16–30. Optical microscopy of microbialites. **Fig. 16** and **Fig. 19**. Consortia at Z20 of *Entophysalis* sp. in purple, *Chroococcidium* sp. in dark pink and reproductive phases of filamentous chlorophyte. **Fig. 17**. Cells of *Entophysalis* sp. at Z20. **Fig. 18**. *Chroococcidium gelatinosum* at Z20. **Fig. 20**. Chlorophytes with filaments of probably *Leptolyngbya* sp. at Z20. **Fig. 21**. Probable cells of *Acaryochloris* sp. or *Prochlorococcus* sp. **Fig. 22**. *Myxosarcina* endolithic at Z20 exhibiting a thick mucilage envelope. **Fig. 23**. *Chroococcidium* sp., most abundant cyanobacteria observed at Z30. **Fig. 24**. *Myxosarcina* at Z30. **Fig. 25**. *Heteroleibleinia* cells at 20 m. **Fig. 26**. Consortia of chlorophytes and *Chroococcidium* at Z30. **Fig. 27**. *Cyanobium* cells with binary fusion. **Fig. 28**. *Chroococcus* at z3. **Fig. 29**. *Halothece* cells at Z10. **Fig. 30**. Empty sheaths of *Rivularia* at z3. Scale bar represents 10 µm.

transparent mucilage membrane and always had a thick cell wall, generally associated with chlorophyte cells. Although the main morphology of Pleurocapsales was amorphous, with fluorescence microscopy we noted some spherical forms buried in the sediments, which is a common morphology in *Chroococcidium* cultures. Solitary picocyanobacteria resembling *Cyanobium* sp. were still present. Besides cyanobacteria, there was a high abundance of filamentous chlorophyte and frustules of diatoms.

### *Monocultures and cyanobacterial isolation*

We obtained eight cyanobacterial monocultures identified as: *Haloleptolyngbya* sp. (AL1), *Oculatella* sp. (AL2), *Nodosilinea* sp. (AL3), *Rivularia* sp. (AL4), *Leptolyngbya* sp. (AL5), *Nodularia* sp. (AL6), *Myxosarcina* sp. (AL7) and *Chroococcidium* sp. (AL8) (Figs 31–38 and Supplementary table S2).

### *Molecular analysis*

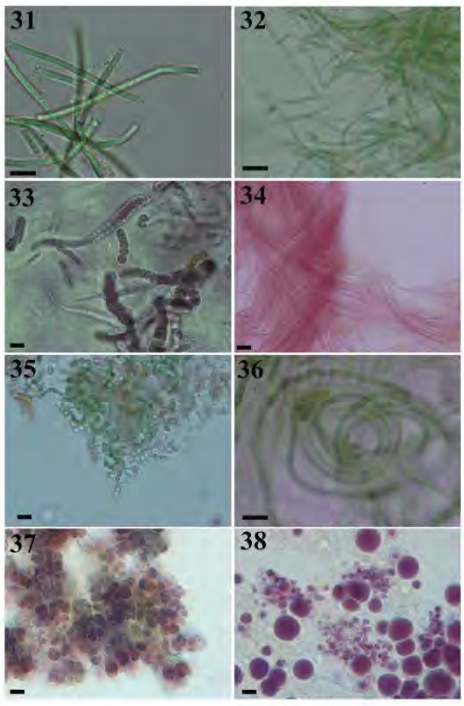
In total, 47 OTUs (23 for clone libraries and 24 MiSeq libraries) were obtained. The OTUs were classified in 14 genera: *Acaryochloris*, *Calothrix*, *Chroococcidium*, *Cyanobium*, *Entophysalis*, *Haloleptolyngbya*, *Halomicronema*, *Halothece*, *Leptolyngbya*, *Myxosarcina*, *Nodosilinea*, *Oculatella*, *Prochlorococcus* and *Tychonema*. Two groups of OTUs were unclassified: filamentous Synechococcales within Leptolyngbyaceae and Chroococcales in the Cyanothrichaceae.

For the clone libraries, a total of 300 clones were obtained from which 206 passed quality sequence control; 69 sequences belonging to chloroplasts were eliminated. The remaining 137 sequences were grouped into 23 OTUs.

Illumina MiSeq libraries generated 1 481 623 ASVs from which 7408 cyanobacterial sequences were recovered. These sequences were grouped into 24 OTUs as described in Materials and Methods.

We obtained sequences for the eight monocultures (AL1-AL8) using Nübel *et al.* (1997) primers. Sequences were BLASTed in NCBI and quality checked, then uploaded to GenBank with accession numbers MN795493–MN795515. A second sequencing effort was carried out using the 27F-1494R primers to obtain nearly complete 16S rRNA sequences. Only AL1, AL4, AL6, AL7 and AL8 passed quality control since AL2 and AL5 cultures were contaminated in 2018. AL2 and AL3 had poor sequence quality, but MiSeq methods confirmed their presence (250 bp) in the lake.

At the genus level (Fig. 39), *Acaryochloris* represented 4.6% of OTUs at Z3, 8.9% at Z10, 16.6% at Z20 and 5% at Z30. *Calothrix* only represented 0.32% of OTUs at Z3. *Chroococcidium* represented 44.7% of OTUs in Z3, 56% in Z10, 49% in Z20 and 21.3% in Z30. *Cyanobium* represented 11.62% of OTUs in Z20 and 21.34% in Z 30. *Entophysalis* represented 1.6% at Z3, 3.4% at Z10, 2.32% at Z20 and 5% at Z30. *Haloleptolyngbya* represented 3.5% at Z3, 8.2% at Z10 and 9% at Z30. *Halomicronema* only represented 0.5% of OTUs at Z3. *Halothece* represented 6.1% in Z10. *Leptolyngbya* represented 7.1% of OTUs at Z3. *Myxosarcina* represented 20.4% of OTUs at Z3, 3.12% at Z10 and 5.81% at Z20. *Nodosilinea* represented 11.1% of OTUs at Z3. *Oculatella* represented 2.2% at Z3. *Prochlorococcus* represented 10.7% of OTUs at Z3, 1.16% at Z10 and 11.7% at Z30. *Tychonema* represented 0.08% of OTUs in Z3 and 12% in Z30. Unknown Chroococcales represented 0.01% at Z3, and 3.12% at Z10. Finally, unknown filamentous Synechococcales represented 3.53% of OTUs in Z3, 12.7% at Z20 and 14.84% at Z30.




Figs 31–38. Optical microscopy of cyanobacterial cultures isolated from microbialites. **Fig. 31**. *Haloleptolyngbya* sp. (AL1). **Fig. 32**. *Oculatella* sp. (AL2). **Fig. 33**. *Rivularia* sp. (AL4). **Fig. 34**. *Leptolyngbya* sp. (AL5). **Fig. 35**. *Nodularia* sp. (AL6). **Fig. 36**. *Nodosilinea* sp. (AL3). **Fig. 37**. *Myxosarcina* sp. (AL7). **Fig. 38**. *Chroococcidium* sp. (AL8). Scale bar represents 10 µm.

### *Phylogenetic analysis*

Eighteen cyanobacterial genera were confirmed from our phylogenetic analysis with clone and MiSeq libraries, cyanobacterial monocultures and sequences determined in previous studies. A phylogenetic tree is presented in Supplementary fig. S3. An association between depth and taxonomic groups was found. For instance, *Rivularia*, *Calothrix*, *Nodularia*, *Tychonema*, *Nodosilinea*, *Haloleptolyngbya*, *Leptolyngbya*, *Halomicronema* and *Oculatella* genera were only found at 3 and 10 m, while *Cyanobium*, *Prochlorococcus* and *Acaryochloris* were specific to 10, 20 and 30 m. The only groups found at all depths were *Chroococcidium*, the possible *Entophysalis* group and an Unknown filamentous Synechococcales group. Each OTU with

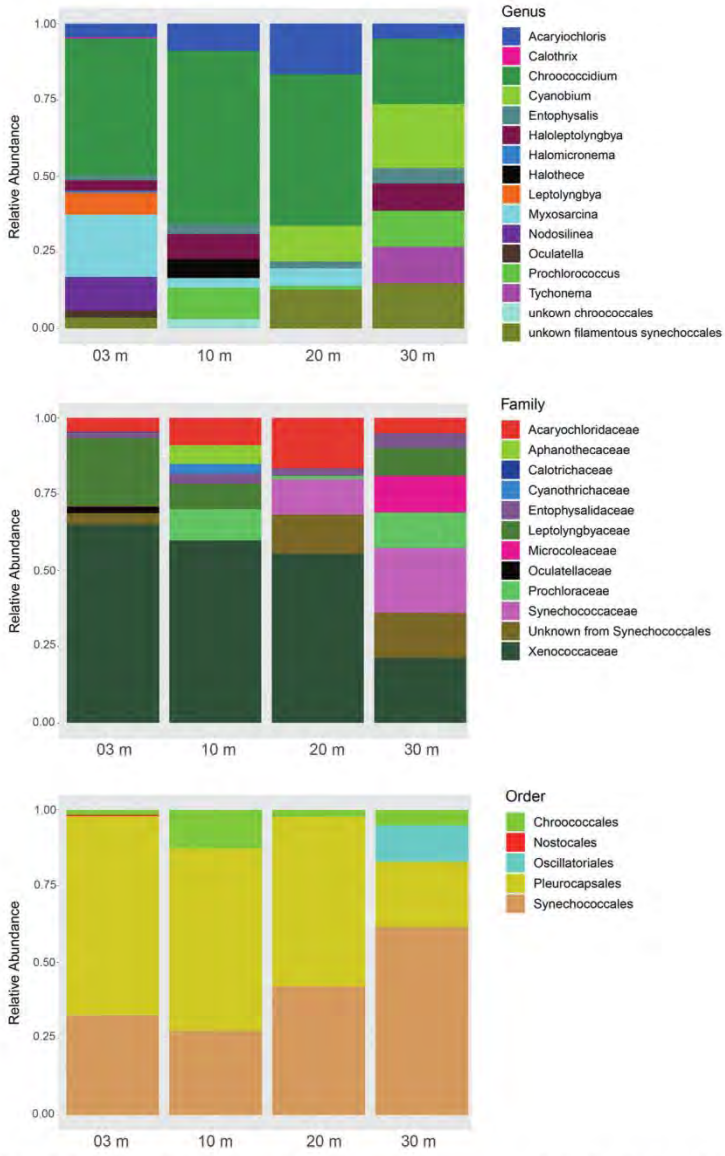



Fig. 39. Bar plots of relative abundances of cyanobacterial 16S rRNA sequences obtained with clones (otu01-otu23) and MiSeq (otu24-otu47) libraries.

classification was plotted in a heat map from most to least abundant in Supplementary fig. S4.

### *Polyphasic characterization*

Cyanobacterial taxa (n = 18) were identified by comparing standard morphometric characterization methods by autofluorescence and DIC microscopy, with 16S rDNA sequences obtained from clone libraries (420 bp), MiSeq libraries (250 bp) and from monocultures (420–1200 bp). Results were compared with the literature (morphometric descriptions and sequence data). Taxon names were based on the phylogenetic analysis which included all data mentioned above. Microscopic abundance, scaled morphotype and identification methods are shown (Fig. 40).

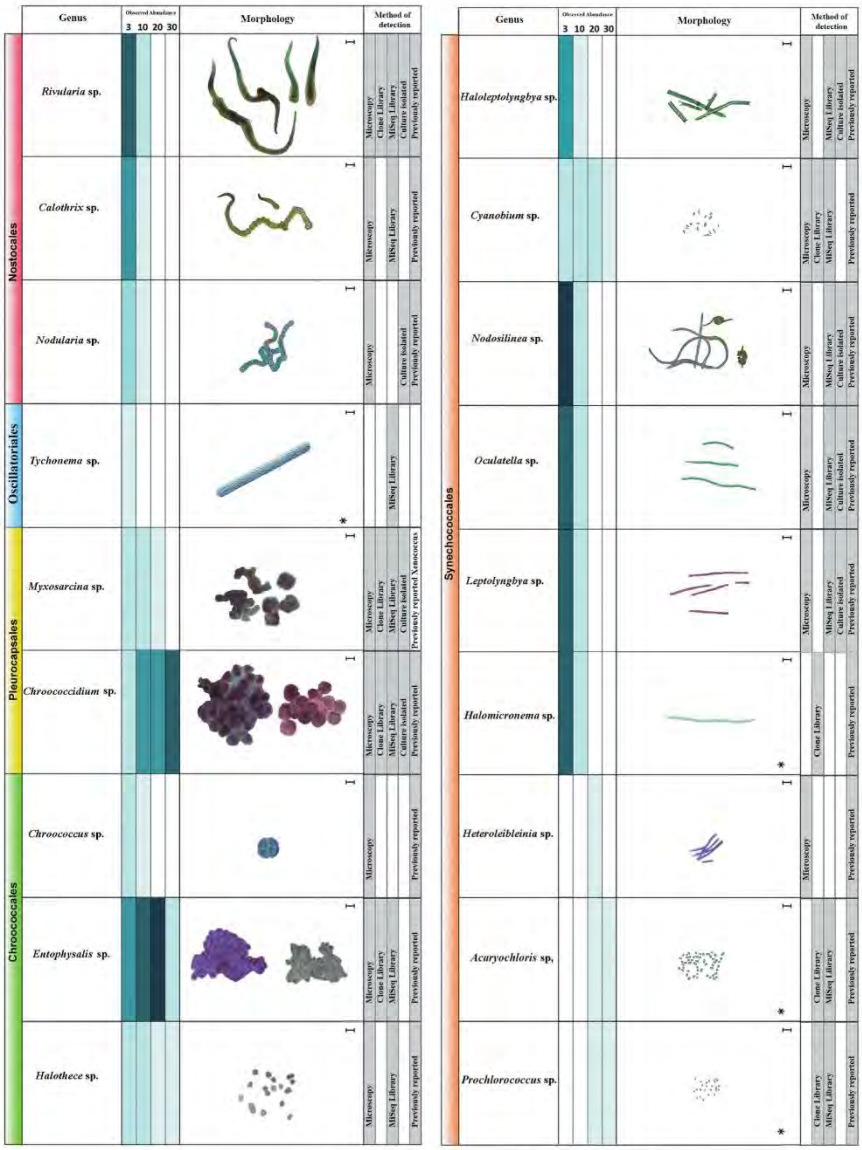



Fig. 40. Relative abundance of taxa observed with microscopy, scale goes from very abundant (dark blue) to no observation (white colour). The grey boxes indicate a positive signal for the different methods of identification. Cyanobacterial images were taken from microscopic analysis in this study except for the ones indicated with a * which will require a revision in future works. The scale bar represents 5 µm.

### *Cyanobacterial descriptions*

All descriptions were made based on material collected from microbialites and cultures obtained.

*Acaryochloris* sp.: Detected by clone libraries at 10, 20 and 30 m. Microscopic analysis found 2 µm spherical cells, but confirmation using other methods is needed. Previously reported in Kaźmierczak *et al.* (2011; Fig. 21).

*Calothrix* sp.: Solitary heteropolar filaments, found in small groups of 3–10 filaments attached to substrate. Filaments 3–12 µm wide at the base. Sheath thick, transparent, 0.3–1 µm. Trichomes longer than

*Rivularia* sp., 160–180 µm, with attenuated apices. Basal ovoid-spherical heterocysts, 3–12 µm wide. Most distinct difference from *Rivularia* sp. is its olive-green, yellow-brown colouration. Only found scarce filaments at green zones from 3 m and 10 m microbialites (Fig. 1, Supplementary fig. S2c).

*Chroococcidium* sp.: Irregular aggregates and colonies form heterogeneous clusters. Sheaths transparent, lamellate due to multiple divisions, 0.2 µm thick. Cells oval and irregular, sometimes polygonal, from 3–12 µm in diameter. Baeocytes 6–7 µm in diameter. Exhibiting green colouration at 3 m but dark pink at 10, 20 and 30 m depth, always in consortia with *Entophysalis* sp. and *Myxosarcina* sp., probably participating in microbialite formation (Figs 2, 9, 10, 13, 16, 18, 19, 23, 26, 27, 38, and Supplementary fig. S2b).

*Chroococcus* sp.: Thallus formed by solitary and spread colonies. Cells oval, blue-green, with two or more symmetrical division planes, 8 µm wide. Previously reported as part of phytoplankton in the water column, scarcely seen at 3 m depth samples (Fig. 28).

*Cyanobium* sp.: Thallus unicellular, formed by small blue-green bacillus, 0.9 µm wide and 1.7 µm long, observed at all depths; due to its size, this kind of picocyanobacteria must be reviewed for precise characterization (Fig. 27).

*Entophysalis* sp.: Thallus formed by colonies densely packed and arranged in rows, forming pseudo-ramifications in a fingerlike structure, growing perpendicular to substrate. Mucilage transparent, 1 µm thick average, forming packages around stacks of 3 to 5 cells. Cells are amorphous-polygonal and oval, with red, grey and purple colouration, 2.5 µm long and 1.5 µm wide, growing in one division plane. Always in association with *Chroococcidium* sp. and chlorophytes. Very abundant from 3 to 20 m depth, its role in carbonate precipitation is remarked in previous studies (Figs 3, 12, 16, 17, 19).

*Haloleptolyngbya* sp.: Filaments thin, blue-green with very fine sheath, 0.3 µm thick. Trichomes isopolar, 2.5 µm wide. Cells 1.7 µm long. Rectangular necridia, abundant hormogonium, only detected in cultures from 3 and 10 m depth (Figs 5, 34).

*Halomicronema* sp.: Detected by clone libraries, and partially by microscopy. Thin filaments were detected similar to *Leptolyngbya*, described as a halotolerant. Trichomes are more less cylindrical. Thylakoids are arranged concentrically and parallel to the plasma membrane, single or stacked. Pores are distributed irregularly on cell walls and septa. Previously detected by Kaźmierczak *et al.* (2011; Fig. 20).

*Halothece* sp.: Solitary and scarce colonies with thin mucilage layer 0.2 µm. Cells oval, 3.5 µm long and 2.5 µm wide, growth by perpendicular division in one plane, very abundant at 3 and 10 m depth, although hard to observe in microscope from original sample collection (Fig. 29 and Supplementary fig. S2d).

*Heteroleibleinia* sp.: Filaments short, composed of 3–9 cells, 12 µm long. Trichomes 4 µm wide but wider at the end, 5 µm. Cells cylindrical, shaped barrel. Very rare in microbialites and only observed in 10 m samples growing on chlorophytes, previously reported as epiphytes on *Cladophora* sp. (Fig. 25).

*Leptolyngbya* sp.: Filaments organized in parallel rows, sometimes intertwined, with transparent sheaths, 0.1 µm thick. Trichomes 1 µm wide and cells rectangular, red-violet, 2.3 µm long. These filaments are found in 3 and 10 m microbialites, although MiSeq methods also found sequences at 20 and 30 m depth (Figs 20, 31 and Supplementary fig. 2a).

*Myxosarcina* sp.: Colonies grow in symmetrical and spherical groups with 8–12 cells, although isolated and irregular cells are sometimes present, colonies tend to form agglomerations and pseudo filaments. Sheath thick, brown and black, 0.4 µm. Baeocytes 5–8 µm in diameter. Probably misclassified as some type of *Entophysalis* in previous studies (Figs 8, 10, 15, 22, 24, 37).

*Nodosilinea* sp.: Entangled filaments, exhibit nodes characterizing the genera. Trichomes 2.5 µm wide and cells 2 µm long. Abundant at 3, 10 and 20 m depth from cultures; sequencing methods showed a high diversity of *Nodosilinea*-like cyanobacteria at 3 and 10 m (Figs 1d, 1n, 3f).

*Nodularia* sp.: Solitary and unbranched filaments sometimes spirally coiled. Sheaths transparent or yellow-brown, 0.2 µm thick. Trichomes isopolar, 120–180 µm long and 4–8 µm wide. Oval cells, mainly blue-green, rarely yellow-brown. Basal heterocysts, yellow or grey-green, spherical, 10–13 µm. Only found in 3 m microbialites from March samples during stratification season, where it probably forms blooms and precipitates above microbialites, meaning it is not part of the microbialite component. Very abundant in cultures where also exhibiting intercalated heterocysts (Fig. 35).

*Oculatella* sp.: Solitary filaments with a thin transparent sheath. Trichomes exhibit red pigmentation at apex, 1.2 µm wide. Cells blue green, 2.2 µm long. Previously misclassified as *Leptolyngbya* sp. in direct microbialite observations, but cultured sequencing methods confirmed this classification. Exhibited chromatic adaptation (Fig. 32).

*Prochlorococcus* sp.: Detected in clone libraries from 10, 20 and 30 m. Microscopic analysis found 1.2 µm

radius spherical cells, but confirmation by other methods is needed. Previously reported in Kaźmierczak *et al.* (2011; Fig. 21).

*Rivularia* sp.: Thallus mucilaginous, radial, formed with filaments heteropolar, basal filaments incrusted in carbonates. Sheaths thick, 0.4 μm. Trichomes rapidly attenuated with a maximum length of 120 μm, with false branching and wide basal and elongated heterocysts, 4–16 μm wide, as well intercalated heterocysts. The cells exhibit dark green colouration in fresh microbialite samples, but are brown, grey and black in culture. Very abundant in black and green regions of microbialites samples from 3 m, although scarce colonies were observed in Z10 (Figs 6, 30, 33).

*Tychonema* sp.: MiSeq libraries detected it in z3 and z30, but not found by microscopic analysis, and there is just one observation that partially confirms this taxon. Filaments solitary, described as free-floating without sheaths. Trichomes cylindrical, isopolar, not attenuated to the ends, unconstructed. Cells mostly cylindrical, heterocysts and akinetes absent (Fig. 7).

## Discussion

### *Cyanobacterial groups found in this study*

In general, the diversity of cyanobacteria from microbialites of crater lake Alchichica found in this study corroborates previous studies up to 15 m in depth. Novel data from 20 and 30 m indicate that Pleurocapsales and unicellular Synechococcales are the most abundant groups at these depths. A general revision and discussion of cyanobacteria reported by all studies including this one, are integrated below.

Nostocales: *Rivularia* sp. colonies were very common at 3 m, but only few *Calothrix* sp. filaments were observed at 3 and 10 m by microscopy, these two genera were confirmed by MiSeq libraries, cultures and previous works (Tavera & Komárek, 1996; Falcón *et al.*, 2002; Kaźmierczak *et al.*, 2011; Beltrán *et al.*, 2012). Although we could not find sequences for *Nodularia* sp. in this study, we were able to observe it in 3 m samples from March 2016 and obtain it in culture. This cyanobacterium has already been reported in bloom formations during mixing seasons (Oliva *et al.*, 2009). It is possible that the *Trichormus* sp. characterizations from previous work were a benthic phase of *Nodularia* sp., since the culture morphology AL6 resembles the original descriptions (Tavera & Komárek, 1996).

Oscillatoriales: We did not find Oscillatoriales *sensu stricto* by microscopic observations or cultures, but MiSeq results suggest that *Tychonema* sequences exist at 3 and 30 m. Its detection at 30 m is unlikely; it is possible that this detection was due to a precipitated bloom, since *Tychonema* is reported to be a planktonic species (Salmaso *et al.*, 2016). Nevertheless, this genus is reported because its ecology agrees with the study site, although it would be necessary to confirm the presence of *Tychonema* sp. in future studies.

Pleurocapsales: We found two groups of very abundant Pleurocapsales in microbialites, which were well represented by phylogenetic analysis, cultures and previous studies. Thanks to the last revision of Pleurocapsales (Shalygin *et al.*, 2019), we propose that what was previously described as *Xenococcus candelariae* was actually a new species within the Xenococcacae family, because the sequences were more affiliated with the *Myxosarcina* group and more separated from *Xenococcus* sensu stricto. Here, this group is temporarily identified as *Myxosarcina* sp., although a more specific analysis would be required for this new genus. *Myxosarcina* was found at 3, 10, 20 and 30 m as an endolithic cyanobacterium with brown, black to purple colouration. It exhibited a thick mucilage layer and had the most amorphous baeocytes, sometimes forming pseudo-filaments in culture. What was previously described as *Chroococcidium gelatinosum* by Tavera & Komárek (1996) has been reclassified as this genus. Although the original description is of a blue-green morphotype, we observed a chromatic plasticity in the culture from blue-green to dark pink. This is the first time this cyanobacterium is obtained in culture and in sequence. Some studies have already postulated the role of Pleurocapsales in microbialite formation by extracellular precipitation (Gérard *et al.*, 2013). These Pleurocapsales cultures will be very valuable for future analysis since *Chroococcidium* and *Myxosarcina* groups are the main cyanobacterial components of spongy microbialites. This pattern of depth abundances for Pleurocapsales has also been described for Lake Pavilion in Canada (Russell *et al.*, 2014), Highborne Cay in Bahamas (Mobberley *et al.*, 2013) and are dominant in some dome shaped microbialites of Lake Dziani-Dzaha in Mayotte (Gérard *et al.*, 2018). The endolithic ecology of Pleurocapsales depends on low light conditions, nonetheless the difficulty of culture isolation has made this group of cyanobacteria historically under-studied (Brito *et al.*, 2017).

Chroococcales: We observed abundant colonies of *Entophysalis* sp., in the brown and red patches of the 3, 10 and 20 m microbialites. They exhibited purple cell pseudo-filaments, growing parallel to the substrate. Although no *Entophysalis* sequences exist to date, the closest relative sequence available within Entophysalidaceae family would be *Chlorogloea* sp. or *Radiocystis* sp. The phylogenetic analysis did not find sequences close to *Chlorogloea*, however of two groups of unique unassigned sequences from Chroococcales

one presented the same distribution pattern as that found in microscopy which partially supports the presence of *Entophysalis*. Previous work mentioned the importance of *Entophysalis litophyla* and *E. atrata* in the formation of microbialites (Tavera & Komárek, 1996), but it is also possible that these genera are actually Pleurocapsales observed in phases in which they do not present baeocytes (Waterbury & Stanier, 1978). Due to the microscopic observations and the unassigned ASVs of Chroococcales, we decided not to discard the presence of *Entophysalis* in case new evidence of this family arises. Solitary *Chroococcus* cells were also observed at 3 and 10 m. Previous work described *C. turgidus* as part of the water column (Tavera & Komárek, 1996). *Halothece*-like cells were observed by microscopy at 3 and 10 m, however it was only confirmed by MiSeq at 10 m representing 20% of sequences at this depth. The ecology of this genus is relevant in the Alchichica crater lake since it has been reported in microbial mats from hypersaline lakes in the Central Pacific (Schneider *et al.*, 2013).

Synechococcales: Was the most abundant and diverse cyanobacterial group in Alchichica. However, they were the most difficult to study and classify. The most abundant cyanobacteria in 3 and 10 m microbialites were filamentous, such as *Nodosilinea* sp., *Haloleptolyngbya* sp., *Oculatella* sp. and *Leptolyngbya* sp. These genera were verified by monocultures, molecular data and previous work. They had been classified within Oscillatoriales (Couradeau *et al.*, 2011; Kaźmierczak *et al.*, 2011; Saghaï *et al.*, 2015). These cyanobacteria were abundant on the outer parts of the microbialites giving them a bluish-green colouration. In the case of *Oculatella* sp., it could only be distinguished thanks to the MiSeq libraries and cultures from 3 m, and it constitutes the first report for Alchichica microbialites. The main problem with classification of filamentous Synechococcales is that most sequences are annotated as *Leptolyngbya* (Becerra-Absalon *et al.*, 2018). Thanks to a recent revision we differentiated all misannotated *Leptolyngbya* sequences (Mai, 2016). The unclassified filamentous Synechococcales group that we found in all samples is probably a new genus affiliated to the *Halomicronema* group. *Halomicronema* sp. was already reported by Kaźmierczak *et al.* (2011). Nevertheless, no evidence of *Halomicronema* was found by microscopy in 20 or 30 m microbialites in this study. Picocyanobacteria measuring less than 2 μm were difficult to classify by microscopy, and the pretreatment of samples with a decalcifying solution may have damaged and discoloured small cells. Yet, sequences of *Cyanobium* sp. were recovered with clone libraries, increasing abundance towards 20 and 30 m. This pattern of distribution of *Cyanobium* has already been described in oligotrophic lakes (Budinoff, 2005), but little is still known regarding their physiology. *Synechococcus* sensu stricto sequences were not found in this study (Pittera *et al.*, 2014). Although *Acaryochloris* could not be directly distinguished under the microscope, we could identify it by clone libraries. This cyanobacterium had a strong signal at 30 m. It is possible that it is a symbiont with chlorophytes, which were very abundant at this depth. Another important picocyanobacteria in depth was *Prochlorococcus* detected with clone libraries at 20 and 30 m. Although this genus is marine, Kaźmierczak *et al.* (2011) also found related sequences in Alchichica, an athalasohaline system. MiSeq methods could not detect the presence of *Prochlorococcus* or *Acaryochloris* groups, probably due to the region that the primers amplify (V4). This has been already discussed as a bias for bacterial characterization in general (Johnson *et al.*, 2019). A few cells of *Heteroleibleinia* sp. were observed as epiphytes on chlorophytes in 20 m; there are still no sequences in the databases for this genus, but since it had already been observed and described (Tavera & Komárek, 1996) it was included in this revision.

### *Cyanobacterial cultures*

Although cultures are important for polyphasic and taxonomic studies to contrast the botanical classifications and bacteriological codes (Palinska *et al.*, 2006), they are very difficult to obtain. More cultures are needed for unicellular Synechococcales (picocyanobacteria) including *Acaryochloris, Cyanobium* and *Prochlorococcus*; the same applies to *Entophysalis* phylotypes. Assemblies from metagenomes could ease the classification for these unknown taxa. Other techniques to be considered are laser microdissection and isolation by centrifugation.

### *Molecular methods*

The high amounts of EPS produced by endolithic taxa such as Nostocales and Chroococcales can hinder DNA extraction (Wu & Xi, 2009). Also, a high concentration of Mg contained in microbialites can interfere with amplification reactions (Wade & Garcia-Pichel, 2003; Gómez-Acata *et al.*, 2019). Therefore, a bias in relative abundances can be expected if only molecular methods are considered. Molecular cyanobacterial signals can vary depending on the species (Fayyad & Dwaish, 2016). Beyond studying cyanobacterial distribution along a depth gradient, our main objective was to characterize and validate cyanobacteria at a genus level in detail. Interestingly, High throughput sequencing methods showed a high sensitivity for detecting Nostocales and Oscillatoriales while clone libraries

exhibit a preference for Synechococcales, specially *Cyanobium* sp., *Acaryochloris* sp. and *Prochlorococcus* sp. Major differences between microscopic and molecular methods included the detection of filamentous genera at 30 m that we could not observe by microscopy, *Haloleptolyngbya* sp., unknown filamentous Synechococcales in 20 and 30 m and *Tychonema* sp. Although the detection of *Tychonema* sp. could be related to precipitation of blooms more work is needed to confirm it. Another important difference is the amount of *Cyanobium* that molecular techniques recovered, 30% of all ASVs at 30 m. Although this cyanobacterium was indirectly detected by autofluorescence microscopy it was not as abundant as molecular techniques showed, instead Pleurocapsales were the most dominant observable cyanobacteria at 30 m.

### *Cyanobacterial classification over time*

Cyanobacterial classification is an ongoing process. The major differences from previous work for Alchichica microbialites is that most of the Oscillatoriales descriptions are now reclassified as filamentous Synechococcales. Oscillatoriales sensu stricto are not major components of microbialites in Alchichica. We did not observe or detect previously reported cyanobacteria such as *Synechococcus, Gloeomargarita, Chamaesiphon, Mantellum* or *Anabaena*. Validation and a fine description of the cyanobacteria is a rigorous, difficult yet important task that is needed for the curation of the constantly growing molecular databases. This work could be useful for a correct annotation of shotgun metagenomic data (Saghaï *et al.*, 2016). As revised here, Alchichica crater lake microbialites contain unclassified sequences associated with filamentous Synechococcales and Chroococcales that still require corroboration. Original cyanobacterial microscopic classifications from 20 years ago continue to be valid, which recalls the importance of natural knowledge of biological groups. The main problem with modern descriptions that rely solely on molecular data is the tendency to rename new species without taxonomic validation, hence the relevance of polyphasic approaches.

The pattern of cyanobacterial taxa distribution along a depth gradient observed in this study strongly suggests that filamentous Synechococcales (*Haloleptolyngbya* sp., *Leptolyngbya* sp., *Nodosilinea* sp. and *Oculatella* sp.) along with Nostocales (*Rivularia* sp. and *Calothrix* sp.) are major components for microbialites developing at 3 m. *Nodularia* sp. are only present in winter but are not a maincomponent of microbialites. Unicellular Synechococcales (*Acaryochloris* sp., *Cyanobium* sp. and *Prochlorococcus* sp.) have a strong association to 20 and 30 m microbialites. Most importantly, Chroococcales (*Entophysalis* sp.) and Pleurocapsales (*Chroococcidium* sp. and *Myxosarcina* sp.) are common in all microbialites but increase their abundance at 20 m. We conclude that *Entophysalis* sp., *Chroococcidium* sp. and *Myxosarcina* sp. have great relevance for understanding the ecology of spongy microbialites and their cultures may shed light to future works with physiological analysis for underlying the processes involved in the formation of these modern microbialites.

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No potential conflict of interest was reported by the authors.

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## Supplementary Information

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at https://doi.org/10.1080/09670262.2020.1853815

**Supplementary table S1**. Primers used in this study.
**Supplementary table S2**. Collection IDs of cultures obtained in this study.
**Supplementary figure S1**. Study site (**S1a**) Alchichica lake is located between the states of Puebla and Veracruz, Mexico. Hypsometric map (Image INEGI 2016) showing Alchichica lake is one of six crater lake systems (Aljojuca and San Miguel Tecuitlapa lakes are not shown in this map). (**S1b**) Microbialite reefs are exposed off shore because of the lake's gradual evaporation. Photographs of microbialites at Z10 and Z30. (**S1c**) Microbialites main morphology are spongy thrombolites, but distinct morphologies are found across a depth gradient, as well a reddish-orange colouration that is visible towards deeper microbialites.
**Supplementary figure S2**. Epifluorescence microscopy. (**S2a**) Thin filaments probably *Haloleptolyngbya* sp. or *Leptolyngbya* sp. were most abundant in Z3. (**S2b**) *Myxosarcina* sp. was very abundant in black-brown patches of the microbialites at Z10. (**S2c**) *Calothrix* sp. filament in Z10. (**S2d**) Probable cells of *Halothece* in Z10. (**S2e**) Spherical cells of *Chroococcidium* at Z20 were only visible with autofluorescence. (**S2f**) Baeocytes of *Chroococcidium* at Z20 exhibited differential production of phycoerythrin.
**Supplementary figure S3**. Phylogenetic tree constructed with OTUs from clone and MiSeq libraries (shown in blue), cyanobacterial monocultures (shown in green) along with other Alchichica's cyanobacterial sequences from Couradeau *et al.*

(2011), Kaźmierczak *et al.* (2011) and complete 16SrDNA cyanobacterial sequences. All sequences were aligned with Seaview v4.7. A Maximum likelihood was constructed with SILVA-ACT (Fast Tree) for the phylogenetic reconstruction.
**Supplementary figure S4**. Heat map of cyanobacterial OTUs ordered from most to least abundant, number indicate relative abundance in percentage.

## Author contributions

B. Águila: original concept, drafting and editing manuscript, culture isolation, analysis of molecular data; R.J. Alcántara-Hernández: original concept, analysis of molecular data, funding, resources; L.I. Falcón: funding, analysis of molecular data; drafting and editing of manuscript; G. Montejano: technical assistance for characterization of cyanobacteria, funding; R. López-Martinez: original concept, sampling of microbialites; I. Becerra-Absalón: analysis of molecular data, technical assistance for characterization of cyanobacteria, funding.

## References


Alcocer, J. & Lugo, A. (2003). Effects of El Niño on the dynamics of Lake Alchichica, central Mexico. *Geofísica Internacional*, **42**: 523–528.

Armienta, M.A., Vilaclara, G., De la Cruz-Reyna, S., Ramos, S., Ceniceros, N., Cruz, O., Aguayo, A. & Arcega-Cabrera, F. (2008). Water chemistry of lakes related to active and inactive Mexican volcanoes. *Journal of Volcanology and Geothermal Research*, **178**: 249–258.

Arp, G., Reimer, A. & Reitner, J. (2003). Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**: 105–127.

Awramik, S.M. (1971). Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **174**: 825–827.

Becerra-Absalon, I., Johansen, J.R., Munoz-Martin, M.A. & Montejano, G. (2018). *Chroakolemma* gen. nov. (Leptolyngbyaceae, Cyanobacteria) from soil biocrusts in the semi-desert Central Region of Mexico. *Phytotaxa*, **367**: 201–218.

Beltrán, Y., Centeno, C.M., García-Oliva, F., Legendre, P. & Falcón, L.I. (2012). $N_2$ fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia from different aquatic environments in Mexico. *Aquatic Microbial Ecology*, **67**: 15–24.

Berelson, W., Corsetti, F., Pepe-Ranney, C., Hammond, D., Beaumont, W. & Spear, J. (2011). Hot spring siliceous stromatolites from Yellowstone National Park: assessing growth rate and laminae formation. *Geobiology*, **9**: 411–424.

Bouton, A., Vennin, E., Pace, A., Bourillot, R., Dupraz, C., Thomazo, C., Brayard, A., Désaubliaux, G. & Visscher, P.T. (2016). External controls on the distribution, fabrics and mineralization of modern microbial mats in a coastal hypersaline lagoon, Cayo Coco (Cuba). *Sedimentology*, **63**: 972–1016.

Brito, A., Ramos, V., Mota, R., Lima, S., Santos, A., Vieira, J., Vieira, C.P., Kaštovský, J., Vasconcelos, V.M. & Tamagnini, P. (2017). Description of new genera and species of marine cyanobacteria from the Portuguese Atlantic coast. *Molecular Phylogenetics and Evolution*, **111**: 18–34.

Budinoff, C.R. (2005). Ecophysiology of a Mono Lake cyanobacterium. PhD thesis, University of Georgia.

Burne, R.V. & Moore, L.S. (1987). Microbialites; organosedimentary deposits of benthic microbial communities. *Palaios*, **2**: 241–254.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, **13**(7): 581–583.

Cam, N., Benzerara, K., Georgelin, T., Jaber, M., Lambert, J.F., Poinsot, M., Skouri-Panet, F., Moreira, D., López-García, P. & Raimbault, E. (2018). Cyanobacterial formation of intracellular Ca-carbonates in undersaturated solutions. *Geobiology*, **16**: 49–61.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. & Gordon, J.I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**: 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L. & Bauer, M. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, **6**: 1621–1624.

Centeno, C.M., Legendre, P., Beltrán, Y., Alcántara-Hernández, R.J., Lidström, U.E., Ashby, M.N. & Falcón, L.I. (2012). Microbialite genetic diversity and composition relate to environmental variables. *FEMS Microbiology Ecology*, **82**: 724–735.

Chagas, A.A., Webb, G.E., Burne, R.V. & Southam, G. (2016). Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy. *Earth-Science Reviews*, **162**: 338–363.

Couradeau, E., Benzerara, K., Moreira, D., Gerard, E., Kaźmierczak, J., Tavera, R. & López-García, P. (2011). Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). *PLoS ONE*, **6**: e28767.

Dupraz, C., Reid, R.P., Braissant, O., Decho, A.W., Norman, R.S. & Visscher, P.T. (2009). Processes of carbonate precipitation in modern microbial mats. *Earth-Science Reviews*, **96**: 141–162.

Edgar, R. (2010). *Usearch*. Lawrence Berkeley National Lab. (LBNL), Berkeley, CA.

Falcón, L.I., Cerritos, R., Eguiarte, L.E. & Souza, V. (2007). Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas, Mexico. *Microbial Ecology*, **54**: 363–373.

Falcón, L.I., Escobar-Briones, E. & Romero, D. (2002). Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico. *Hydrobiologia*, **467**: 71–78.

Falcón, L.I., Valdespino-Castillo, P.M., Alcántara-Hernández, R.J., Gómez-Acata, E.S., Yanez-Montalvo, A. & Águila, B. (2020). Stromatolites in Crater-Lake Alchichica and Bacalar Lagoon. In *Astrobiology and Cuatro Ciénegas Basin as an Analog of Early Earth* (Foster, J.S., Segura, A. & Souza, V., editors), 183–201. Springer, Cham.

Fayyad, R.J. & Dwaish, A.S. (2016). New modified protocol of DNA extraction comparison with other extraction methods for polymerase chain reaction analysis of gnomic DNA from Cyanophyceae isolates. *Advances in Environmental Biology*, **10**: 77–83.

Flannery, D.T. & Walter, M.R. (2012). Archean tufted microbial mats and the Great Oxidation Event: new insights into an ancient problem. *Australian Journal of Earth Sciences*, **59**: 1–11.

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

Garcia-Pichel, F., Zehr, J.P., Bhattacharya, D. & Pakrasi, H. B. (2020) What's in a name? The case of cyanobacteria. *Journal of Phycology*, **56**: 1–5.

Gérard, E., De Goeyse, S., Hugoni, M., Agogué, H., Richard, L., Milesi, V., Guyot, F., Lecourt, L., Borensztajn, S. & Joseph, M.-B. (2018). Key role of alphaproteobacteria and cyanobacteria in the formation of stromatolites of Lake Dziani Dzaha (Mayotte, Western Indian Ocean). *Frontiers in Microbiology*, **9**: 796.

Gérard, E., Ménez, B., Couradeau, E., Moreira, D., Benzerara, K., Tavera, R. & López-García, P. (2013). Specific carbonate–microbe interactions in the modern microbialites of Lake Alchichica (Mexico). *ISME Journal*, **7**: 1997–2009.

Gómez-Acata, E.S., Centeno, C.M. & Falcón, L.I. (2019). Methods for extracting 'omes from microbialites. *Journal of Microbiological Methods*, **160**: 1–10.

Johnson, D., Beddows, P.A., Flynn, T. & Osburn, M.R. (2018). Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar, Mexico. *Geobiology*, **16**: 319–337.

Johnson, J.S., Spakowicz, D.J., Hong, B.-Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O. & Gerstein, M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, **10**: 1–11.

Kamennaya, N.A., Ajo-Franklin, C.M., Northen, T. & Jansson, C. (2012). Cyanobacteria as biocatalysts for carbonate mineralization. *Minerals*, **2**: 338–364.

Kaźmierczak, J., Kempe, S., López-Garcia, P., Tavera, R., Kremer, B. & Moreira, D. (2008). Modern and subrecent carbonate microbialites from the alkaline crater lake Alchichica, Mexico. In *Geobiology of Stromatolites* (Reitner, J., Quéric, N. & Reich, M., editors), 85–88. International Kalkowsky-Symposium, Abstract Volume and Field Guide to Excursions, Gottingen.

Kaźmierczak, J., Kempe, S., Kremer, B., López-García, P., Moreira, D. & Tavera, R. (2011). Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico. *Facies*, **57**: 543–570.

Kempe, S. & Kaźmierczak, J. (2012). Terrestrial analogues for early planetary oceans: Niuafo 'ou Caldera Lakes (Tonga) and their geology, water chemistry, and stromatolites. In *Life on Earth and other Planetary Bodies* (Hanslmeier, A., Kempe, S. & Seckbach, J., editors), 195–234. Springer, Dordrecht.

Kent, W.J. (2002). BLAT – the BLAST-like alignment tool. *Genome Research*, **12**: 656–664.

Komárek, J. (2006). Cyanobacterial taxonomy: current problems and prospects for the integration of traditional and molecular approaches. *Algae*, **21**: 349–375.

Komárek, J. (2016). A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. *European Journal of Phycology*, **51**: 346–353.

Komárek, J. & Anagnostidis, K.C. (1998). *Süßwasserflora von Mitteleuropa*. Jena, Stuttgart.

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

Krumbein, W.E., Brehm, U., Gerdes, G., Gorbushina, A.A., Levit, G. & Palinska, K.A. (2003). Biofilm, biodictyon, biomat microbialites, oolites, stromatolites geophysiology, global mechanism, parahistology. In *Fossil and Recent Biofilms* (Krumbein, W.E., Paterson, D.M. & Zavarzin, G.A., editors), 1–27. Springer, Dordrecht.

Laval, B., Cady, S.L., Pollack, J.C., McKay, C.P., Bird, J.S., Grotzinger, J.P., Ford, D.C. & Bohm, H.R. (2000). Modern freshwater microbialite analogues for ancient dendritic reef structures. *Nature*, **407**: 626–629.

López-García, P., Kaźmierczak, J., Benzerara, K., Kempe, S., Guyot, F. & Moreira, D. (2005). Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey. *Extremophiles*, **9**: 263–274.

Mai, T. (2016). Revision of the Synechococcales (cyanobacteria) through recognition of four families including Oculatellaceae fam. nov. and Trichocoleaceae fam nov. and seven new genera containing 14 species. Masters dissertation, JCU.

Mancilla Villa, O.R., Bautista Olivas, A.L., Ortega Escobar, H.M., Sánchez Bernal, E.I., Can Chulim, Á., Guevara Gutiérrez, R.D. & Ortega Mikolaev, Y.M. (2014). Hidrogeoquímica de salinas Zapotitlán y los lagos-cráter Alchichica y Atexcac, Puebla. *Idesia (Arica)*, **32**: 55–69.

Merz, M.U. (1992). The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**: 81–101.

Mobberley, J.M., Khodadad, C.L. & Foster, J.S. (2013). Metabolic potential of lithifying cyanobacteria-dominated thrombolitic mats. *Photosynthesis Research*, **118**: 125–140.

Neilan, B.A., Jacobs, D., Blackall, L.L., Hawkins, P.R., Cox, P.T. & Goodman, A.E. (1997). rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus Microcystis. International Journal of Systematic and Evolutionary Microbiology, **47**(3): 693–697.

Nübel, U., Garcia-Pichel, F. & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and Environmental Microbiology*, **63**: 3327–3332.

Nutman, A.P., Bennett, V.C., Friend, C.R., Van Kranendonk, M.J. & Chivas, A.R. (2016). Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. *Nature*, **537**: 535–538.

Obst, M., Dynes, J., Lawrence, J., Swerhone, G., Benzerara, K., Karunakaran, C., Kaznatcheev, K., Tyliszczak, T. & Hitchcock, A. (2009). Precipitation of amorphous CaCO3 (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. *Geochimica et Cosmochimica Acta*, **73**: 4180–4198.

Oliva, M.G., Lugo, A., Alcocer, J., Peralata, L. & Oseguera, L.A. (2009). Planktonic bloom-forming *Nodularia* in the saline Lake Alchichica, Mexico. *Natural Resources and Environmental Issues*, **15**: 22.

Oren, A. & Ventura, S. (2017). The current status of cyanobacterial nomenclature under the "prokaryotic" and the "botanical" code. *Antonie van Leeuwenhoek*, **110**: 1257–1269.

Palinska, K.A., Thomasius, C.F., Marquardt, J. & Golubic, S. (2006). Phylogenetic evaluation of cyanobacteria preserved as historic herbarium exsiccata. *International Journal of Systematic and Evolutionary Microbiology*, **56**: 2253–2263.

Pereira, S., Micheletti, E., Zille, A., Santos, A., Moradas-Ferreira, P., Tamagnini, P. & De Philippis, R. (2011). Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional

groups and also accumulate inside the cell? *Microbiology*, **157**: 451–458.

Pittera, J., Humily, F., Thorel, M., Grulois, D., Garczarek, L. & Six, C. (2014). Connecting thermal physiology and latitudinal niche partitioning in marine *Synechococcus*. *ISME Journal*, **8**: 1221–1236.

Power, I., Wilson, S., Dipple, G. & Southam, G. (2011). Modern carbonate microbialites from an asbestos open pit pond, Yukon, Canada. *Geobiology*, **9**: 180–195.

Pruesse, E., Peplies, J. & Glöckner, F.O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics*, **28**: 1823–1829.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, **41**: D590–D596.

Ragon, M., Benzerara, K., Moreira, D., Tavera, R. & López-García, P. (2014). 16S rDNA-based analysis reveals cosmopolitan occurrence but limited diversity of two cyanobacterial lineages with contrasted patterns of intracellular carbonate mineralization. *Frontiers in Microbiology*, **5**: 331.

Riding, R. (2011). Microbialites, stromatolites, and thrombolites. In *Encyclopedia of Geobiology* (Reitner, J. & Thiel, V., editors). Springer, Dordrecht.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. & Stanier, R.Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, **111**: 1–61.

Russell, J.A., Brady, A., Cardman, Z., Slater, G., Lim, D. & Biddle, J. (2014). Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada. *Geobiology*, **12**: 250–264.

Russell, M.J. (1996). The generation at hot springs of sedimentary ore deposits, microbialites and life. *Ore Geology Reviews*, **10**: 199–214.

Saghaï, A., Zivanovic, Y., Moreira, D., Benzerara, K., Bertolino, P., Ragon, M., Tavera, R., López-Archilla, A. I. & López-García, P. (2016). Comparative metagenomics unveils functions and genome features of microbialite-associated communities along a depth gradient. *Environmental Microbiology*, **18**: 4990–5004.

Saghaï, A., Zivanovic, Y., Zeyen, N., Moreira, D., Benzerara, K., Deschamps, P., Bertolino, P., Ragon, M., Tavera, R. & López-Archilla, A.I. (2015). Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. *Frontiers in Microbiology*, **6**: 797.

Salmaso, N., Cerasino, L., Boscaini, A. & Capelli, C. (2016). Planktic *Tychonema* (Cyanobacteria) in the large lakes south of the Alps: phylogenetic assessment and toxigenic potential. *FEMS Microbiology Ecology*, **92**: fiw155.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H. & Robinson, C.J. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, **75**: 7537–7541.

Schneider, D., Arp, G., Reimer, A., Reitner, J. & Daniel, R. (2013). Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of the Kiritimati Atoll, Central Pacific. *PLoS ONE*, **8**: e66662.

Shalygin, S., Kavulic, K.J., Pietrasiak, N., Bohunická, M., Vaccarino, M.A., Chesarino, N.M. & Johansen, J.R. (2019). Neotypification of *Pleurocapsa fuliginosa* and epitypification of *P. minor* (Pleurocapsales): resolving a polyphyletic cyanobacterial genus. *Phytotaxa*, **392**: 245.

Soo, R.M., Hemp, J., Parks, D.H., Fischer, W.W. & Hugenholtz, P. (2017). On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria. *Science*, **355**: 1436–1440.

Souza, V., Siefert, J.L., Escalante, A.E., Elser, J.J. & Eguiarte, L.E. (2012). The cuatro ciénegas basin in Coahuila, Mexico: an astrobiological Precambrian park. *Astrobiology*, **12**: 641–647.

Tavera, R. & Komárek, J. (1996). Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla State, Mexico. *Archiv für Hydrobiologie*, **117**: 511–538.

Valdespino-Castillo, P.M., Alcántara-Hernández, R.J., Alcocer, J., Merino-Ibarra, M., Macek, M. & Falcón, L. I. (2014). Alkaline phosphatases in microbialites and bacterioplankton from Alchichica soda lake, Mexico. *FEMS Microbiology Ecology*, **90**: 504–519.

Wade, B.D. & Garcia-Pichel, F. (2003). Evaluation of DNA extraction methods for molecular analyses of microbial communities in modern calcareous microbialites. *Geomicrobiology Journal*, **20**, 549–561.

Waterbury, J.B. & Stanier, R.Y. (1978). Patterns of growth and development in Pleurocapsalean cyanobacteria. *Microbiological Reviews*, **42**: 2.

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis*. Springer, Dordrecht.

Wilmotte, A., Van der Auwera, G. & De Wachter, R. (1993). Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *Chlorogloeopsis* (HTF) *Mastigocladus laminosus* (HTF) strain PCC7518 and phylogenetic analysis. *FEBS Letters*, **317**: 96–100.

Wu, J. & Xi, C. (2009). Evaluation of different methods for extracting extracellular DNA from the biofilm matrix. *Applied and Environmental Microbiology*, **75**: 5390–5395.

Yanez-Montalvo, A., Gómez-Acata, S., Águila, B., Hernández-Arana, H. & Falcón, L.I. (2020). The microbiome of modern microbialites in Bacalar Lagoon, Mexico. *PLoS ONE*, **15**: e0230071.

Zhu, T. & Dittrich, M. (2016). Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. *Frontiers in Bioengineering and Biotechnology*, **4**: 4.

### 5.3.2 Microbialites show a distinct cyanobacterial phylogenetic structure and functional redundancy in Bacalar lagoon and Cenote Azul sinkhole, Yucatan Peninsula, Mexico.

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# Microbialites show a distinct cyanobacterial phylogenetic structure and functional redundancy in Bacalar lagoon and Cenote Azul sinkhole, Yucatan Peninsula, Mexico

B. Águila[1,2,3], A. Yanez-Montalvo[4], R. A. Mercado-Juárez[1,2,3], G.A. Montejano[5], I. Becerra-Absalón[5], L.I. Falcón[1,2,*]

[1]Instituto de Ecología, UNAM Campus Yucatán, Parque Científico y Tecnológico de Yucatán, 97302, México
[2]Instituto de Ecología, UNAM, Coyoacán, CdMx, 04510, México
[3]Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1° Piso, Circuito de Posgrados CU, Coyoacán, CdMx, 04510, México
[4]El Colegio de la Frontera Sur Unidad Chetumal, Av. Centenario Km. 5.5, Chetumal, Quintana Roo 77014, México
[5]UNAM, Laboratorio de Ficología, Facultad de Ciencias, Av. Universidad 3000, CdMx, 04510, México
*Corresponding author: UNAM, Instituto de Ecología, Campus Yucatán, Yucatán, 97302, México. Tel: +52 5521837123; E-mail: falcon@ecologia.unam.mx
**One sentence summary:** This manuscript describes cyanobacterial taxonomic and functional diversity within microbialites in BL and the CA sinkhole Quintana Roo, Mexico.
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## Abstract

Cyanobacterial components of microbialites from two geographically close systems, the Bacalar lagoon (BL) and the Cenote Azul sinkhole (CA) in Quintana Roo, Mexico, were characterized. BL and CA systems were studied along a longitudinal gradient (north to south) and a depth gradient (5–30 m), respectively. Microscopic observations, 16S rRNA amplicon sequencing, and shotgun metagenomics were used to characterize Cyanobacteria. Both systems showed similar metabolic/functional profiles but harbored completely different cyanobacterial taxa. BL was dominated by Nostocales, including a population of previously undescribed *Chakia* sp., while CA was dominated by an unknown taxon of Chroococcales, comprising 70% of relative abundance through all depths. Interestingly, cyanobacterial assemblages in microbialites exhibited phylogenetic overdispersion in most of the BL sites, while CA sites exhibited phylogenetic clustering, these differences were attributed to depth/light conditions and possibly different times of geological formation for BL and CA systems.

**Keywords:** Cyanobacteria, functional redundancy, Bacalar, Cenote Azul, microbial ecology, microbialites, phylogenetic structure

## Introduction

Microbialites are organosedimentary structures where microbial communities favor the accretion, precipitation, and binding of minerals (Burne and Moore 1987, Riding 2011) Microbialites have also been defined as microbial mats that undergo a lithification process (Dupraz *et al.* 2009). Modern occurrences of microbialites are unusual, with only 20–30 documented sites in the world. Sites include soda lakes, hypersaline lakes, oligotrophic lakes or pools, pits, brackish waters, and marine environments (Russell 1996, Laval *et al.* 2000, Arp *et al.* 2003, Gischler *et al.* 2008, Foster *et al.* 2009, Kempe and Kazmierczak 2012, Souza *et al.* 2012), within these sites, microbialites are confined in zones where conditions are harsh for microbial grazers. On average, modern microbialites originated in the Holocene and are dated between 2000 and 9000 years old. Yet these structures have been hypothesized to be analogous to fossil stromatolites, which have been dated up to 3.7 billion years (Flannery and Walter 2012, Nutman *et al.* 2016).

Several studies have identified important microorganisms for the formation of microbialites, including Cyanobacteria, sulfate reducing bacteria, alphaproteobacteria, and viruses (Chagas *et al.* 2016, White *et al.* 2016, Zhu and Dittrich 2016). Cyanobacteria are fundamental for microbialite formation because of their role as: (1) the main diazotrophs in microbialites, (2) are the principal primary producers, (3) photosynthesis changes microhabitat conditions, elevating the pH which in turn increases extracellular precipitation, and (4) extracellular polymeric substances (EPS) formation that trap cations (e.g. $Mg^{2+}$ or $Ca^{2+}$) to regulate the formation of minerals (Obst *et al.* 2009, Pereira *et al.* 2011, Kamennaya *et al.* 2012). Some studies have also suggested a process of intracellular precipitation in Cyanobacteria that could favor microbialite formation (Ragon *et al.* 2014, Cam *et al.* 2018).

Mexico has important systems with microbialites. For instance, Bacalar Lagoon (BL) system is a 40-km long oligotrophic lagoon rich in sulfates and carbonates, which contains the largest reef of freshwater microbialites in the world (Gischler *et al.* 2008, Beltrán *et al.* 2012, Centeno *et al.* 2012, Valdespino-Castillo *et al.* 2018). Cenote Azul (CA) system is a 70-m sinkhole with 200 m in diameter, separated from BL by 100 m, where microbialites have been described in its west wall, from a depth of 3–30 m (Yanez-Montalvo *et al.* 2021). Both systems are located in the Yucatan Peninsula (YP), a karst platform dominated by evaporitic rock and an interconnected underground aquifer that extends over 165 000 $km^2$ in Mexico, Guatemala and Belize (Bauer-Gottwein *et al.* 2011). Although BL and CA share similar hydrogeochemi-

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cal characteristics, several studies have demonstrated major differences and isolation of their biological components (Perry *et al.* 2018, Montes-Ortiz and Elías-Gutiérrez 2020, Schmitter-Soto 2020, Yanez-Montalvo *et al.* 2021). Moreover, geologic data supports differences in isotopic firms, which indicate that the CA system predates the formation of the BL system (Schmitter-Soto *et al.* 2002, Perry *et al.* 2009)

Microbial ecology studies in microbialites from BL, have identified *Leptolyngbya* sp., *Limnothrix* sp., *Scytonema* sp., and *Calothrix* sp., which exhibit different processes of carbonate deposition (Gischler *et al.* 2008, 2011). Also, Nostocales have been postulated to be the main diazotrophs in BL (Beltrán *et al.* 2012). Microbial diversity studies in BL have suggested that microbialites in the north region are more diverse (including Cyanobacteria) than those in the central and south regions. This microbial dysbiosis in southern microbialites has been attributed to eutrophication processes because of anthropogenic activities and a recent NOx levels increase (Rosado Varela and Medina Argueta 2014, Yanez-Montalvo *et al.* 2020).

To date, no cyanobacterial descriptions exist in microbialites from CA. Microbial diversity studies in CA, show that microbialites from 5 to 10 m are more closely related phylogenetically with each other, while microbialites from 20 to 30 m are also closely related. Microbialites from different depths are suggested to represent different times of formation, where deeper microbialites represent the oldest structures (Yanez-Montalvo *et al.* 2021).

In this work, we aimed to characterize the cyanobacterial component of microbialites from BL and the CA sinkhole. For this, we used microscopic observations, 16S rRNA sequence analysis, and functional genomics. We wanted to test if the Cyanobacteria of microbialites in these two systems were phylogenetically or functionally related, since both systems share abiotic characteristics and are geographically close.

## Materials and methods

### Sample collection

Microbialites from BL were collected from shallow waters (> 1 m) in 15 sites along a north–south gradient in the lagoon. A total of five subsamples (cores of approximately 2.5 cm in diameter) were obtained from individual microbialite, and three to five individuals per site were sampled. Samples were taken with gloves and sterile material to avoid cross-site contamination. For CA, microbialites were obtained in a bathymetric profile, at 30, 20, 10, and 5 m by scuba diving. A total of five subsamples (10 × 5 × 2 cm) were collected in each depth, with separation of approximately 1 m per sample, using a sterile chisel and a sledgehammer. The fragments were placed individually in nets previously labeled for each depth. The sampling was carried out during the spring and winter of 2018 (BL) and winter of 2019 (CA). All microbialite samples were placed in refrigeration at 4°C for transport to the laboratory, then stored in a freezer at −80°C. All microbialites were sampled under collector permit PPF/DGOPA-113/14. Field studies did not involve endangered or protected species.

### Physicochemical data

Conductivity/temperature and pH were measured *in situ* using a YSI Professional handheld (YSI model Pro 30) and a pH-meter (Hanna HI 9146). NOx (nitrites and nitrates) were measured with a UV–visible spectrophotometer (SHIMADZU, Model UV-1700) in the Chemistry Laboratory at ECOSUR-Chetumal, Mexico. $SO_4$ (sulfates) were determined by ion chromatography with a Waters 1525 binary HPLC pump, autosampler (model number 717) and conductivity detector (model number 432) in the LANGEM-PT-LCL laboratory at the Institute of Geology UNAM, Mexico. Calcium and bicarbonates were determined with an EMPYREAN Diffractometer equipped with an Fe filter, a cobalt thin tube focus and PIXcel3D detector in the X-Ray Diffraction Laboratory at the Instituto of Geology UNAM, Mexico. Light intensity for each site was calculated as a proportion of light flux at certain depth with the formula Id = I0 e^(-kd), where d is depth, I0 is 100% of light intensity at 0 m, and k is the vertical extinction coefficient (k = 1.7/Secchi depth). Secchi depths from BL and CA were recovered from the bibliography (Cervantes-Martínez *et al.* 2009, Smirnov and Elías-Gutiérrez 2011). A supplementary table with physicochemical values, type of microbialite, type of analysis, and the region assigned for each site is presented (Table 1).

### Microscopic methods

Subsamples of 15 sites in BL ($n$ = 120) and four depths in CA ($n$ = 60) were examined with optical microscopy in semipermanent preparations made with phenol–glycerin (20/20 g/l) and dry microbialites fragments previously treated with acetic acid (5%) for demineralization of carbonates. Observations were made in a DIC Olympus BXX51 microscope and a BX51 microscope coupled to a Hg fluorescence lamp, equipped with a U-MNG2 Green filter (excitation 530–550 nm; emission 590 nm) for autofluorescence of phycoerythrin. The Sigma Scan Pro V5 software (Systat Software Inc., SJ) was used for morphometric analysis. Classification of Cyanobacteria was made according to the scheme proposed by Komárek from the latest revisions (Komárek and Anagnostidis 1998, Komarek 2006, Komárek *et al.* 2014). In addition, taxonomic characterizations of Cyanobacteria in alkaline wetland systems in the Caribbean region and the YP, were used as reference (Komárek and Komárková-Legnerová 2007, Turicchia *et al.* 2009, Komárková *et al.* 2013, Komárek *et al.* 2017).

### DNA extraction

DNA extractions of each microbialite subsample were done in triplicate using the DNeasy PowerSoil® Kit (Qiagen) following the manufacturer's instructions. DNA was visualized on a 1% agarose gel and quantified by spectrophotometry (EPOCH™ BioTek) and fluorometry (Qubit® dsDNA HS Assay Kit, Thermo Scientific™).

### DNA amplification and sequencing

Amplicon libraries of the V4 region of the 16S rRNA were prepared for each subsample in triplicate using primers 515F-806R and following previous protocols (Caporaso *et al.* 2012). The polymerase chain reaction (PCR) was performed in a volume of 25 μl containing total DNA (1–20 ng), 1 U Taq DNA polymerase (Invitrogen™), $MgCl_2$ (1.5 mM), Taq Pol Buffer (200 mM Tris-HCl, pH 8.4, and 500 mM KCl), primers (0.4 μM), and dNTPs (200 mM). PCR conditions included: 98°C for 30 s followed by 35 cycles of 95°C for 30 s, 52°C for 40 s, and 72°C for 90 s, and a final elongation step of 12 min at 72°C, then kept at 4°C. PCR products were pooled and purified with Ampliclean carboxyl-coated magnetic beads (NimaGen, NDL). The purified amplicon library was quantified with a QUBIT fluorometer (Promega). The amplicon library with 20 ng/μl per sample was sequenced on an Illumina MiSeq 2 × 250 platform. Whole genome sequencing was run in a 2 × 150 NovaSeq system with gDNA libraries prepared after pooling the following samples (1 μg/μl): (1) BL northern and center regions (BL-NC) included sites BL1, BL2, BL3, BL6, BL7, and, BL8. (2) CA shallow region (CA-S) included CA05 and CA10, and (3) CA depth re-

**Table 1.** Physicochemical values and type of analysis for each site.

| Site | Region | Miseq | Shotgun | Depth (m) | pH | Conductivity (μS/cm) | NOx (mg/l) | Sulfate (mg/l) | Calcium (mg/l) | Bicarbonate (mg/l) | Morphology | Longitude | Latitude |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BL01 | North | x | BL-NC | 1 | 6.9 | 4620 | 2.09 | 1170 | 338 | 110 | Crusts | −88.236789 | 18.877908 |
| BL02 | North | x | BL-NC | 1 | 7.2 | 4436 | 2.05 | 1170 | 338 | 110 | Heads | −88.248486 | 18.860753 |
| BL03 | North | x | BL-NC | 1 | 7.3 | 4340 | 1.75 | 1030 | 310 | 104 | Heads | −88.254152 | 18.85268 |
| BL04 | North | x | - | 1 | 7.9 | 2950 | 3.54 | 1140 | 388 | 120 | Heads | −88.308938 | 18.76191 |
| BL05 | North | x | - | 1 | 7.1 | 2700 | 1.44 | 1140 | 400 | 120 | Dome | −88.354056 | 18.728306 |
| BL06 | Center | x | BL-NC | 1 | 7.3 | 2040 | 7.31 | 1190 | 404 | 140 | Heads | −88.367901 | 18.722989 |
| BL07 | Center | x | BL-NC | 1 | 7.2 | 2150 | 8.41 | 1190 | 404 | 140 | Heads | −88.373181 | 18.718401 |
| BL08 | Center | x | BL-NC | 1 | 7.3 | 2300 | 16.43 | 1190 | 404 | 140 | Domes | −88.381167 | 18.709669 |
| BL09 | Center | x | - | 1 | 7.5 | 2280 | 4.05 | 1154 | 313 | 104 | Crusts | −88.385422 | 18.635899 |
| BL10 | Center | x | - | 1 | 7.4 | 2470 | 7.04 | 1190 | 404 | 140 | Domes | −88.385142 | 18.695144 |
| BL11 | Center | x | - | 1 | 7.9 | 2480 | 7.99 | 1190 | 404 | 140 | Spherical | −88.384379 | 18.684996 |
| BL12 | South | x | - | 1 | 7.5 | 2550 | 13.93 | 1072 | 325 | 183 | Heads | −88.408765 | 18.651161 |
| BL13 | South | x | - | 1 | 7.5 | 2130 | 24.51 | 1185 | 329 | 220 | Ledges | −88.436822 | 18.587259 |
| BL14 | South | x | - | 1 | 7.7 | 2460 | 22.73 | 1038 | 314 | 238 | Ledges | −88.44439 | 18.572474 |
| BL15 | South | x | - | 1 | 7.2 | 2580 | 21.87 | 1031 | 309 | 232 | Ledges | −88.444376 | 18.563962 |
| CA05 | Shallow | x | CA-S | 5 | 7.9 | 2330 | 28.1 | 1226.4 | 426.6 | 193.8 | Ledges | −88.412624 | 18.647086 |
| CA10 | Shallow | x | CA-S | 10 | 7.9 | 2320 | 0.01 | 1231.8 | 404.9 | 220.2 | Ledges | −88.412624 | 18.647086 |
| CA20 | Depth | x | CA-D | 20 | 8.1 | 2290 | 0.01 | 1234.9 | 432.2 | 231.6 | Domes | −88.412624 | 18.647086 |
| CA30 | Depth | x | CA-D | 30 | 8.1 | 2300 | 0.01 | 1245.4 | 432.6 | 217 | Domes | −88.412624 | 18.647086 |

gion (CA-D) included CA20 and CA30. All sequencing and gDNA libraries were done in the Yale Center for Genome Analysis, CT, USA.

## Sequence analysis

Illumina MiSeq amplicon 16S rRNA raw sequences were analyzed with the QIIME2 2018.8 (Hall and Beiko 2018) workflow. Pair end reads were demultiplexed into single reads, and sequences were grouped in ASVs with DADA2 v1.16 (Callahan *et al.* 2016). Taxonomy classification was assigned with the Silva 132 99% OTUs DATAbase (Quast *et al.* 2012). Oxyphotobacterial ASVs were filtered from the ASV table excluding chloroplasts. ASVs were grouped in OTUs with a Denovo 97% cutoff. The OTU table was constructed with the Vsearch plugin, chimaeric OTUs were eliminated by USEARCH v6.1 software (Edgar 2010). Criteria for selection of the V4 region of the 16S rRNA sequences in this analysis is described below. For BL, a total of 147 439 Oxyphotobacteria sequences with no chloroplasts (250 bp) were filtered out from taxa tables (SILVA classification), sequences were grouped in 347 ASVs, and collapsed in 216 *de novo* OTUs with a 97% cut off; OTUs representing less than 1% of relative abundance per sample were filtered out, resulting in 39 OTUs for BL, which were assigned to 32 genera. The same procedure was done for CA, where a total of 538 479 Oxyphotobacteria sequences with no chloroplasts (250 bp) were filtered out from taxa tables (SILVA classification), sequences were grouped in 262 ASVs and collapsed in 197 OTUs with a 97% cut off, then OTUs representing less than 1% of relative abundance by sample were filtered out, resulting in 37 OTUs for CA which were assigned to 31 genera.

Whole genome sequences from BL-NC, CA-S, and CA-D were randomly filtered to 20 million sequences and submitted to be analyzed in the MG-RAST pipeline version 4.0.3 (Meyer *et al.* 2019) for assembly and annotation. Parallel to this, 16S rRNA related to Cyanobacteria from unassembled sequences was predicted. For this, quality control of raw reads of the whole genome libraries was done with BBMAP 38.86 (Bushnell 2014). Reads were assembled with MEGAHIT v1.2.9 (Li *et al.* 2015) with standard options. Sequence contigs were filtered to 1000 pb with PRINSEQ-lite 0.20.4 (Schmieder and Edwards 2011). Ribosomal genes were located and predicted with barrnap 0.9 (Seeman 2018). Predicted 16S rRNA sequences were blasted with BLASTn 2.10.0+ (Altschul *et al.* 1990) against SILVA database SSU 138 Ref NR 99 (Quast *et al.* 2012), resulting sequences related to Cyanobacteria were screened for phylogenetic analysis. Criteria for selection of the complete 16S rRNA sequences in this analysis is described below: A total of 72 16S rRNA sequences were obtained from assembled metagenomes (25 for BL-NC, 24 for CA-S, and 23 for CA-D). A total of 39 16S rRNA sequences could be aligned to the V3–V4 region and were trimmed to 743 bp and collapsed in 16 OTUs with a 97% cut off for later phylogenetic analysis.

## Phylogenetic analysis

A Neighbor Joining (NJ) phylogenetic tree was built in SeaView (Gouy *et al.* 2010), the parameters used were: Model: BioNJ, Distance method: K2P, ignoring gap sites, and 1000 Bootstrap. A Maximum Likelihood phylogenetic tree was built in ARBSilva (Pruesse *et al.* 2012), the parameters used were: RAxML GTR Model, Gamma rate for likelihood, Denovo construction with user sequences only, 10 neighbors per query sequence, and 0.95 of minimum identity with query sequence. Fixed positional variation was set at highly and moderate variable positions (1–9). SILVA, RDP, GTDB, LTP, and EMBL-EBI/ENA databases were used for the 16S rRNA phylogeny. Sequences used for both trees consisted of: (1) extracted cyanobacterial 16S rRNA sequences from assembled metagenomes (780–1220 bp), (2) V4 region of the 16S rRNA (250 bp), (3) curated 16S rRNA cyanobacterial sequences from bibliography (1400 bp). Sequences used in this study are available in GenBank under accession numbers MZ872836-MZ872911 and MZ874782-MZ874797. Aligned sequences and phylogenetic trees are available at the Github repository: https://github.com/Burn121212/BL-CA_Phy_trees_seqs

## Reclassification of molecular data

In order to classify Cyanobacteria according to the latest nomenclature (Komárek *et al.* 2014), ASVs (previously classified with SILVA 132 rRNA database) from previous microbialite studies in BL and CA (Yanez-Montalvo *et al.* 2020, 2021) were converted to OTUs and reclassified. The reclassification was based on: BLAST analysis, phylogenetic trees, microscopic observations, and taxonomic studies from similar ecological systems (Komárek and Komárková-Legnerová 2007, Komárková *et al.* 2013, Komárek *et al.* 2017). The phylogenetic tree used for the classification of taxa is presented (see Supplementary Figure 1) and the criteria used for reclassified OTUs is available at the GitHub repository: https://github.com/Burn121212/BL-CA_microbialites/blob/main/FLAX%20reclasificated%20OTUs.xlsx.

## Statistical analysis

With reclassified OTUs at genus level, data exploration was performed in the R environment (v 4.1.1). The following software packages were used: Ampvis2 (v 2.7.10; Andersen *et al.* 2018), Vegan (v 2.5; Oksanen *et al.* 2013), and PhyloMeasures (v 2.1; Tsirogiannis and Sandel 2016). For functional analysis, Cyanobacteria were filtered with the "filter by taxa" option in the MG-RAST and exported for analysis in STAMP (v 2.1.3; Parks *et al.* 2014). Files used for R statistical analysis are available at the Github repository: https://github.com/Burn121212/BL-CA_Cyano_R.

Files used for STAMP are available at the Github repository: https://github.com/Burn121212/BL-CA_Cyano-Functional-Profile.

# Results

## Site description

Microbialites were classified according to previous descriptions (Gischler *et al.* 2008) as heads (thrombolytic structures with widened top), spherical (Oncolites), ledges (finger-like structures), domes (semicircular thrombolites), and crusts (flatten layered thrombolites; see Supplementary Figure 2). Sampling sites are shown in Fig. 1. Significant physicochemical values that separated BL from CA were depth ($P < .001$) and light availability ($P < .01$; Table 2). Cyanobacterial communities of north and south regions from BL were associated with high conductivity and high NOx concentrations respectively, while communities of shallow and depth regions from CA were associated with high pH and high $SO_4$ respectively (see Supplementary Figure 3).

## Cyanobacterial classification

A total of 44 cyanobacterial taxa were identified in this study, of which 29 were classified to known genus and 15 were classified as putative new taxa (unknown Cyanobacteria). Within unknown Cyanobacteria, six were corroborated by shotgun assembly (780–1220 bp), and nine were obtained by 16S rRNA amplicons (250 bp). On average, unclassified taxa had less than 94% of similarity with reference sequences (GenBank; Benson *et al.* 2012).

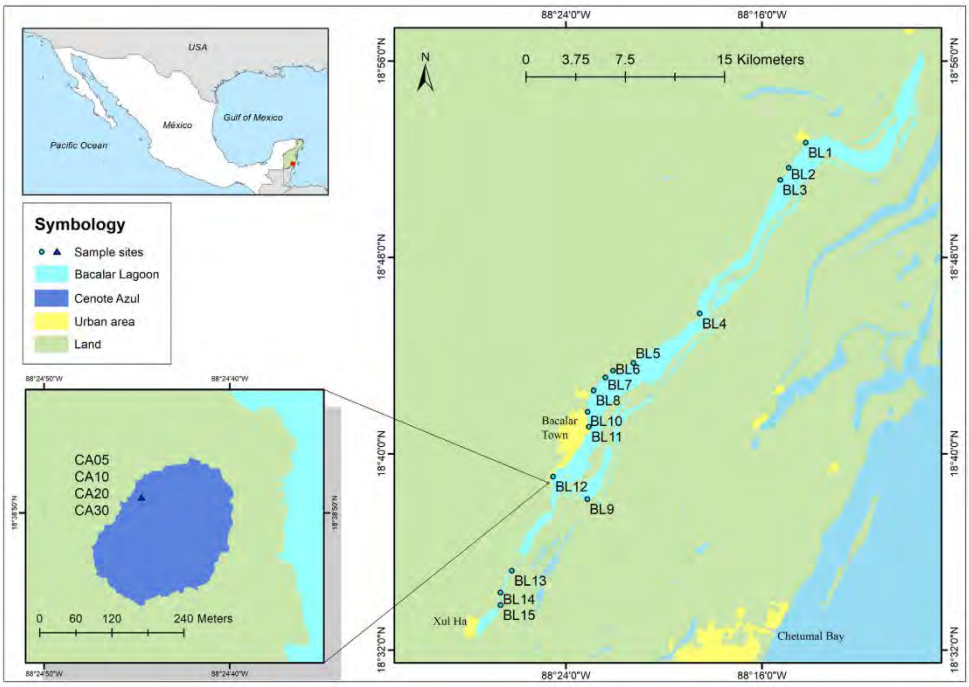


**Figure 1.** Map of the study site. BL and the CA sinkhole are located in the state of Quintana Roo, Mexico. BL was sampled along a north–south gradient while CA was sampled along a depth gradient on the west wall of the sinkhole.

**Table 2.** Statistical significance for physicochemical variables. '***' $P = 0$, '**' $P < .001$. (Adonis test).

| Value | f Su | msOfSqs M | eanSqs F | .Model | R2 P | r(> F) |
|---|---|---|---|---|---|---|
| Depth | 1 | 1.8226 | 1.82255 | 17.8986 | 0.47538 | 0.001 *** |
| Light intensity | 1 | 0.4296 | 0.42961 | 6.2218 | 0.11206 | 0.008 ** |
| pH | 1 | 0.2171 | 0.21712 | 2.1323 | 0.05663 | 0.138 |
| Conductivity | 1 | 0.1186 | 0.11862 | 1.1649 | 0.03094 | 0.291 |
| NOx | 1 | 0.0634 | 0.06337 | 0.6223 | 0.01653 | 0.537 |
| Sulfate | 1 | 0.1987 | 0.1987 | 1.9513 | 0.05183 | 0.147 |
| Calcium | 1 | 0.1615 | 0.16153 | 1.5863 | 0.04213 | 0.174 |
| Bicarbonate | 1 | 0.1319 | 0.13187 | 1.2951 | 0.0344 | 0.261 |
| Residuals | 11 | 1.1201 | 0.10183 | | 0.29216 | |
| Total | 18 | 3.8339 | | | 1 | |

## Cyanobacterial distribution

Distribution of cyanobacterial taxa (44) included 32 in BL and 31 in CA, of which 13 were unique to BL and 12 to CA, sharing 43% of the taxa. Alpha diversity indexes (Shannon and Simpson) suggested Cyanobacteria in BL are more diverse than those in CA ($P < .01$; Table 3), while cyanobacterial alpha diversity decreased with depth in CA (see Supplementary Figure 4). Diversity indexes and a Venn diagram for taxa in BL and CA are shown (Fig. 2). Microscopic observations corroborated 15 cyanobacterial genera previously found by molecular methods. Most commonly observed Cyanobacteria in microbialites from BL and CA are shown (Fig. 3). Predicted and assembled 16S rRNA sequences from metagenomes corroborated 11 cyanobacterial taxa. A supplementary table with relative abundances and method for genus validation is presented (see Supplementary Figure 5).

In BL, *Scytonema* sp. and *Xenococcus* sp. exhibited preference for microbialites in the northern region while *Gloeobacter* sp., filamentous Synechococcales, *Elainella* sp. and Synechococcales VI, were abundant in southern microbialites. *Chakia* sp., *Rivularia* sp., and unknown Nostocales I, were the most abundant Cyanobacteria in BL microbialites, showing a homogeneous distribution in all samples. *Chroococcidiopsis* sp., was abundant in samples from central BL. For CA, *Xenococcus* sp., *Cyanosarcina*, sp., *Aphanocapsa* sp., and *Mastigocladus* sp., increased in abundance towards depth microbialites (20 and 30 m), while unknown filamentous Synechococcales V, and *Leptolyngbya* sp., were associated with shallow micro-

**Table 3.** Diversity index means, ** = $P < .001$.

| System | Chao1 | SD | CV | Shannon | SD | CV | Simpson | SD | CV |
|---|---|---|---|---|---|---|---|---|---|
| BL | 19.56667 | 6.614 | 0.338 | 2.00163176 | 0.175 | 0.087 | 0.30646724 | 0.11 | 0.358 |
| CA | 23.8125 | 6.176 | 0.259 | 1.07274618 | 0.29 | 0.27 | 0.08258689 | 0.016 | 0.191 |

$P = .27$, $P = .00052$**, and $P = .00052$**.

| Diversity index | BL | CA | |
|---|---|---|---|
| Total individuals (OTU abundance) | 44395 | 48237 | |
| Taxa number | 32 | 31 | |
| Richness (Chao1) | 19.566 | 23.812 | p= 0.27 |
| Alpha diversity (Shannon) | 3.2911 | 1.6411 | ** |
| Evenness (Simpson) | 0.9633 | 0.3123 | ** |
| Beta diversity | 0.6031 | | |

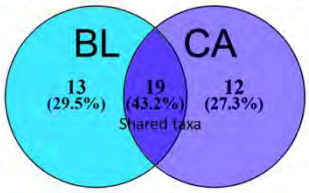


**Figure 2.** Chart with average diversity indexes from BL and CA. A Venn diagram shows the shared cyanobacterial genera recovered.

bialites (5 and 10 m). A previously undescribed Chroococcales I was the most abundant Cyanobacteria in all CA samples. *Acaryochloris* sp. and *Chroococcopsis* sp., were distributed homogeneously in all depths. *Planktothrix* sp. was equally distributed in BL and CA. A barplot of taxa by site is presented (Fig. 4).

An NMDS analysis of the most abundant genera (top 22) was made to represent distribution patterns between BL and CA (Fig. 5) showing a clear separation and a dominance of Nostocales in BL and Chroococcales in CA (solution reached at run 20, stress: 0.1104989). A supplementary figure of NMDS with all 44 taxa is presented (see Supplementary Figure 6).

### Cyanobacterial functional profile comparison

The cyanobacterial functional profiles of BL-NC, CA-S, and CA-D were similar (Fig. 6). All microbialites had on average 30% of unknown functional regions. Protein and lipids biosynthesis, nucleic acid processing, central carbohydrate metabolic pathways were common to all metagenomes of microbialites analyzed. A comparison of genetic regions associated to energy metabolic pathways including C, N, P, and S, suggested that the microbialites in CA-S and CA-D had a higher signal for sulfur assimilation, proteorhodopsin, PSI, phycobilisome synthesis, ammonia assimilation, and ammonification than microbialites from BL-NC, while Carboxysome, $CO_2$ uptake, and $N_2$ fixation had a stronger signal in BL-NC (Fig 7). No statistical differences were found for the functional profile of BL and CA microbialites ($P < .05$). The relative abundance of Cyanobacteria with respect to all bacteria and archaea in microbialites was: 15% in BL-NC, 10% in CA-S, and 12% in CA-D (data not shown).

### Phylogenetic structure

Standardized values of net relatedness index (NRI; SES.MPD), showed that Cyanobacteria exhibited phylogenetic overdispersion in all BL sites with the exception of BL12. For the rest of CA sites and BL12, Cyanobacteria exhibited phylogenetic clustering. Standardized value for nearest taxon index (NTI) the SES.MNTD, showed that Cyanobacteria had random phylogenetic structure in all sites (Table 4). The phylogenetic tree used for phylogenetic diversity (PD) analysis is shown (see Supplementary Figure 7).

## Discussion

### Putative new taxa of Cyanobacteria

BL microbialites harbor interesting putative new taxa including unknown Nostocales I, unknown filamentous Synechococcales III and VI. Due to their high abundance, it is possible they have important roles within microbialites, especially Nostocales (Beltrán *et al.* 2012). The rest of unknown genera from BL were not confirmed by shotgun sequencing data or did not represent more than 1% of relative abundance. For microbialites of the CA sinkhole, the most important Cyanobacteria by abundance was an unknown Chroococcales I, classified within the Merismopediaceae family, which was also found abundantly by microscopic analysis growing in colonies. Its morphology resembled *Aphanocapsa* sp., but bibliographic revision of Chroococcales of the YP, suggested it could be a form of *Eucapsis* sp. (Komárek and Komárková-Legnerová 2007, Krings and Sergeev 2019), unfortunately no sequence of *Eucapsis* sp. exists to date. Another interesting Cyanobacteria was unknown filamentous Synechococcales V, which could not be assigned to known families and increased its abundance toward 30 m. More work is required to corroborate unknown taxa identified solely by the V4 region of the 16S rRNA. For this, it is important a polyphasic approach, isolating and sequencing cultures (Palinska *et al.* 2006), which will validate new taxa and improve metagenomic databases with single-amplified genomes (SAGs; Alneberg *et al.* 2018). Nonetheless, sequence information provided by this work will ease future taxonomic works for freshwater Cyanobacteria in the YP.

### Functional redundancy or lack of information?

It is expected that no major differences exist in functional profiles when comparing Cyanobacteria from different systems, since they share basic metabolisms and have the genetic potential to produce similar metabolites (Yin *et al.* 2000, Yu and Whalen 2020), hence major differences are likely at the transcriptomic level (Rousk *et al.* 2009, Voorhies *et al.* 2016). Still, we wanted to test if some minor differential patterns were appreciated at the DNA level for two reasons: (1) CAP analysis proved that cyanobacterial communities in BL and CA had significant differences in depth and light conditions, (2) previous works (Yanez-Montalvo *et al.* 2021) had postulated microbial communities in CA are older than the ones on BL. Both assumptions should reflect minor changes in the DNA and a differential adaptation of Cyanobacteria (Klatt *et al.* 2011, Alvarenga *et al.* 2017). To this end, an interesting minor pattern in the energy profile was detected. For instance, the fixation of $N_2$ and $CO_2$ profiles were higher in BL, where Nostocales were abundant, while CA had higher relative abundance of genes related to photosynthesis, ammonification, and sulfur as-

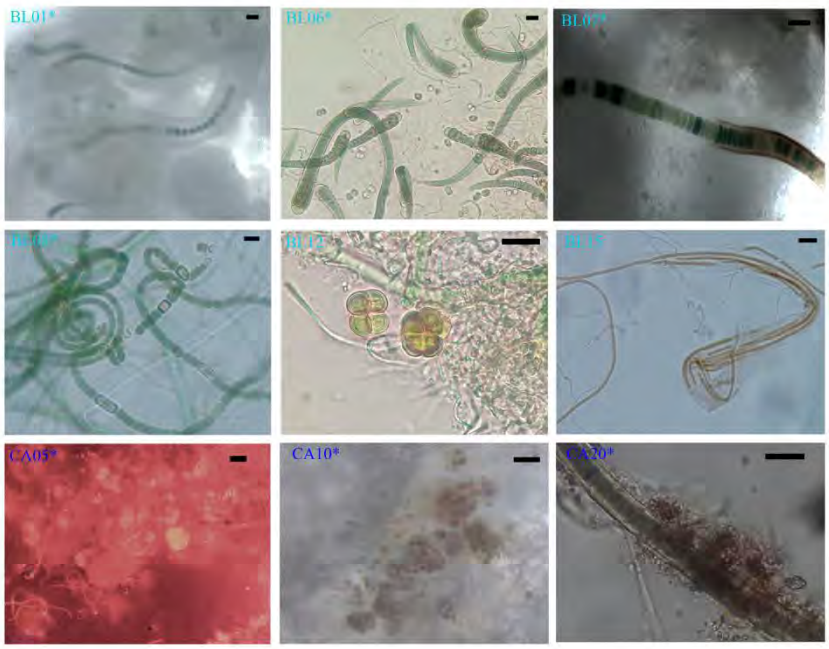


**Figure 3.** Optical and fluorescence microscopy from microbialites showing the most abundant taxa. BL01 *Chakia* sp., BL06 *Rivularia* sp., BL07 unknown Nostocales., BL08 Scytonema sp. BL12 *Chroococcidiopsis* sp., BL15 *Elainella* sp., CA05 colonies of unknown Chroococcales I along with filamentous Synechococcales, CA10 *Chroococcopsis* sp., and CA20 colonies of *Xenococcus* sp. growing epiphytic on an unknown Oscillatoriales.

**Table 4.** Phylogenetic distances for each cyanobacterial community by site.

| Site | PD | MPD | SES.PD | SES.MPD (-NRI) | SES.MNTD (-NTI) |
|---|---|---|---|---|---|
| BL01 | 1.7552 | 0.1695 | 0.4133 | 0.9090 | 0.0000 |
| BL02 | 1.6797 | 0.1694 | 0.1171 | 0.8503 | 0.0000 |
| BL03 | 1.7199 | 0.1699 | −0.1833 | 0.9966 | 0.0000 |
| BL04 | 1.1798 | 0.1738 | 0.7940 | 1.0966 | 0.0000 |
| BL05 | 0.8722 | 0.1741 | 1.0649 | 0.8571 | 0.0000 |
| BL06 | 1.8552 | 0.1712 | 0.1629 | 1.3593 | 0.0000 |
| BL07 | 1.5390 | 0.1653 | −0.2707 | 0.0804 | 0.0000 |
| BL08 | 1.7499 | 0.1677 | 0.3231 | 0.5524 | 0.0000 |
| BL09 | 1.8242 | 0.1719 | 0.6114 | 1.4497 | 0.0000 |
| BL10 | 0.9851 | 0.1702 | 0.7353 | 0.5636 | 0.0000 |
| BL11 | 0.9866 | 0.1719 | 0.7604 | 0.7338 | 0.0000 |
| BL12 | 0.6374 | 0.1577 | −0.7778 | **−0.5748** | 0.0000 |
| BL13 | 1.3050 | 0.1767 | 1.8285 | 1.5183 | 0.0000 |
| BL14 | 1.1216 | 0.1692 | 0.8734 | 0.5106 | 0.0000 |
| BL15 | 1.2757 | 0.1680 | 0.3349 | 0.4216 | 0.0000 |
| CA05 | 1.7430 | 0.1584 | −0.7753 | **−1.3245** | 0.0000 |
| CA10 | 1.7268 | 0.1618 | −0.0664 | **−0.5992** | 0.0000 |
| CA20 | 1.4198 | 0.1612 | −0.2753 | **−0.5649** | 0.0000 |
| CA30 | 1.0824 | 0.1556 | −0.8178 | **−1.1263** | 0.0000 |

similation. These adaptations have been reported for unicellular Synechococcales, Pleurocapsales, and Chroococcales of microbialites (Tandeau De Marsac 1977, Schwaderer *et al.* 2011, Roldán *et al.* 2013, Shalygin *et al.* 2019, Águila *et al.* 2021), which could mean a differential adaptation to lowlight conditions in CA. Although these minor differential patterns were not statistically significant, data should be considered in the context of two major biases when comparing functional profiles: (1) databases for metagenomes are still incomplete (Temperton and Giovannoni 2012). An important impediment to distinguishing functional differences on DNA profiles are the unclassified or unannotated sequences (Micallef *et al.* 2015). Between 65% and 75% of se-

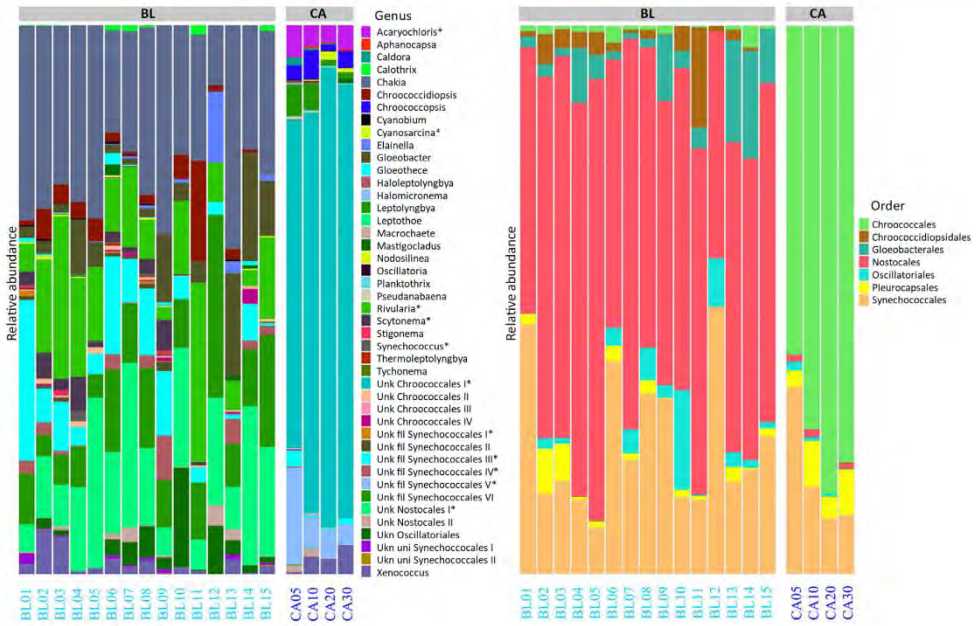


**Figure 4.** Barplots from BL and CA at genus, family and order level.

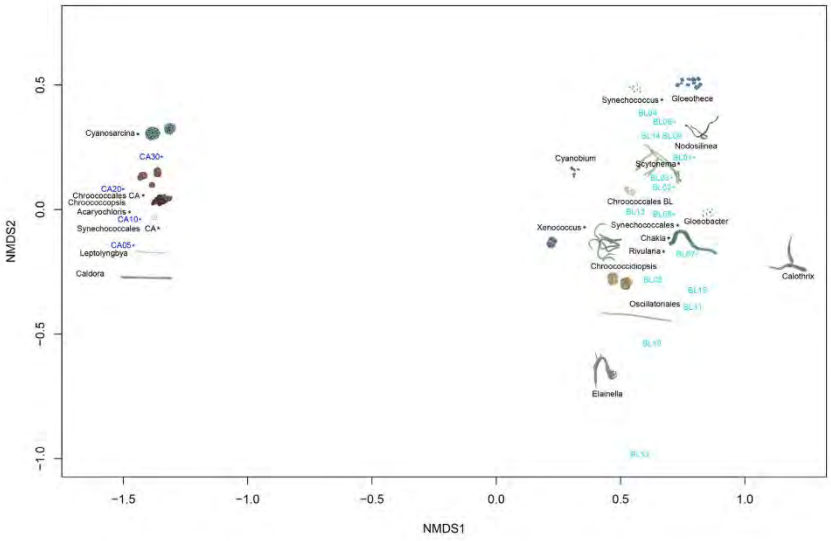


**Figure 5.** NMDS analysis showing the most abundant cyanobacterial genera in BL and CA. Solution reached at run 20, stress value: 0.1104989.

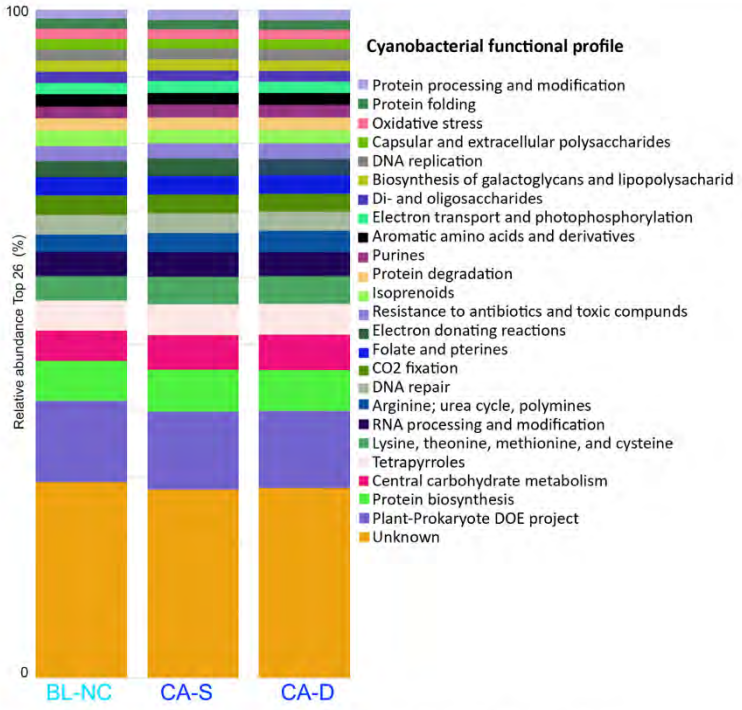


**Figure 6.** Relative abundance of cyanobacterial functional profiles in microbialites from BL-NC, CA-S, and CA-D.

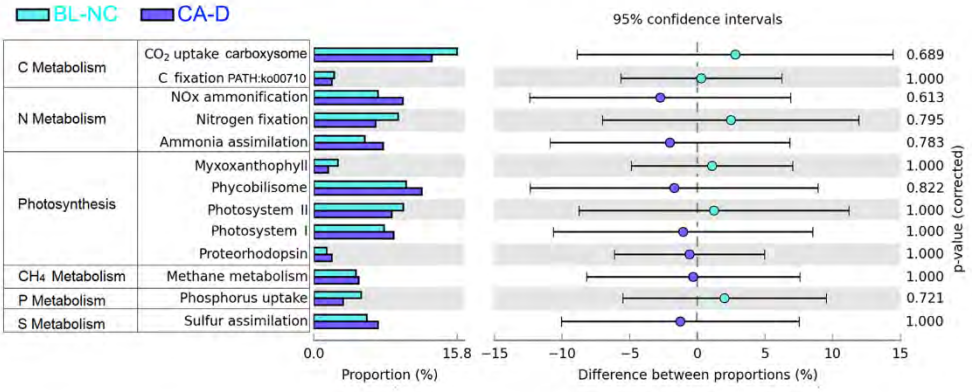


**Figure 7.** Relative abundance of cyanobacterial energy profiles in microbialites from BL-NC and BL-D.

quences from metagenomes in BL and CA were related to undescribed proteins. The real percentage of novel cyanobacterial proteins was unknown because databases show only known proteins from known taxa, and most of the Cyanobacteria described in this work are putative new taxa. This is a recurring problem for metagenomes from poorly studied environments such as microbialites (Prabha and Singh 2014, Mclaren *et al.* 2019, Browne *et al.* 2020). So, the actual functional differences of Cyanobacteria may be within the undescribed proteins and unknown annotations (Garcia-Pichel *et al.* 2020). (2) Functional profile comparisons between communities are fragile to low sampling effects (Pettengill *et al.* 2012, Prabha *et al.* 2016, Chen *et al.* 2021): BL-NC, CA-S, and

CA-D metagenomes were constructed pooling nine sites with five replicas each one, but no additional replicas were made for the three metagenomes. This gave high sequence coverage for assembling metagenomes *de novo* but sacrificed metagenome replicas for statistical analysis. Transcriptomic analysis will help understand the different patterns found at the energy level signal.

## Mechanistic explanations for differences between systems

Approximately, half of the cyanobacterial taxa recovered from microbialites were shared between BL and CA. Major taxonomic differences were observed when considering abundance. It is expected that different light niches are occupied by different types of Cyanobacteria. To this regard, our findings are consistent with literature, where Nostocales, Chroococcidiopsidales, and filamentous Synechococcales occupy microhabitats with high light concentration (i.e. BL) producing high amounts of pigments to avoid UV stress (Mai 2016, Perez *et al.* 2016, Wang *et al.* 2019). While Pleurocapsales, Chroococcales, and unicellular Synechococcales occupy low light conditions (i.e. CA; Waterbury and Stanier 1978, Angeler *et al.* 2005). The same shift in taxonomic composition of Cyanobacteria has been reported in microbialites from Alchichica crater lake (Águila *et al.* 2021), Pavilion lake (Russell *et al.* 2014), and Satonda lake (Arp *et al.* 2003). Instead, Oscillatoriales and some filamentous Synechococcales were related to regions with high NOx concentration, a taxonomic pattern also reported by other works (Nixdorf *et al.* 2003, Kokociński *et al.* 2011, Salmaso *et al.* 2016). Also, the hardness of the microbialites that develop in BL and CA are different: BL microbialites are granular, soft, and are made up of calcite (87%) and plagioclase (9%) while CA microbialites are harder and richer in calcite (90%–97%) with traces of magnesite, quartz, and hematite (Valdespino-Castillo *et al.* 2018).

Nevertheless, a mechanistic explanation should not only address physicochemical conditions, but also time and history of the different systems. Regarding this, not only light conditions, but light exposure is important for Cyanobacteria in CA, because of the angle of the Sun, which changes during daytime over a sinkhole with a bowl-cave shape. It is possible that Cyanobacteria in CA are also adapted to less hours of light with respect to BL, and may have different regulations in their circadian clocks.

Another major difference beyond physicochemical conditions is the geologic history between BL and CA. Although, BL microbialites are dated between 2000 years and CA microbialites have not been dated, surface rocks of BL have been mapped as Eocene and Miocene–Pliocene, while CA rock bed is associated with a granite boulder (Perry *et al.* 2002) previous to the Tertiary (Perry *et al.* 2009). BL, like most of the YP, is covered by expulsion material from the Chicxulub crater, while CA at depths greater than 3 m do not have this material. This difference in geological origin could conditionate differently the communities of organisms that form microbialites. Although it is rare that microbialites are present in a sinkhole, the possibility of microbialites in other sinkholes in the southern region of Quintana Roo remains to be explored. The same for BL, where it is possible that microbialites develop in in the southern region of Xulha within BL, where depth reaches 90 m. This data could help to elucidate if Cyanobacteria from BL and CA systems are different only by physicochemical variables (i.e. light) or also by different historical events.

Other methods to elucidate differences between systems are PD indexes. For instance, the NRI index (SES.MPD) can help determine whether bacterial communities are actively changing the microenvironment (phylogenetic overdispersion) or are being selected by environmental factors (phylogenetic clustering). The PD of the microbial communities associated with microbialites has been suggested to be driven by both niche construction (Centeno *et al.* 2016) and habitat conditions, such as light availability, nutrient concentration, water saturation index, and conductivity (Hanson *et al.* 2012, Cerqueda-García and Falcón 2016, Chagas *et al.* 2016). Cyanobacterial communities generally show phylogenetic overdispersion in microbialites and microbial mats (Chase 2010), whereas heterotrophic components mostly show phylogenetic clustering (Armitage *et al.* 2012).

For BL, it was interesting that only site BL12 showed phylogenetic clustering, which could mean Cyanobacteria are being selected by high NOx concentrations. The rest of BL showed phylogenetic overdispersion, which means Cyanobacteria are actively changing their microenvironment or building niche, these findings are consistent with previous literature (Centeno *et al.* 2016). For CA, all sites showed phylogenetic clustering, but higher values were found in CA05 and CA30, which could be explained since Cyanobacteria from 10 and 20 m could be better adapted to specific conditions than those in 5 and 30 m. Although NTI (SES.MNTD) index values indicated random dispersion for both systems, this was attributable to the low number of taxa (44) recovered in this study.

## Concluding remarks

This work will ease future taxonomic characterizations on freshwater Cyanobacteria of the YP. Isolation of Cyanobacteria for sequencing will be necessary to validate new taxa. Transcriptomic analysis is needed to elucidate major functional differences between BL and CA, especially for the energy profiles, circadian clock, and light adaptations. It is crucial to compare other similar systems near BL and CA to address if cyanobacterial biogeography is explained solely by environmental factors or different geohistorical processes.

In this study, two systems with microbialites, which share some physicochemical variables, are close geographically, yet are separated by their geologic origin, have differences in their cyanobacterial composition, light availability and depth. BL exhibited a dominance of Nostocales, while in CA sinkhole, Chroococcales were dominant. Yet, at the functional level, both sites shared genetic components, which makes these systems interesting models to study functional ecology in Cyanobacteria.

## Supplementary data

Supplementary data are available at FEMSEC online.

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## References

Águila B, Alcántara-Hernándezb RJ, Montejano G *et al.* Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization. *Eur J Phycol* 2021;**56**:1–16.

Alneberg J, Karlsson CMG, Divne A-M *et al.* Genomes from uncultivated prokaryotes: a comparison of metagenome-assembled and single-amplified genomes. *Microbiome* 2018;**6**:1–14.

Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *J Mol Biol* 1990;**215**:403–10.

Alvarenga DO, Fiore MF, Varani AM. A metagenomic approach to cyanobacterial genomics. *Front Microbiol* 2017;**8**:809.

Andersen KS, Kirkegaard R, Karst SM ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. *Biorxiv* 2018; 299537.

Angeler DG, Sánchez-Carrillo S, Rodrigo MA *et al.* On the importance of water depth, macrophytes and fish in wetland picocyanobacteria regulation. *Hydrobiologia* 2005;**549**:23–32.

Armitage DW, Gallagher KL, Youngblut ND *et al.* Millimeter-scale patterns of phylogenetic and trait diversity in a salt marsh microbial mat. *Front Microbiol* 2012;**3**:293.

Arp G, Reimer A, Reitner J. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *J Sediment Res* 2003;**73**:105–27.

Bauer-Gottwein P, Gondwe BRN, Charvet G *et al.* The Yucatán Peninsula karst aquifer, *Hydrol J* 2011;**19**:507–24.

Beltrán Y, Centeno Cm, García-Oliva F *et al.* N2 fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia from different aquatic environments in Mexico. *Aquat Microb Ecol* 2012;**67**:15–24.

Benson DA, Cavanaugh M, Clark K *et al.* GenBank. *Nucleic Acids Res* 2012;**41**:D36–42.

Browne PD, Nielsen TK, Kot W *et al.* GC bias affects genomic and metagenomic reconstructions, underrepresenting GC-poor organisms. *GigaScience* 2020;**9**:giaa008.

Burne RV, Moore LS. Microbialites; organosedimentary deposits of benthic microbial communities. *PALAIOS* 1987;**2**:241–54.

Bushnell B. *BBMap: A Fast, Accurate, Splice-Aware Aligner*. 2014, Berkeley, CA: Lawrence Berkeley National Lab. (LBNL).

Callahan BJ, Mcmurdie PJ, Rosen MJ *et al.* DADA2: high-resolution sample inference from illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Cam N, Benzerara K, Georgelin T *et al.* Cyanobacterial formation of intracellular Ca-carbonates in undersaturated solutions. *Geobiology* 2018;**16**:49–61.

Caporaso JG, Lauber CL, Walters WA *et al.* Ultra-high-throughput microbial community analysis on the illumina hiseq and miseq platforms. *ISME J* 2012;**6**:1621–4.

Centeno CM, Legendre P, Beltrán Y *et al.* Microbialite genetic diversity and composition relate to environmental variables. *FEMS Microbiol Ecol* 2012;**82**:724–35.

Centeno CM, Mejía O, Falcón LI. Habitat conditions drive phylogenetic structure of dominant bacterial phyla of microbialite communities from several locations in Mexico. *Rev Biol Trop* 2016;**64**:1057–66.

Cerqueda-García D, Falcón LI. Metabolic potential of microbial mats and microbialites: autotrophic capabilities described by an in silico stoichiometric approach from shared genomic resources. *J Bioinform Comput Biol* 2016;**14**:1650020.

Cervantes-Martínez A, Mezeta-Barrera M, Gutiérrez-Aguirre MA. Basic limnology of the karstic tourist lake Cenote Azul in Quintana Roo, Mexico. *Hidrobiológica* 2009;**19**:177–80.

Chagas AAP, Webb GE, Burne RV *et al.* Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy. *Earth Sci Rev* 2016;**162**:338–63.

Chase JM. Stochastic community assembly causes higher biodiversity in more productive environments. *Science* 2010;**328**:1388–91.

Chen M-Y, Teng W-K, Zhao L *et al.* Comparative genomics reveals insights into cyanobacterial evolution and habitat adaptation. *ISME J* 2021;**15**:211–27.

Dupraz C, Reid RP, Braissant O *et al.* Processes of carbonate precipitation in modern microbial mats. *Earth Sci Rev* 2009;**96**:141–62.

Edgar R. *Usearch*. Berkeley, CA: Lawrence Berkeley National Lab. (LBNL), 2010.

Flannery DT, Walter MR. Archean tufted microbial mats and the great oxidation event: new insights into an ancient problem. *Aust J Earth Sci* 2012;**59**:1–11.

Foster JS, Green SJ, Ahrendt SR *et al.* Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. *ISME J* 2009;**3**:573–87.

García-Pichel F, Zehr JP, Bhattacharya D *et al.* What's in a name? The case of cyanobacteria. *J Phycol* 2020;**56**:1–5.

Gischler E, Gibson MA, Oschmann W, Giant holocene freshwater microbialites, Laguna Bacalar, Quintana Roo, Mexico. *Sedimentology* 2008;**55**:1293–309.

Gischler E, Golubic S, Gibson MA *et al.* Microbial mats and microbialites in the freshwater Laguna Bacalar, Yucatan Peninsula, Mexico. In: *Advances in Stromatolite Geobiology*. Berlin, Heidelberg: Springer, 2011, 187–205.

Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010;**27**:221–4.

Hall M, Beiko RG. *16S rRNA Gene Analysis with QIIME2, in Microbiome Analysis*. Berlin, Heidelberg: Springer, 2018, 113–29.

Hanson CA, Fuhrman JA, Horner-Devine MC *et al.* Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* 2012;**10**:497–506.

Kamennaya N, Ajo-Franklin C, Northen T *et al.* Cyanobacteria as biocatalysts for carbonate mineralization. *Minerals* 2012;**2**:338–64.

Kempe S, Kazmierczak J. Terrestrial analogues for early planetary oceans: NIUAFO 'OU CALDERA LAKES (Tonga) and their geology, water chemistry, and stromatolites, In: *Life on Earth and other Planetary Bodies*. Berlin, Heidelberg: Springer, 2012, 195–234.

Klatt CG, Wood JM, Rusch DB *et al.* Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. *ISME J* 2011;**5**:1262–78.

Kokociński M, Stefaniak K, Izydorczyk K *et al.* Temporal variation in microcystin production by *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek (Cyanobacteria, oscillatoriales) in a temperate lake. In: *Annales de Limnologie-International Journal of Limnology*. Les Ulis: EDP sciences, 2011.

Komárek J, Anagnostidis KC, Stuttgart J *Süßwasserflora von Mitteleuropa: Cyanoprokaryota*. Berlin, Heidelberg: Springer. 1998.

Komárek J, Kaštovský J, Mares J *et al.* Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* 2014;**86**:295–335.

Komárek J, Komárková J, Ventura S *et al.* Taxonomic evaluation of cyanobacterial microflora from alkaline marshes of northern Belize. 3. Diversity of heterocytous genera. *Nova Hedwigia* 2017;**105**:445–86.

Komárek J, Komárková-Legnerová J. Taxonomic evaluation of the cyanobacterial microflora from alkaline marshes of northern Belize. 1. Phenotypic diversity of coccoid morphotypes. *Nova Hedwigia* 2007;**84**:65–111.

Komarek J. Cyanobacterial taxonomy: current problems and prospects for the integration of traditional and molecular approaches. *Algae* 2006;**21**:349–75.

Komárková J, Zapomělová E, Komárek J. Chakia (cyanobacteria), a new heterocytous genus from Belizean marshes identified on the basis of the 16S rRNA gene. *Fottea* 2013;**13**:227–33.

Krings M, Sergeev VN. A coccoid, colony-forming cyanobacterium from the lower Devonian Rhynie Chert that resembles Eucapsis (Synechococcales) and Entophysalis (Chroococcales). *Rev Palaeobot Palynol* 2019;**268**:65–71.

Laval B, Cady SL, Pollack JC *et al.* Modern freshwater microbialite analogues for ancient dendritic reef structures. *Nature* 2000;**407**: 626–9.

Li D, Liu C-M, Luo R *et al.* MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 2015;**31**:1674–6.

Mai T. Revision of the Synechococcales (cyanobacteria) through recognition of four families including Oculatellaceae fam. nov. and Trichocoleaceae fam nov. and seven new genera containing 14 species. *Phytotaxa* 2016;**1**:001–059.

Mclaren MR, Willis AD, Callahan BJ. Consistent and correctable bias in metagenomic sequencing experiments. *Elife* 2019;**8**:e46923.

Meyer F, Bagchi S, Chaterji S *et al.* MG-RAST version 4—lessons learned from a decade of low-budget ultra-high-throughput metagenome analysis. *Briefings Bioinf* 2019;**20**:1151–9.

Micallef ML, D'agostino PM, Al-Sinawi B *et al.* Exploring cyanobacterial genomes for natural product biosynthesis pathways. *Mar Geonomics* 2015;**21**:1–12.

Montes-Ortiz L, Elías-Gutiérrez M. Water mite diversity (Acariformes: prostigmata: parasitengonina: hydrachnidiae) from karst ecosystems in southern of Mexico: a barcoding approach. *Diversity* 2020;**12**:329.

Nixdorf B, Mischke U, Rücker J. Phytoplankton assemblages and steady state in deep and shallow eutrophic lakes—an approach to differentiate the habitat properties of oscillatoriales, In: *Phytoplankton and Equilibrium Concept: The Ecology of Steady-State Assemblages*. Berlin, Heidelberg: Springer, 2003, 111–21.

Nutman AP, Bennett VC, Friend CRL *et al.* Rapid emergence of life shown by discovery of 3700-million-year-old microbial structures. *Nature* 2016;**537**:535–8.

Obst M, Dynes JJ, Lawrence JR *et al.* Precipitation of amorphous caco3 (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. *Geochim Cosmochim Acta* 2009;**73**:4180–98.

Oksanen J, Blanchet FG, Kindt R *et al.* Package 'vegan' Community Ecology Package. CRAN. 2013;**2**: 1–295.

Palinska KA, Thomasius CF, Marquardt J *et al.* Phylogenetic evaluation of cyanobacteria preserved as historic herbarium exsiccata. *Int J Syst Evol Microbiol* 2006;**56**:2253–63.

Parks DH, Tyson GW, Hugenholtz P *et al.* STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 2014;**30**:3123–4.

Pereira S, Micheletti E, Zille A *et al.* Using extracellular polymeric substances (EPS)-producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and also accumulate inside the cell?. *Microbiology* 2011;**157**:451–8.

Perez R, Forchhammer K, Salerno G *et al.* Clear differences in metabolic and morphological adaptations of akinetes of two nostocales living in different habitats. *Microbiology* 2016;**162**:214–23.

Perry E, Paytan A, Pedersen B *et al.* Groundwater geochemistry of the Yucatan Peninsula, Mexico: constraints on stratigraphy and hydrogeology. *J Hydrol* 2009;**367**:27–40.

Perry E, Velazquez-Oliman G, Marin L. The hydrogeochemistry of the karst aquifer system of the northern Yucatan Peninsula, *Int Geol Rev* 2002;**44**:191–221.

Perry EC, Leal-Bautista RM, Velazquez-Oliman G *et al.* The icaiche formation: unacknowledged contributor to yucatán hydrogeology and geomorphology. In: *Proceedings of the GSA Annual Meeting in Indianapolis*. Indiana: GSA. 2018.

Pettengill JB, Mcavoy E, White JR *et al.* Using metagenomic analyses to estimate the consequences of enrichment bias for pathogen detection. *BMC Res Notes* 2012;**5**:1–7.

Prabha R, Singh DP, Somvanshi P *et al.* Functional profiling of cyanobacterial genomes and its role in ecological adaptations. *Genomics Data* 2016;**9**:89–94.

Prabha R, Singh DP. Analysis of dinucleotide bias and genomic signatures across cyanobacterial genomes. *J Adv Biotechnol* 2014;**3**. DOI: 10.24297/jbt.v3i3.5029.

Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;**28**:1823–9.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012;**41**:D590–6.

Ragon M, Benzerara K, Moreira D *et al.* 16S rDNA-based analysis reveals cosmopolitan occurrence but limited diversity of two cyanobacterial lineages with contrasted patterns of intracellular carbonate mineralization. *Fronti Microbiol* 2014;**5**:331.

Riding R. Microbialites, stromatolites, and thrombolites. In: *Encyclopedia of Geobiology*. Heidelberg: Springer. 2011.

Roldán M, Ramírez M, Del Campo J *et al.* *Chalicogloea cavernicola* gen. nov., sp. nov.(Chroococcales, cyanobacteria), from low-light aerophytic environments: combined molecular, phenotypic and ecological criteria. *Int J Syst Evol Microbiol* 2013;**63**: 2326–33.

Rosado Varela ÁA, Medina Argueta G. Ciclo de vida turístico de bacalar, pueblo mágico, Quintana Roo. *TeorÃa y Praxis* 2014;**10**: 96–120.

Rousk J, Brookes PC, BaATh E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl Environ Microbiol* 2009;**75**:1589–96.

Russell JA, Brady AL, Cardman Z *et al.* Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada. *Geobiology* 2014;**12**:250–64.

Russell MJ. The generation at hot springs of sedimentary ore deposits, microbialites and life. *Ore Geol Rev* 1996;**10**:199–214.

Salmaso N, Cerasino L, Boscaini A *et al.* Planktic tychonema (Cyanobacteria) in the large lakes south of the Alps: phylogenetic assessment and toxigenic potential. *FEMS Microbiol Ecol* 2016;**92**:fiw155.

Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 2011;**27**:863–4.

Schmitter-Soto JJ, Comín FA, Escobar-Briones E *et al.* Hydrogeochemical and biological characteristics of cenotes in the Yucatan Peninsula (SE Mexico). *Hydrobiologia* 2002;**467**:215–28.

Schmitter-Soto JJ. La ictiofauna cenotícola (peces de cenote) más relevante de la península de yucatán. *Bioagrociencias* 2020;**13**:1.

Schwaderer AS, Yoshiyama K, De Tezanos Pinto P *et al.* Eco-evolutionary differences in light utilization traits and distributions of freshwater phytoplankton. *Limnol Oceanogr* 2011;**56**: 589–98.

Shalygin S, Kavulic KJ, Pietrasiak N *et al.* Neotypification of *Pleurocapsa fuliginosa* and epitypification of *P. minor* (Pleurocapsales): resolving a polyphyletic cyanobacterial genus. *Phytotaxa* 2019; **392**:245.

Smirnov NN, Elías-Gutiérrez M. Biocenotic characteristics of some Yucatan lentic water bodies based on invertebrate remains in sediments. *Inland Water Biol* 2011;**4**:211–7.

Souza V, Siefert JL, Escalante AE *et al.* The cuatro ciénegas basin in Coahuila, Mexico: an astrobiological precambrian park. *Astrobiology* 2012;**12**:641–7.

Tandeau De Marsac N. Occurrence and nature of chromatic adaptation in cyanobacteria. *J Bacteriol* 1977;**130**:82–91.

Temperton B, Giovannoni SJ. Metagenomics: microbial diversity through a scratched lens. *Curr Opin Microbiol* 2012;**15**:605–12.

Tsirogiannis C, Sandel B. PhyloMeasures: a package for computing phylogenetic biodiversity measures and their statistical moments. *Ecography* 2016;**39**:709–14.

Turicchia S, Ventura S, Komárková J *et al.* Taxonomic evaluation of cyanobacterial microflora from alkaline marshes of northern Belize. 2. Diversity of oscillatorialean genera. *Nova Hedwigia* 2009;**89**:165–200.

Valdespino-Castillo PM, Hu P, Merino-Ibarra M *et al.* Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. *Front Microbiol* 2018;**9**:510.

Voorhies AA, Eisenlord SD, Marcus DN *et al.* Ecological and genetic interactions between cyanobacteria and viruses in a low-oxygen mat community inferred through metagenomics and metatranscriptomics. *Environ Microbiol* 2016;**18**:358–71.

Wang Y, Cai F, Jia N *et al.* Description of a novel coccoid cyanobacterial genus and species *Sinocapsa zengkensis* gen. nov. sp. nov.(Sinocapsaceae, incertae sedis), with taxonomic notes on genera in chroococcidiopsidales. *Phytotaxa* 2019;**409**:146–60.

Waterbury JB, Stanier RY. Patterns of growth and development in pleurocapsalean cyanobacteria. *Microbiol Rev* 1978;**42**:2.

White RA, Chan AM, Gavelis GS *et al.* Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community. *Front Microbiol* 2016;**6**:1531.

Yanez-Montalvo A, Águila B, Gómez-Acata S *et al.* Depth related structure and microbial composition of microbialites in a karst sinkhole, *Geomicrobiol J* 2021;**38**:237–51.

Yanez-Montalvo A, Gómez-Acata S, Águila B *et al.* The microbiome of modern microbialites in Bacalar Lagoon. *PLoS ONE* 2020;**15**:e0230071.

Yin B, Crowley D, Sparovek G *et al.* Bacterial functional redundancy along a soil reclamation gradient. *Appl Environ Microbiol* 2000;**66**:4361–5.

Yu J, Whalen JK. A new perspective on functional redundancy and phylogenetic niche conservatism in soil microbial communities. *Pedosphere* 2020;**30**:18–24.

Zhu T, Dittrich M. Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. *Front Bioeng Biotechnol* 2016;**4**:4.

### 5.3.3 Cyanobacteria in microbialites: A lithobiontic perspective

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# Cyanobacteria in microbialites: A lithobiontic perspective

**Bernardo Águila [1,2], Miriam Guerrero-Jacinto[1,3], Itzel Becerra-Absalón [4], Gustavo Montejano-Zurita [4], Alfredo Yanez-Montalvo[1], Arit S. De León-Lorenzana[1], Luisa I Falcón [1]***

[1]Instituto de Ecología, UNAM Campus Yucatán, Parque Científico y Tecnológico de Yucatán, 97302, México
[2]Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1° Piso, Circuito de Posgrados CU, Coyoacán, CdMx 04510, México
[3]Posgrado en Ciencias del Mar, UNAM, Coyoacán, CdMx 04510, México
[4]Laboratorio de Ficología, Facultad de Ciencias, UNAM, Coyoacán, CdMx 04510, México
* Corresponding author: falcon@ecologia.unam.mx Tel: +52 5521837123

**Abstract:** Cyanobacteria have been described as one of the keystone organisms for the formation of microbialites. In this work, we analyze the role of Cyanobacteria with a lithobiontic perspective. For this, we propose 5 different lithobiontic groups based on their microhabitat and adaptations. We assign cyanobacterial genera of microbialites to the lithobiontic groups and analyze their distribution across the cyanobacterial clades. Next, we analyze the apparition of these groups in the context of the microbialite geologic record. Finally, we remark the importance of using a lithobiontic perspective to ease the finding of possible patterns within modern microbialites.

**Keywords:** Cyanobacteria; Microbialites; Evolution; Mesolith; Metalith; Lithobiontic; Taxonomy.

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## 1. Role of Cyanobacteria in the formation of microbialites

Microbialites are organosedimentary structures formed by microbial communities [1]. They are also defined as microbial mats that lithify [2]. Lithification of a mat consists of mineralization (formation of mineral crystals), cementation (the filling of a porous structure), and compaction (the pressure placed on a porous structure) [3, 4]. Modern microbialites are metabolically active [5], still they can become fossilized by petrification processes or degraded by demineralization processes [6]. Both abiotic and biotic factors influence the formation of microbialites (Figure 1). Abiotic factors include: High saturation index (SI) [7], which is the concentration of cations in the water (i.e. $Ca^{2+}$ $Mg^{2+}$); Elevated water alkalinity[8]; Oligotrophy, meaning low nitrogen or phosphorous availability[8-10]. Biotic factors include a low abundance of microbial grazers[11] , and high abundance of bacteria (90% of total composition), with coupled metabolisms in favor of carbonate precipitation (i.e. Photosynthesis, Nitrification, Sulfate reduction, Methane oxidation, Ammonification, Ureolysis or Fermentation) [2, 12]. Most relevant bacterial linages for microbialite formation are Sulfate Reducing Bacteria (SRB), Proteobacteria, and Cyanobacteria [13, 14]. Cyanobacteria influence directly and/or indirectly the formation of microbialites due to four properties:

1) Biomineral precipitation. Cyanobacteria are the major primary producers, synthesizing large amounts of organic matter (OM), mucilage, and extracellular polymeric substances (EPS), which can become saturated with ions or degraded by other bacteria, promoting mineralization [5]; Also, their photosynthesis coupled with carbon fixation increases pH and promotes *in situ* extracellular mineral precipitation and organomineralization[15].

2) Accretion. Their EPS and mucilaginous sheaths stabilize unconsolidated sediments, actively trapping and binding minerals and sediment [16, 17].

3) Biocementation facilitation. Some groups of Cyanobacteria degrade carbonates inside the microbialites, creating porous structures [18, 19], facilitating an ecological succession for heterotrophic bacteria, which cement and confer different hardness to the microbialite [20, 21].

4) Biocompaction. Organic matter (OM) produced by Cyanobacteria transforms into inorganic carbon by organomineralization [22], promoting carbon burial, which compacts layers of sediment [23].

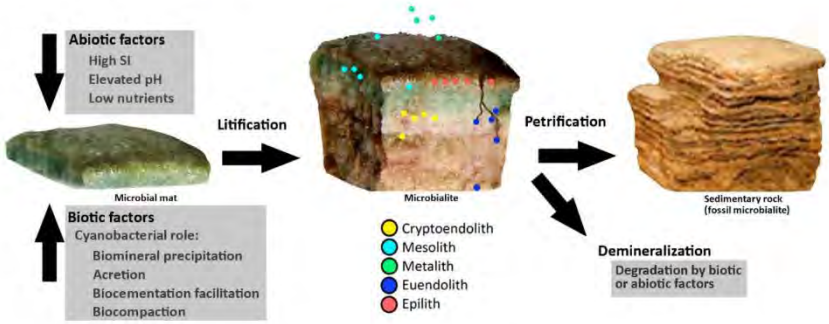


**Figure 1.** Proposed life cycle for microbialites; specific biotic and abiotic facttors cause microbial mats to lithify, thus froming a microbialite; Microbialites could contain different lithobiontic groups of Cyanobacteria; When petrification processes occur a sedimentary rock is formed. Microbialites can become degradated by specific biotic and abiotic factors.

## 2. Lithobiontic cyanobacterial groups

There is not a unique species or genus of Cyanobacteria which is cosmopolitan to all microbialites [24], yet there are specific lithobiontic groups of Cyanobacteria inhabiting specific micro regions of microbialites, each with different adaptations.

### *2.1. Cryptoendoliths: Group I*

Cryptoendoliths cyanobacteria cannot penetrate rock or sedimentary structures, but they bury themselves and form colonies within the pores of fenestrae structures, or under carbonates precipitated by themselves [25, 26]. They are sessile communities adapted to live in low light conditions, produce carotenoids to avoid UV stress, live encrusted in sediments [27-29], and also use minerals as protection [30]. They form colonies enveloped by mucilage that passively precipitate carbonates [31]. Some have specialized reproductive cells for hypersaline environments [32, 33]. Some others have thicker S-layers than the average Cyanobacteria [34]. Generally, extracellular precipitation of cryptoendolithic Cyanobacteria produce well-laminated submillimeter fabrics that do not contain sediment [35]. All cryptoendolithic Cyanobacteria exhibit slow growth, using most of their energy in maintenance of cell structure [36, 37]. They are reported in microbialites at depths from 2 up to 30 m [14, 38-46].

### *2.2. Mesoliths: Group II*

Mesoliths (term proposed in this study) move between the outer and internal parts of unlithified sediment of the microbialite (meso= middle) (Fig. 2). They form mats and move according to light conditions, nutrient concentration or presence of grazers [47-52] They are mostly alkalophiilic, adapted to ion saturated environments. They precipitate carbonates in their sheaths by ion saturation and become completely buried or encrusted, still their cells can move inside their mucilaginous sheath, leaving behind their incrusted

sheaths [53-56]. Some mesolithic Cyanobacteria completely cover the external microbialite structure with their EPS [57]. They constantly produce EPS [58]. When EPS becomes saturated with ions, they produce irregular sparitic minerals [59] Some are still capable of obtaining energy by anoxygenic photosynthesis, using $H_2$ or $H_2S$ as electron donors [60, 61]. Mesoliths are cosmopolitan to all modern microbialites.

### *2.3. Metaliths: Group III*

Metaliths (term proposed in this study) are planktonic or metaphytic Cyanobacteria, floating in the water column, but when precipitated, are found abundantly in the outer parts of microbialites becoming buried in the external layers of the microbial mats [49, 62-64]. They are adapted to growth between 2 to 15 m depth in the water column [65]. They can also regulate their buoyancy to reach the optimum light conditions[66]. Most metaliths form blooms when nutrients concentrations are favorable and cause whitening events, which increases pH and a subsequent precipitation of organic matter in the water column [67, 68]. Most exhibit genome reduction with low G-C content and streamlined genomes[69]. Other adaptations include chlorophyll D [70]or chlorophyll F[71]. All metalithic Cyanobacteria have well-regulated circadian clocks [72] to separate oxygenic photosynthesis from nitrogen fixation [73]. They are not considered part of microbialites until they precipitate and become buried or trapped by microbialites [74].

### *2.4. Euendoliths: Group IV*

Euendoliths actively penetrate rock, carbonates, and sedimentary structures to carry out their life cycle, excavating tunnels in hard mineral substrates and soft carbonates [18] Euendoliths are adapted to move and rearrange the elements of the carbonate matrix [75]. They have adapted to reduce their photosynthetic capacity [76] since oxygenic photosynthesis produces alkalization and promotes precipitation of minerals instead of carbonate dissolution [75]. They can use fermentation to obtain energy [77]. Still little is known of the mechanisms to alternate between phototrophic and heterotrophic metabolism. They have complex genetic regulation [78] and it is speculated they facilitate the presence of heterotrophic bacteria, which cement the pores left by euendoliths [76, 79].

### *2.5. Epiliths: Group V*

Epilithic Cyanobacteria are sessile, inserting themselves into the substrate of microbialites, forming homogeneous mats of circular/radial colonies (Fig. 5). They are adapted to high light intensities, producing thick mucilaginous sheets and pigments, to avoid photic stress and damage by UV radiation [28, 36, 80]. They also produce large amounts of EPS to trap debris and detrital material. Their EPS also confers adhesion to the substrate, UV protection, and forms a three-dimensional matrix where other bacteria localize activities[81]. Because of their large cell size, they are considered the primary producers [82] and the main diazotrophs in microbialites [47, 83]. Due to their ability to fix nitrogen, they can survive in extremely oligotrophic environments. Some epiliths form specialized cells like akinetes, to reserve nutrients [84]. They have the largest genome sizes of Cyanobacteria, with high G-C content and complex genetic regulation [85]. Genomic analysis suggests they have multiple horizontal gene transfer events [86]. Epilithic Cyanobacteria influence the availability of nutrients and therefore attract other bacteria that use the OM they produce [87]. They have been described in shallow microbialites from oligotrophic lakes and pools [48, 49, 88-93].

## 3. Taxonomy of lithobiontic groups

A revision for all Cyanobacterial genera reported in microbialites was made. Assignation was based on their microhabitat and adaptations as stated in the previous section. Taxonomic nomenclature used was based on the latest revisions [94, 95].

**Table 1.** Most common cyanobacterial genera of Cryptoendoliths in microbialites

| Group | Order | Family | Genus | Reference |
|---|---|---|---|---|
| I | Chroococcales | Aphanothecaceae | *Aphanothece* | [96, 97] |
| I | Chroococcales | Aphanothecaceae | *Halothece* | [43, 98] |
| I | Chroococcales | Chroococcaceae | *Gloeothece* | [89, 99] |
| I | Chroococcales | Entophysalidaceae | *Entophysalis* | [49, 89] |
| I | Chroococcidiopsidales | Aliterellaceae | *Aliterella* | [10] |
| I | Chroococcidiopsidales | Chroococcidiopsidaceae | *Chroococcidiopsis* | [10, 100, 101] |
| I | Gloeobacterales | Gloeobacteraceae | *Gloeobacter* | [10, 40] |
| I | Pleurocapsales | Dermocarpellaceae | *Stanieria* | [82, 102, 103] |
| I | Pleurocapsales | Hyellaceae | *Pleurocapsa* | [48] |
| I | Pleurocapsales | Xenococcaceae | *Chroococcidum* | [49] |
| I | Pleurocapsales | Xenococcaceae | *Xenococcus* | [14, 41, 104] |
| I | Pleurocapsales | Xenococcaceae | *Myxosarcina* | [49, 91] |

**Table 2.** Most common cyanobacterial genera of Mesoliths in microbialites

| Group | Order | Family | Genus | Reference |
|---|---|---|---|---|
| II | Oscillatoriales | Homoeotrichacea | *Homoeothrix* | [105, 106] |
| II | Oscillatoriales | Microcoleaceae | *Microcoleus* | [47, 58, 62, 107] |
| II | Oscillatoriales | Microcoleaceae | *Hydrocoleum* | [98] |
| II | Oscillatoriales | Oscillatoriaceae | *Blennothrix* | [98, 108] |
| II | Oscillatoriales | Oscillatoriaceae | *Lyngbya* | [98] |
| II | Oscillatoriales | Oscillatoriaceae | *Phormidium* | [109] |
| II | Oscillatoriales | Schizotrichaceae | *Schizothrix* | [107, 110, 111] |
| II | Synechococcales | Leptolyngbyaceae | *Haloleptolyngbya* | [49, 112] |
| II | Synechococcales | Leptolyngbyaceae | *Halomicronema* | [49, 101] |
| II | Synechococcales | Leptolyngbyaceae | *Leptolyngbya* | [43, 47, 62, 89] |
| II | Synechococcales | Pseudanabaenaceae | *Geitlerinema* | [113] |
| II | Synechococcales | Pseudanabaenaceae | *Pseudanabaena* | [93] |

**Table 3.** Most common cyanobacterial genera of Metaliths in microbialites

| Group | Order | Family | Genus | Reference |
|---|---|---|---|---|
| III | Chroococcales | Chroococcaceae | *Chroococcus* | [96] |
| III | Chroococcales | Gomontiellaceae | *Cyanothece* | [43] |
| III | Nostocales | Aphanizomenonaceae | *Nodularia* | [47] |
| III | Nostocales | Nostocaceae | *Nostoc* | [90] |
| III | Oscillatoriales | Oscillatoriaceae | *Oscillatoria* | [114] |
| III | Synechococcales | Acaryochloridaceae | *Acaryochloris* | [40, 62, 115] |
| III | Synechococcales | Merismopediaceae | *Aphanocapsa* | [41] |
| III | Synechococcales | Merismopediaceae | *Eucapsis* | [116] |
| III | Synechococcales | Merismopediaceae | *Synechocystis* | [117] |

| | | | | |
|---|---|---|---|---|
| III | Synechococcales | Prochlorococcaceae | *Cyanobium* | [118] |
| III | Synechococcales | Prochlorococcaceae | *Prochlorococcus* | [49, 119] |
| III | Synechococcales | Synechococcaceae | *Synechococcus,* | [120] |

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136 **Table 4.** Most common cyanobacterial genera of Euendoliths in microbialites

| Group | Order | Family | Genus | Reference |
|---|---|---|---|---|
| IV | Pleurocapsales | Hyellaceae | *Solentia* | [104] |
| IV | Pleurocapsales | Hyellaceae | *Hyella* | [109] |
| IV | Pleurocapsales | Hyellaceae | *Cyanosaccus* | [121] |
| IV | Pleurocapsales | Hydrococcaceae | *Hormathonema* | [122] |
| IV | Nostocales | Rivulariaceae | *Kyrtuthrix* | [123] |
| IV | Nostocales | Hapalosiphonaceae | *Matigocoleus* | [124] |

137 **Table 5.** Most common cyanobacterial genera of Epiliths in microbialites

| Group | Order | Family | Genus | Reference |
|---|---|---|---|---|
| V | Nostocales | Calotrichaceae | *Calothrix* | [125] |
| V | Nostocales | Rivulariaceae | *Dichothrix* | [121] |
| V | Nostocales | Rivulariaceae | *Rivularia* | [126-128] |
| V | Nostocales | Scytonemataceae | *Chakia* | [10] |
| V | Nostocales | Scytonemataceae | *Scytonema* | [89] |
| V | Nostocales | Stigonemataceae | *Fischerella* | [62, 114] |
| V | Nostocales | Stigonemataceae | *Stigonema* | [129] |
| V | Nostocales | Tolypotrichaceae | *Tolypothrix* | [130] |

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139 To find phylogenetic affiliations among genera and the distribution of the
140 lithobiontic groups across clades, a cladogram was constructed with available 16S rRNA
141 sequences from known cyanobacterial genera described in microbialites. The cladogram
142 was generated using a Neighbour Joining method in ARBSilva [131]; parameters used
143 were: FastTree GTR Model, Gamma rate for likelihood, Denovo construction with user
144 sequences only, 10 neighbours per query sequence and 0.95 of minimum identity with
145 query sequence. Fixed Positional variation was set at highly and moderately variable
146 positions (1–9). SILVA, RDP, GTDB, LTP and EMBL-EBI/ENA. In total 61 sequences
147 (1127 pb.) were analyzed.

148 Lithobiontic groups were polyphyletic at Order level (Figure 2). Altough most of the
149 families (25 of 29) showed a pattern of association for specific lithobiontic groups, 4
150 exceptions were found: Rivulariaceae contained in groups IV and V; Hyellaceae
151 contained in groups I and IV; Chroococceae contained in groups I and III;
152 Oscillatoriaceae contained in groups II and III (Table 1-5). For this reason, identifying
153 Cyanobacteria at genus level is necessary to diferenciate their lithobiontic group.

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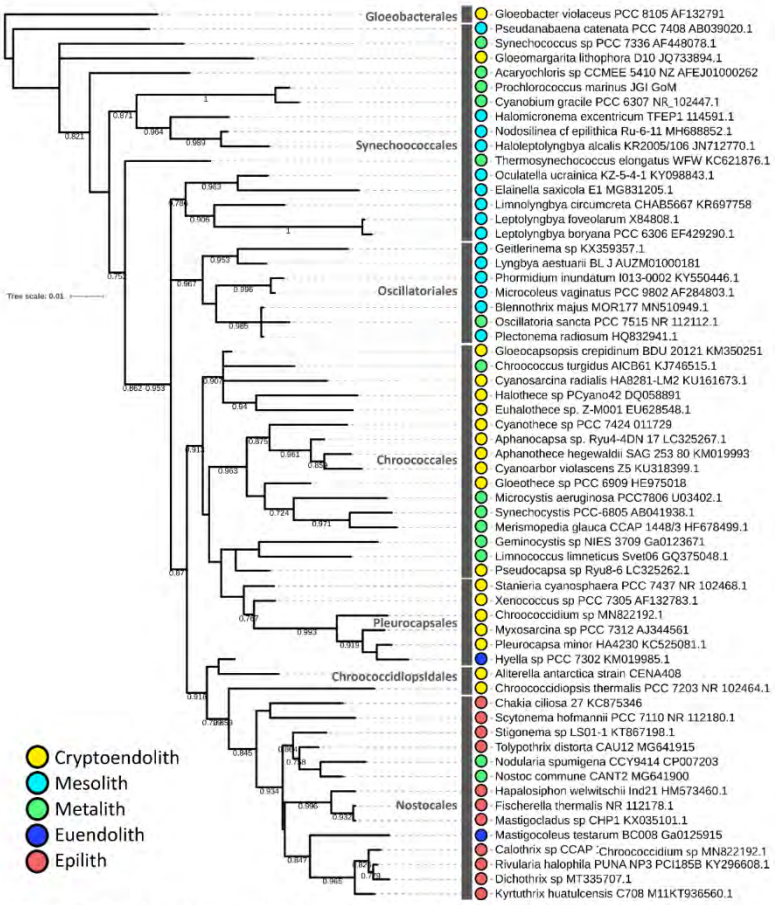


**Figure 2.** Neighbor-joining phylogenetic tree of the most common Cyanobacteria reported in modern microbialites (Bootstrap =1000, K2P), indicating the proposed lithobiotic groups.

## 4. Cyanobacterial lithobiotic groups in the context of microbialite geologic record

Fossil microbialites constitute the earliest evidence of life, the oldest being dated between ~3.5 billion years [132] and ~3.7 billion years [133]. Microbialites have played an important role in the evolution of the Earth's atmosphere, providing niches for microbial metabolisms since the Archean [13]. According to their age, microbialites are divided into Archean microbialites (3.5-2.5 Gya.) [134, 135], Proterozoic microbialites (2.5 Gya. -538 Ma) [136], Phanerozoic microbialites (538 Ma .02 Ma)[137], and Modern microbialites (less than 20 thousand years) [5] (Figure 3). It has been documented how their macro structure and inner fabrics have changed across the geologic record, with stromatolites, as the oldest structures, thrombolites in the mid Proterozoic and dendrolites in the Phanerozoic [13, 138].

Altough the origin of the first metabolisms are debatable [139], the first oxygenic photosynthetic organisms may have originated before 3.0 Ga [140, 141]; molecular clock analysis suggest the first cyanobacteria were a sister group separated from a Gloeobacterales ancestor at 2.8 gya., with cryptoendolithic characteristics [142, 143];

mesoliths would be affiliated to the evolution of multicellular filamentous *Pseudanabaena*-like cyanobacteria, 2.5 Gya [144, 145].

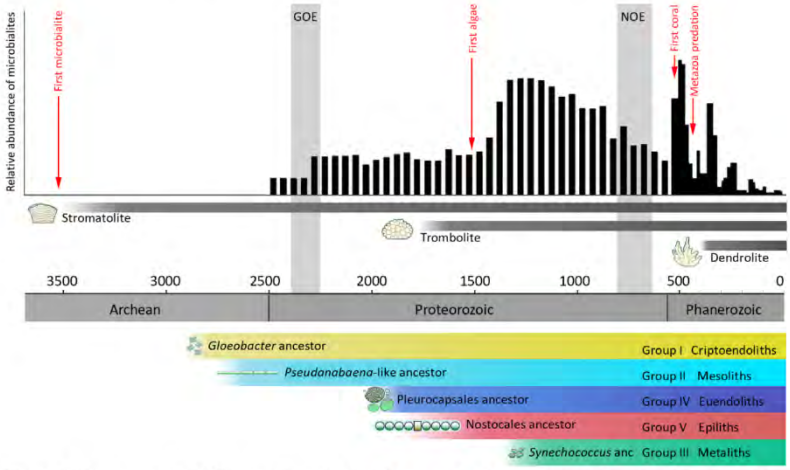


**Figure 3**. Appearance of different cyanobacterial groups in the context of the microbialite geologic record. Microbialite relative abundance modified from [13]. Calibrated ages for Cyanobacterial taxa were obtained from [142, 143].

Microbialite fossils from 2.7 Gya show the first coccal and filamentous cyanobacteria-like structures [146]. Its possible that both cryptoendoliths and mesoliths were responsible for the accumulation of oxygen during the Archean, until the oxygen reached its maximum peak 2.47 Ga ago in the Proterozoic, which caused the Great Oxidation Event (GOE) [147]. Later, the evolution of Pleurocapsales and Nostocales in the mid Proterozoic [148] brought the apparition of euendoliths and epiliths lifestyles. Coincidentally, these lithobiontic groups correlate to the record of the first thrombolytic structures [13]. Microbialites had their maximum abundance at the end of the Mesoproterozoic in the Stateric (1.3 Gya), when the supercontinent Columbia separated in Rodinia and several other island systems, creating a larger coastal area for the development of benthic communities [149, 150]. Planktonic cyanobacteria, including *Synechococcus*, *Prochlorococcus*, and *Cyanobium* made the first metaliths observable in microbialites [151], which correlates to the origin of algae and photosynthetic eukaryonts [152] and the decrease of microbialite populations, since planktonic lifestyles competed with benthic communities [153]. Microbialite abundances increased again in the early Phanerozoic, associated to diatoms and other green microalgae which associated to microbialites, increasing primary productivity, trapping more carbonates more efficiently [154]. The emergence of corals [155] in the Cambrian coincides with dendrolite microbialitic structures in the Phanerozoic [156]. It is speculated that corals and sponges outcompeted microbialites from their photic niche [157]. Also, microbialites in the Phanerozoic suffered predation by foraminifera and other heterotrophic metazoans that evolved during the Cambrian explosion [153, 158]. The last peaks of microbialite abundances correlate to eukaryotic mass extinctions [137], which could corroborate the theory for metazoan predation and eukaryotic niche competition.

Modern microbialites are rare, and are confined to environments which are harsh for microbial grazers to grow [8, 48, 93, 159], still they are some of the more biologically complex extant communities [5, 159], and they contain mostly all lithobiontic cyanobacterial groups. These groups coexist in the microbialite and have different

functions and interactions with other microorganisms, ranging from competence to mutualism (Figure 4).

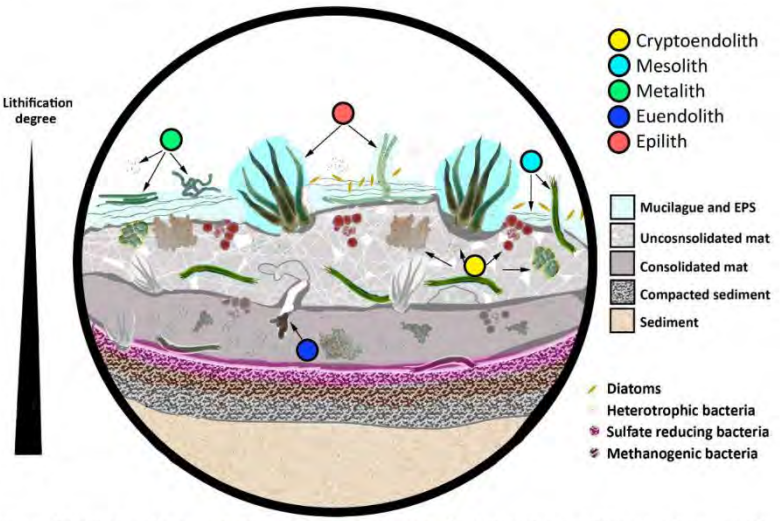


**Figure 4**. Lithobiontic cyanobacterial groups have complex interactions with each other and other microorganisms in microbialites. The lithification degree changes across the microbialite layers. In the outer layers, microbial mats are soft, growing on uncosolidated sediment and a porous carbonate matrix, while inner parts of the microbialite have mats that have been cemented, compacted or completely lithified.

## 5. Concluding remarks and perspectives

Cyanobacteria have demonstrated a high adaptability to different environments with wide range of variables such as pH, temperature, salinity. They represent a high percentage in biomass of the microbial community in microbialites, providing support through EPS production, extracellular precipitation, bioturbation or degradation. Due to these high diversity and functional versatility of Cyanobacteria, its important to differentiate which Cyanobacteria is doing what in microbialites.

In this review we highlight the most common and important Cyanobacteria builders that promote bioformation of microbialites such as Cryptoendoliths, Mesoliths and Epiliths. Within these builders there are differences in movement (sessile or mobile), growth rate (slow or continuous), and specific cellular adaptations (akinetes, heterocites, baeocytes). Also we describe the Cyanobacteria that do not form microbialite per se, such as Metaliths (squatters) and Euendoliths (tenants that promote bioturbation). To address comparative studies of microbialites these differences within Cyanobacteria are crucial to take in consideration.

Lithobiontic groups of Cyanobacteria are polyphyletic, yet families are more consistent with this grouping. The lithobiontic groups presented here are not a reclassification of taxa rather a perspective that could be used to detect indirectly specific habitat characteristics of unknown Cyanobacteria, and will help us understand more about their function and putative role within microbialites. Much more studies are required to classify Cyanobacteria at genus level, for instance euendolithic Cyanobacteria are understudied.

The revisions on evolution of Cyanobacteria has shown a benthic origin with cryptoendoliths and mesoliths in stromatolites, which led to an accumulation of oxygen in the sea and in the atmosphere, then the appearance of euendoliths and epiliths were associated with the appearance of thrombolitic structures. Finally, the evolution of metaliths was associated with a decrease in microbialite populations and an increase in planktonic clades towards the Neoproterozoic Oxidation Event.

Reports of the presence of modern microbialites are scarce, but metagenomic studies have shown a high biodiversity and metabolic potential for the cyanobacterial component. Cyanobacterial communities are the main biosigns of modern fossils, elucidating these groups may be relevant for astrobiology and paeleontology studies, because lithobiontic photosynthetic bacteria leave fossil evidence.

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## References

1. Burne, R.V. and L.S. Moore, *Microbialites; organosedimentary deposits of benthic microbial communities.* Palaios, 1987. **2**(3): p. 241-254.
2. Dupraz, C., et al., *Processes of carbonate precipitation in modern microbial mats.* Earth-Science Reviews, 2009. **96**(3): p. 141-162.
3. Golubic, S., *Modern stromatolites: a review.* Calcareous algae and stromatolites, 1991: p. 541-561.
4. Dupraz, C. and P.T. Visscher, *Microbial lithification in marine stromatolites and hypersaline mats.* Trends in microbiology, 2005. **13**(9): p. 429-438.
5. Dupraz, C., et al., *Microbialites, modern.* Encyclopedia of geobiology, 2011: p. 617-635.
6. Schindler, M. and M.F. Hochella Jr, *Soil memory in mineral surface coatings: Environmental processes recorded at the nanoscale.* Geology, 2015. **43**(5): p. 415-418.
7. Zeyen, N., et al., *Geochemical conditions allowing the formation of modern lacustrine microbialites.* Procedia Earth and Planetary Science, 2017. **17**: p. 380-383.
8. Chagas, A.A., et al., *Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy.* Earth-Science Reviews, 2016. **162**: p. 338-363.
9. Breitbart, M., et al., *Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico.* Environmental Microbiology, 2009. **11**(1): p. 16-34.
10. Yanez-Montalvo, A., et al., *The microbiome of modern microbialites in Bacalar Lagoon, Mexico.* PloS one, 2020. **15**(3): p. e0230071.
11. Lim, D.S., et al., *Limnology of Pavilion Lake, BC, Canada—Characterization of a microbialite forming environment.* Fundamental and Applied Limnology, 2009. **173**(4): p. 329-351.
12. Zhu, T. and M. Dittrich, *Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review.* Frontiers in bioengineering and biotechnology, 2016. **4**: p. 4.
13. Riding, R., *Microbialites, stromatolites, and thrombolites*, in *Encyclopedia of geobiology*. 2011.

14. Gérard, E., et al., *Key role of alphaproteobacteria and cyanobacteria in the formation of stromatolites of Lake Dziani Dzaha (Mayotte, Western Indian Ocean).* Frontiers in microbiology, 2018. **9**: p. 796.
15. Martinez, R.E., et al., *Surface charge and zeta-potential of metabolically active and dead cyanobacteria.* Journal of colloid and interface science, 2008. **323**(2): p. 317-325.
16. Reid, R.P., et al., *The role of microbes in accretion, lamination and early lithification of modern marine stromatolites.* Nature, 2000. **406**(6799): p. 989-992.
17. Decho, A.W. and T. Gutierrez, *Microbial extracellular polymeric substances (EPSs) in ocean systems.* Frontiers in microbiology, 2017. **8**: p. 922.
18. Le Campion-Alsumard, T. and A. Pantazidou, *On the euendolithic genus Solentia Ercegović (Cyanophyta/Cyanobacteria).* Algological Studies/Archiv für Hydrobiologie, Supplement Volumes, 1996: p. 107-127.
19. Radtke, G. and S. Golubic, *Microbial euendolithic assemblages and microborings in intertidal and shallow marine habitats: insight in cyanobacterial speciation*, in *Advances in stromatolite geobiology*. 2011, Springer. p. 233-263.
20. Riding, R., *Calcified cyanobacteria*, in *Calcareous algae and stromatolites*. 1991, Springer. p. 55-87.
21. Cacchio, P. and M. Del Gallo, *A novel approach to isolation and screening of calcifying bacteria for biotechnological applications.* Geosciences, 2019. **9**(11): p. 479.
22. Reitner, J., *Organomineralization*, in *Origins*. 2004, Springer. p. 195-212.
23. Kamennaya, N.A., et al., *High pCO2-induced exopolysaccharide-rich ballasted aggregates of planktonic cyanobacteria could explain Paleoproterozoic carbon burial.* Nature communications, 2018. **9**(1): p. 1-8.
24. Suarez-Gonzalez, P., et al., *'Trapping and binding': A review of the factors controlling the development of fossil agglutinated microbialites and their distribution in space and time.* Earth-Science Reviews, 2019. **194**: p. 182-215.
25. Schneider, J. and T. Le Campion-Alsumard, *Construction and destruction of carbonates by marine and freshwater cyanobacteria.* European Journal of Phycology, 1999. **34**(4): p. 417-426.
26. Macintyre, I.G., L. Prufert-Bebout, and R.P. Reid, *The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites.* Sedimentology, 2000. **47**(5): p. 915-921.
27. Montejano, G., et al., *Gloeobacter violaceus: primitive reproductive scheme and its significance.* Plant Systematics and Evolution, 2018. **304**(10): p. 1221-1229.
28. Büdel, B., et al., *Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes.* Geobiology, 2004. **2**(4): p. 261-268.
29. Saw, J.H., T. Cardona, and G. Montejano, *Complete Genome Sequencing of a Novel Gloeobacter Species from a Waterfall Cave in Mexico.* Genome Biology and Evolution, 2021. **13**(12).
30. Mosca, C., et al., *Over-expression of UV-damage DNA repair genes and ribonucleic acid persistence contribute to the resilience of dried biofilms of the desert cyanobacetrium Chroococcidiopsis exposed to Mars-like UV flux and long-term desiccation.* Frontiers in Microbiology, 2019: p. 2312.
31. Komárek, J. and K.C. Anagnostidis, *Teil 1/Part 1: Chroococcales.* Süßwasserflora von Mitteleuropa; Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D., Eds, 2008: p. 1-556.
32. Pinevich, A., et al., *Baeocytes in the cyanobacterium Pleurocapsa sp.: Characterization of the differentiated cells produced by multiple fission.* Microbiology, 2008. **77**(1): p. 62-68.
33. Waterbury, J.B. and R.Y. Stanier, *Patterns of growth and development in pleurocapsalean cyanobacteria.* Microbiological reviews, 1978. **42**(1): p. 2.
34. Šmarda, J., *S-layer in cyanobacteria*, in *Crystalline Bacterial Cell Surface Layers*. 1988, Springer. p. 127-132.
35. Kempe, S. and J. Kazmierczak, *Hydrochemical key to the genesis of calcareous nonlaminated and laminated cyanobacterial microbialites*, in *Algae and cyanobacteria in extreme environments*. 2007, Springer. p. 239-264.
36. Potts, M. and E.I. Friedmann, *Effects of water stress on cryptoendolithic cyanobacteria from hot desert rocks.* Archives of Microbiology, 1981. **130**(4): p. 267-271.
37. Rexroth, S., et al., *The Plasma Membrane of the Cyanobacterium Gloeobacter violaceus Contains Segregated Bioenergetic Domains* The Plant Cell, 2011. **23**(6): p. 2379-2390.
38. Gérard, E., et al., *Specific carbonate–microbe interactions in the modern microbialites of Lake Alchichica (Mexico).* The ISME journal, 2013. **7**(10): p. 1997-2009.

344 39. Power, I., et al., *Modern carbonate microbialites from an asbestos open pit pond, Yukon, Canada.* Geobiology,
345 2011. **9**(2): p. 180-195.
346 40. White III, R.A., et al., *Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core*
347 *microbial community.* Frontiers in microbiology, 2016. **6**: p. 1531.
348 41. Yanez-Montalvo, A., et al., *Depth Related Structure and Microbial Composition of Microbialites in a Karst*
349 *Sinkhole, Cenote Azul, Mexico.* Geomicrobiology Journal, 2021. **38**(3): p. 237-251.
350 42. Pace, A., et al., *Microbial and diagenetic steps leading to the mineralisation of Great Salt Lake microbialites.*
351 Scientific Reports, 2016. **6**(1): p. 1-12.
352 43. Schneider, D., et al., *Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of*
353 *the Kiritimati Atoll, Central Pacific.* PloS one, 2013. **8**(6): p. e66662.
354 44. Golubic, S. and R.M. Abed, *Entophysalis mats as environmental regulators*, in *Microbial Mats*. 2010, Springer.
355 p. 237-251.
356 45. Dupraz, C. *Microbe-carbonate mineral interactions in modern microbialite formation (open marine and*
357 *hypersaline environments, Bahamas)*. in *The 2nd Swiss Geoscience Meeting, Lausanne*. 2004.
358 46. Reitner, J., et al., *Lake Thetis domal microbialites; a complex framework of calcified biofilms and*
359 *organomicrites (Cervantes, Western Australia).* Global and regional controls on biogenic sedimentation, 1996.
360 **1**: p. 85-89.
361 47. Beltrán, Y., et al., *N2 fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia*
362 *from different aquatic environments in Mexico.* Aquatic microbial ecology, 2012. **67**(1): p. 15-24.
363 48. Russell, J., et al., *Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British*
364 *Columbia, Canada.* Geobiology, 2014. **12**(3): p. 250-264.
365 49. Águila, B., et al., *Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization.*
366 European Journal of Phycology, 2021: p. 1-16.
367 50. Corman, J. and J. Elser, *Life on a stoichiometric knife edge: biogeochemical interactions and trophic interactions*
368 *in stromatolites in Rio Mesquites*, in *Ecosystem Ecology and Geochemistry of Cuatro Cienegas*. 2018, Springer.
369 p. 55-65.
370 51. Last, F.M., W.M. Last, and N.M. Halden, *Carbonate microbialites and hardgrounds from Manito Lake, an*
371 *alkaline, hypersaline lake in the northern Great Plains of Canada.* Sedimentary Geology, 2010. **225**(1-2): p. 34-
372 49.
373 52. Warthmann, R., et al. *A unique model system of microbial carbonate precipitation: stromatolites of Lagoa*
374 *Vermelha, Brazil*. in *EGS-AGU-EUG Joint Assembly*. 2003.
375 53. Hoiczyk, E., *Gliding motility in cyanobacteria: observations and possible explanations.* Archives of
376 microbiology, 2000. **174**(1): p. 11-17.
377 54. Shepard, R. and D. Sumner, *Undirected motility of filamentous cyanobacteria produces reticulate mats.*
378 Geobiology, 2010. **8**(3): p. 179-190.
379 55. Yamamoto, H., et al., *Scattered migrating colony formation in the filamentous cyanobacterium,*
380 *Pseudanabaena sp. NIES-4403.* BMC microbiology, 2021. **21**(1): p. 1-17.
381 56. Jiménez, J.C., Y.B. Magos, and L. Collado-Vides, *Taxonomy and distribution of freshwater Blennothrix ganeshii*
382 *Watanabe et Komárek (Oscillatoriaceae, Cyanophyceae) from central Mexico.* Nova Hedwigia, 2005. **80**(3-4):
383 p. 323-334.
384 57. Fattom, A. and M. Shilo, *Hydrophobicity as an adhesion mechanism of benthic cyanobacteria.* Applied and
385 Environmental Microbiology, 1984. **47**(1): p. 135-143.
386 58. Payandi-Rolland, D., et al., *Carbonate Precipitation in Mixed Cyanobacterial Biofilms Forming Freshwater*
387 *Microbial Tufa.* Minerals, 2019. **9**(7): p. 409.
388 59. Paulo, C., *Organomineralization of Cyanobacteria Cell Envelopes: Insights from Laboratory Studies and*
389 *Implications to Carbonates Nucleation, Mineralogy and Carbon Cycling*. 2016.
390 60. Klatt, J.M., et al., *Cyanobacteria in sulfidic spring microbial mats can perform oxygenic and anoxygenic*
391 *photosynthesis simultaneously during an entire diurnal period.* Frontiers in microbiology, 2016. **7**: p. 1973.
392 61. Padan, E., *Facultative anoxygenic photosynthesis in cyanobacteria.* Annual review of plant physiology, 1979.
393 **30**(1): p. 27-40.

62. Chan, O.W., et al., *Phylogenetic diversity of a microbialite reef in a cold alkaline freshwater lake.* Canadian journal of microbiology, 2014. **60**(6): p. 391-398.
63. Oh, S., et al., *Evolution and adaptation of SAR11 and Cyanobium in a saline Tibetan lake.* Environmental Microbiology Reports, 2016. **8**(5): p. 595-604.
64. Gischler, E., M.A. Gibson, and W. Oschmann, *Giant holocene freshwater microbialites, laguna bacalar, quintana roo, Mexico.* Sedimentology, 2008. **55**(5): p. 1293-1309.
65. Ohki, K., et al., *Morphological, phylogenetic and physiological studies of pico-cyanobacteria isolated from the halocline of a saline meromictic lake, Lake Suigetsu, Japan.* Microbes and environments, 2009: p. 1112210347-1112210347.
66. Reynolds, C.S., R.L. Oliver, and A.E. Walsby, *Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments.* New Zealand journal of marine and freshwater research, 1987. **21**(3): p. 379-390.
67. Hipsher, C., J. Barker, and A. MacKay, *Impact of bloom events on dissolved organic matter fluorophore signatures in Ohio waters.* Science of the Total Environment, 2020. **699**: p. 134003.
68. Iwamoto, K., et al., *Cryopreservation of the chlorophyll d-containing cyanobacterium Acaryochloris marina.* Procedia Environmental Sciences, 2012. **15**: p. 118-125.
69. Gao, Z.-M., et al., *Symbiotic adaptation drives genome streamlining of the cyanobacterial sponge symbiont "Candidatus Synechococcus spongiarum".* MBio, 2014. **5**(2): p. e00079-14.
70. Kühl, M., et al., *A niche for cyanobacteria containing chlorophyll d.* Nature, 2005. **433**(7028): p. 820-820.
71. Chen, M., et al., *A cyanobacterium that contains chlorophyll f–a red-absorbing photopigment.* FEBS letters, 2012. **586**(19): p. 3249-3254.
72. Swan, J.A., et al., *Structure, function, and mechanism of the core circadian clock in cyanobacteria.* Journal of Biological Chemistry, 2018. **293**(14): p. 5026-5034.
73. Wasmund, N., M. Voss, and K. Lochte, *Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and re-calculation of a budget of nitrogen fixation.* Marine Ecology Progress Series, 2001. **214**: p. 1-14.
74. Jacob-Lopes, E., L.Q. Zepka, and M.I. Queiroz, *Cyanobacteria and carbon sequestration.* Cyanobacteria: An Economic Perspective, 2014: p. 65-71.
75. Ramírez-Reinat, E. and F. Garcia-Pichel, *Prevalence of Ca2+-ATPase-mediated carbonate dissolution among cyanobacterial euendoliths.* Applied and environmental microbiology, 2012. **78**(1): p. 7-13.
76. Guida, B.S., *Unique Cellular, Physiological, and Metabolic Adaptations to the Euendolithic Lifestyle in a Boring Cyanobacterium*. 2016: Arizona State University.
77. Yu, H., S. Jia, and Y. Dai, *Growth characteristics of the cyanobacterium Nostoc flagelliforme in photoautotrophic, mixotrophic and heterotrophic cultivation.* Journal of Applied Phycology, 2009. **21**(1): p. 127-133.
78. Olsson-Francis, K. and C.S. Cockell, *Use of cyanobacteria for in-situ resource use in space applications.* Planetary and Space Science, 2010. **58**(10): p. 1279-1285.
79. Roush, D. and F. Garcia-Pichel, *Succession and colonization dynamics of endolithic phototrophs within intertidal carbonates.* Microorganisms, 2020. **8**(2): p. 214.
80. Shields, T.J., *Lipid biomarker and pigment signature analysis of Fayetteville Green Lakes microbialites*. 2017, State University of New York College of Environmental Science and Forestry.
81. Mlewski, E.C., et al., *Characterization of pustular mats and related rivularia-rich laminations in oncoids from the Laguna Negra lake (Argentina).* Frontiers in microbiology, 2018. **9**: p. 996.
82. López-García, P., et al., *Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey.* Extremophiles, 2005. **9**(4): p. 263-274.
83. De Anda, V., et al., *Understanding the mechanisms behind the response to environmental perturbation in microbial mats: a metagenomic-network based approach.* Frontiers in microbiology, 2018. **9**: p. 2606.
84. Perez, R., et al., *Clear differences in metabolic and morphological adaptations of akinetes of two Nostocales living in different habitats.* Microbiology, 2016. **162**(2): p. 214-223.
85. Lassalle, F., et al., *GC-content evolution in bacterial genomes: the biased gene conversion hypothesis expands.* PLoS genetics, 2015. **11**(2): p. e1004941.

86. Wolfe, J.M. and G.P. Fournier, *Horizontal gene transfer constrains the timing of methanogen evolution.* Nature ecology & evolution, 2018. **2**(5): p. 897-903.
87. Pentecost, A. and H.G. Edwards, *Raman spectroscopy and light microscopy of a modern and sub-fossil microstromatolite: Rivularia haematites (cyanobacteria, Nostocales).* International Journal of Astrobiology, 2002. **1**(4): p. 357-363.
88. Mobberley, J.M., C.L. Khodadad, and J.S. Foster, *Metabolic potential of lithifying cyanobacteria-dominated thrombolitic mats.* Photosynthesis research, 2013. **118**(1-2): p. 125-140.
89. Gischler, E., et al., *Microbial mats and microbialites in the freshwater Laguna Bacalar, Yucatan Peninsula, Mexico*, in *Advances in stromatolite geobiology*. 2011, Springer. p. 187-205.
90. Graham, L.E., et al., *Lacustrine N ostoc (N ostocales) and associated microbiome generate a new type of modern clotted microbialite.* Journal of phycology, 2014. **50**(2): p. 280-291.
91. Kazmierczak, J. and S. Kempe, *Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga.* Naturwissenschaften, 2006. **93**(3): p. 119-126.
92. Souza, V., et al., *The cuatro ciénegas basin in Coahuila, Mexico: an astrobiological precambrian park.* Astrobiology, 2012. **12**(7): p. 641-647.
93. Valdespino-Castillo, P.M., et al., *Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba.* Frontiers in microbiology, 2018. **9**: p. 510.
94. Komárek, J., et al., *Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach.* Preslia, 2014. **86**(4): p. 295-335.
95. Komárek, J., *A polyphasic approach for the taxonomy of cyanobacteria: principles and applications.* European Journal of Phycology, 2016. **51**(3): p. 346-353.
96. Smith, M.D., et al., *Effects of recent increases in salinity and nutrient concentrations on the microbialite community of Lake Clifton (Western Australia): are the thrombolites at risk?* Hydrobiologia, 2010. **649**(1): p. 207-216.
97. Baskin, R.L., G. Della Porta, and V.P. Wright, *Characteristics and controls on the distribution of sublittoral microbial bioherms in Great Salt Lake, Utah: Implications for understanding microbialite development.* 2022.
98. Abed, R.M., et al., *Characterization of microbialite-forming cyanobacteria in a tropical lagoon: Tikehau Atoll, Tuamotu, French Polynesia.* Journal of Phycology, 2003. **39**(5): p. 862-873.
99. Alcántara-Hernández, R.J., et al., *Genetic diversity associated with N-cycle pathways in microbialites from Lake Alchichica, Mexico.* Aquatic Microbial Ecology, 2017. **78**(2): p. 121-133.
100. Goh, F., et al., *Determining the specific microbial populations and their spatial distribution within the stromatolite ecosystem of Shark Bay.* The ISME journal, 2009. **3**(4): p. 383-396.
101. Nguyen, S.T., et al., *Bacterial community structure and metabolic potential in microbialite-forming mats from South Australian saline lakes.* Geobiology, 2022.
102. Garby, T.J., et al., *Diversity of cyanobacterial biomarker genes from the stromatolites of Shark Bay, Western Australia.* Environmental microbiology, 2013. **15**(5): p. 1464-1475.
103. Burns, B.P., M.R. Walter, and B.A. Neilan, *Microbial communities of stromatolites*, in *From Fossils to Astrobiology*. 2009, Springer. p. 143-158.
104. Foster, J.S., et al., *Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas.* The ISME journal, 2009. **3**(5): p. 573-587.
105. Johnson, D., et al., *Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar, Mexico.* Geobiology, 2018. **16**(3): p. 319-337.
106. Roche, A., et al., *The Role of the Substrate on the Mineralization Potential of Microbial Mats in A Modern Freshwater River (Paris Basin, France).* Minerals, 2019. **9**(6): p. 359.
107. Tarhan, L.G., et al., *Microbial mat controls on infaunal abundance and diversity in modern marine microbialites.* Geobiology, 2013. **11**(5): p. 485-497.
108. Beltrán-Magos, Y., et al., *Calcification of the filamentous cyanobacterium Blennothrix ganeshii in calcareous tropical streams of central Mexico region.* Hidrobiológica, 2013. **23**: p. 17-27.
109. Arp, G., A. Reimer, and J. Reitner, *Arp G, Reimer A, Reitner J.. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. J Sediment Res 73: 105-127.* Journal of Sedimentary Research - J SEDIMENT RES, 2003. **73**: p. 105-127.

110. Reid, R.P., I.G. Macintyre, and R.S. Steneck, *A microbialite/algal ridge fringing reef complex, Highborne Cay, Bahamas.* Atoll Research Bulletin, 1999.
111. Gautret, P., et al., *Biochemical control of calcium carbonate precipitation in modern lagoonal microbialites, Tikehau Atoll, French Polynesia.* Journal of Sedimentary Research, 2004. **74**(4): p. 462-478.
112. Cellamare, M., et al., *Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean).* FEMS microbiology ecology, 2018. **94**(8): p. fiy108.
113. Johnson, M.E., et al., *Lagoon microbialites on Isla Angel de la Guarda and associated peninsular shores, Gulf of California (Mexico).* Sedimentary Geology, 2012. **263**: p. 76-84.
114. Laval, B., et al., *Modern freshwater microbialite analogues for ancient dendritic reef structures.* Nature, 2000. **407**(6804): p. 626-629.
115. Kazmierczak, J., et al. *Modern and sub-recent carbonate microbialites from the alkaline crater lake Alchichica, Mexico*. in *Geobiology of Stromatolites. International Kalkowsky-Symposium, Abstract Volume and Field Guide to excursions, Gottingen, Germany*. 2008.
116. Krings, M. and V.N. Sergeev, *A coccoid, colony-forming cyanobacterium from the Lower Devonian Rhynie chert that resembles Eucapsis (Synechococcales) and Entophysalis (Chroococcales).* Review of Palaeobotany and Palynology, 2019. **268**: p. 65-71.
117. Han, Z., et al., *Bio-precipitation of calcite with preferential orientation induced by Synechocystis sp. PCC6803.* Geomicrobiology Journal, 2014. **31**(10): p. 884-899.
118. Valdespino-Castillo, P.M., et al., *Microbialites: Diversity Hotspots in the Mexican Plateau*, in *Lake Alchichica Limnology*. 2022, Springer. p. 375-390.
119. Saghaï, A., et al., *Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites.* Frontiers in microbiology, 2015. **6**: p. 797.
120. Ferris, F.G., J.B. Thompson, and T.J. Beveridge, *Modern freshwater microbialites from Kelly Lake, British Columbia, Canada.* Palaios, 1997: p. 213-219.
121. Reid, R.P., et al., *Modern marine stromatolites of Little Darby Island, Exuma Archipelago, Bahamas: environmental setting, accretion mechanisms and role of euendoliths*, in *Advances in stromatolite geobiology*. 2011, Springer. p. 77-89.
122. Pamela Reid, R., et al., *Shark Bay stromatolites: microfabrics and reinterpretation of origins.* Facies, 2003. **49**(1): p. 299-324.
123. Díez, B., K. Bauer, and B. Bergman, *Epilithic cyanobacterial communities of a marine tropical beach rock (Heron Island, Great Barrier Reef): diversity and diazotrophy.* Applied and environmental microbiology, 2007. **73**(11): p. 3656-3668.
124. Ramírez-Reinat, E.L. and F. Garcia-Pichel, *Characterization of a marine cyanobacterium that bores into carbonates and the redescription of the genus Mastigocoleus.* Journal of phycology, 2012. **48**(3): p. 740-749.
125. Couradeau, E., et al., *Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico).* PloS one, 2011. **6**(12): p. e28767.
126. Boidi, F.J., et al., *Characterization of microbialites and microbial mats of the Laguna Negra hypersaline lake (Puna of Catamarca, Argentina)*, in *Microbial Ecosystems in Central Andes Extreme Environments*. 2020, Springer. p. 183-203.
127. Kaźmierczak, J., et al., *Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico.* Facies, 2011. **57**(4): p. 543-570.
128. Pentecost, A. and U. Franke, *Photosynthesis and calcification of the stromatolitic freshwater cyanobacterium Rivularia.* Eur. J. Phycol., 2010. **45**(4): p. 345-353.
129. Doddy, P., C.M. Roden, and M.P. Gammell. *Microbialite crusts in Irish limestone lakes reflect lake nutrient status*. in *Biology and Environment: Proceedings of the Royal Irish Academy*. 2019. JSTOR.
130. Kremer, B., et al., *Calcification and silicification: fossilization potential of cyanobacteria from stromatolites of Niuafo 'ou's Caldera Lakes (Tonga) and implications for the early fossil record.* Astrobiology, 2012. **12**(6): p. 535-548.

131. Pruesse, E., J. Peplies, and F.O. Glöckner, *SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes.* Bioinformatics, 2012. **28**(14): p. 1823-1829.
132. Awramik, S.M., *Precambrian columnar stromatolite diversity: reflection of metazoan appearance.* Science, 1971. **174**(4011): p. 825-827.
133. Dodd, M.S., et al., *Evidence for early life in Earth's oldest hydrothermal vent precipitates.* Nature, 2017. **543**(7643): p. 60-64.
134. Schopf, J.W., *Fossil evidence of Archaean life.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2006. **361**(1470): p. 869-885.
135. Schopf, J.W., et al., *Evidence of Archean life: stromatolites and microfossils.* Precambrian Research, 2007. **158**(3-4): p. 141-155.
136. Butterfield, N.J., *Proterozoic photosynthesis–a critical review.* Palaeontology, 2015. **58**(6): p. 953-972.
137. Mata, S.A. and D.J. Bottjer, *Microbes and mass extinctions: paleoenvironmental distribution of microbialites during times of biotic crisis.* Geobiology, 2012. **10**(1): p. 3-24.
138. Riding, R., *Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic–Cambrian changes in atmospheric composition.* Geobiology, 2006. **4**(4): p. 299-316.
139. Becerra, A., *The Semi-Enzymatic Origin of Metabolic Pathways: Inferring a Very Early Stage of the Evolution of Life.* Journal of Molecular Evolution, 2021. **89**(3): p. 183-188.
140. Fischer, W.W., J. Hemp, and J.E. Johnson, *Evolution of Oxygenic Photosynthesis.* Annual Review of Earth and Planetary Sciences, 2016. **44**(1): p. 647-683.
141. Delwiche, C.F., *Microbial biodiversity: A newly isolated cyanobacterium sheds light on the evolution of photosynthesis.* Current Biology, 2021. **31**(13): p. R843-R845.
142. Sánchez-Baracaldo, P. and T. Cardona, *On the origin of oxygenic photosynthesis and Cyanobacteria.* New Phytologist, 2020. **225**(4): p. 1440-1446.
143. Sánchez-Baracaldo, P., et al., *Cyanobacteria and biogeochemical cycles through Earth history.* Trends in Microbiology, 2022. **30**(2): p. 143-157.
144. Schirrmeister, B.E., A. Antonelli, and H.C. Bagheri, *The origin of multicellularity in cyanobacteria.* BMC Evolutionary Biology, 2011. **11**(1): p. 45.
145. Schirrmeister, B.E., et al., *Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event.* Proceedings of the National Academy of Sciences, 2013. **110**(5): p. 1791-1796.
146. Schopf, J.W., *The Fossil Record of Cyanobacteria*, in *Ecology of Cyanobacteria II: Their Diversity in Space and Time*, B.A. Whitton, Editor. 2012, Springer Netherlands: Dordrecht. p. 15-36.
147. Gumsley, A.P., et al., *Timing and tempo of the Great Oxidation Event.* Proceedings of the National Academy of Sciences, 2017. **114**(8): p. 1811.
148. Schirrmeister, B.E., M. Gugger, and P.C.J. Donoghue, *Cyanobacteria and the Great Oxidation Event: evidence from genes and fossils.* Palaeontology, 2015. **58**(5): p. 769-785.
149. Wang, W., et al., *Enhanced terrestrial input into Paleoproterozoic to Mesoproterozoic carbonates in the southwestern South China Block during the fragmentation of the Columbia supercontinent.* Precambrian Research, 2018. **313**: p. 1-17.
150. Planavsky, N. and K. Grey, *Stromatolite branching in the Neoproterozoic of the Centralian Superbasin, Australia: an investigation into sedimentary and microbial control of stromatolite morphology.* Geobiology, 2008. **6**(1): p. 33-45.
151. Sánchez-Baracaldo, P., *Origin of marine planktonic cyanobacteria.* Scientific Reports, 2015. **5**(1): p. 17418.
152. Yoon, H.S., et al., *A Molecular Timeline for the Origin of Photosynthetic Eukaryotes.* Molecular Biology and Evolution, 2004. **21**(5): p. 809-818.
153. Knoll, A.H., I.J. Fairchild, and K. Swett, *Calcified Microbes in Neoproterozoic Carbonates: Implications for Our Understanding of the Proterozoic/Cambrian Transition.* PALAIOS, 1993. **8**(6): p. 512-525.
154. Woo, J. and S.K. Chough, *Growth patterns of the Cambrian microbialite: Phototropism and speciation of Epiphyton.* Sedimentary Geology, 2010. **229**(1): p. 1-8.
155. Stanley, G.D., *Photosymbiosis and the Evolution of Modern Coral Reefs.* Science, 2006. **312**(5775): p. 857-858.
156. Shapiro, R.S. and J.K. Rigby, *First occurrence of an in situ Anthaspidellid sponge in a dendrolite mound (Upper Cambrian; Great Basin, USA).* Journal of Paleontology, 2004. **78**(4): p. 645-650.

596 157. Guido, A., et al., *Dataset of biogenic crusts from submarine caves of the Aegean Sea: An example of sponges*
597 *vs microbialites competition in cryptic environments.* Data in Brief, 2019. **27**: p. 104745.
598 158. Foster, W.J., et al., *Suppressed competitive exclusion enabled the proliferation of Permian/Triassic boundary*
599 *microbialites.* The Depositional Record, 2020. **6**(1): p. 62-74.
600 159. Souza, V., et al., *The lost world of Cuatro Ciénegas Basin, a relictual bacterial niche in a desert oasis.* eLife,
601 2018. **7**: p. e38278.

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# 6 DISCUSIÓN GENERAL

## 6.1 Integración polifásica

El marco de trabajo polifásico desarrollado en esta tesis puede aplicarse en el estudio de cianobacterias en otros sistemas. Con este acercamiento se obtienen datos morfológicos, taxonómicos, ecológicos y funcionales, además de que los consorcios y cultivos aislados pueden usarse posteriormente para ensayos bioquímicos y fisiológicos, para describir nuevas especies o clados y corroborar genes putativos nuevos encontrados por metagenómica ambiental (**Figura 12**).

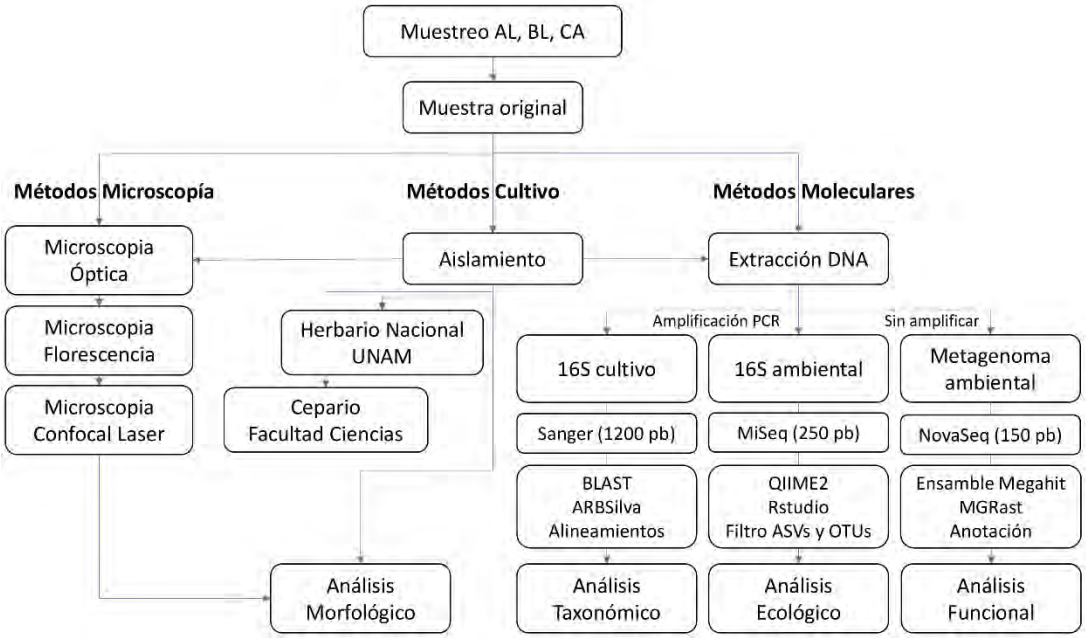


***Figura 12.*** *Diagrama de flujo de la metodología para los análisis morfológicos, taxonómicos, ecológicos y funcionales de microbialitas de AL, BL y CA.*

## 6.2 Grupos funcionales

Las similitudes funcionales encontradas en AL, BL y CA pueden estar dadas por que son el mismo tipo de comunidad formadora de microbialitas, algo que ya se ha descrito en tapetes microbianos y en suelo (Yin, Crowley et al. 2000, Dupraz, Reid et al. 2009, Rousk, Brookes et al. 2009). Aun así, no se descarta una falta de información en la base de datos, ya que muchas cianobacterias descritas en estos sistemas, son nuevas para las bases genómicas y no pueden anotarse correctamente, es decir falta resolución funcional para organismos poco conocidos.

Gracias a la información recabada en este trabajo y una extensa revisión bibliográfica, se agruparon a las cianobacterias en cinco grupos funcionales de acuerdo a su ecología litobiónticа y sus adaptaciones particulares, como a la luz, patrón de movimiento, tasa de crecimiento, tamaño genómico, tipo de precipitación mineral, entre otras. Esta agrupación funcional será útil para futuros estudios de las comunidades de cianobacterias de microbialitas de otras regiones. A continuación, se enlistan los grupos y sus características generales.

**Grupo I. Criptoendolitos**

Cianobacterias adaptadas a hábitats con baja intensidad lumínica y de crecimiento lento. Son comunidades sésiles, sin capacidad de perforar, viven dentro de los poros de sedimento o enterradas en material detrítico que ellas mismas precipitan. La precipitación extracelular de estas cianobacterias produce carbonatos laminados que no contienen sedimentos. Entre los criptoendolitos más extendidos en microbialitas se encuentran los órdenes Gloeobacterales, Chroococcales bentónicas y Pleurocapsales (**Figura 13**). Se encuentran en cuevas y microbialitas de profundidad hasta los 30 metros.

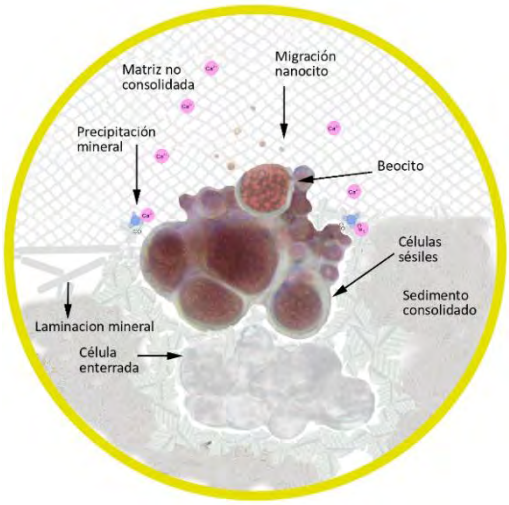


***Figura 13.*** *Los criptoendolitos incluyen a los géneros como Gloeobacter, Aphanothece, Halothece, Gloeothece, Entophysalis, Cyanothece, Cyanoarbor, Aliterella, Chroococcidiopsis, Staneria, Pleurocapsa, Chroococcidum, Xenococcus, Myxosarcina.*

**Grupo II. Mesolitos**

Cianobacterias adaptadas a moverse entre la matriz microbialítica blanda o no litificada, de acuerdo a las condiciones de luz, concentración de nutrientes o para evitar depredación por herbivoria. Tienen alta producción de EPS con resistencia a la alcalinidad y a la hipersalinidad. Es común que estén asociados en las capas más externas de microbialitas a veces cubriendo completamente la estructura. La precipitación extracelular de estas cianobacterias produce minerales amorfos esparíticos. Incluye a cianobacterias filamentosas del orden Synechococcales y Oscillatoriales bentónicas (**Figura 14**). Son

el único grupo cosmopolita en microbialitas modernas, aunque están en mayor abundancia en microbialitas de sistemas oligotróficos.

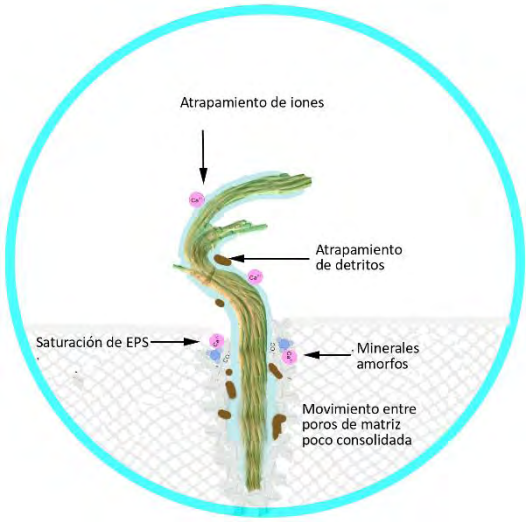


***Figura 14.*** *Los mesolitos incluyen a los géneros como Homoeothrix, Microcoleus, Blennothrix, Lyngbya, Phormidium, Plectonema, Schizothrix, Haloleptolyngbya, Halomicronema, Leptolyngbya, Limnolingbya, Geitlerinema, Pseudanabaena.*

**Grupo III. Metalitos**

Son cianobacterias planctónicas y del metafiton. Están adaptadas a nichos de 2 hasta los 15 metros de profundidad y se mueven en la columna de agua dependiendo sus necesidades fotosintéticas. Al florecer, precipitan carbonato cayendo sobre la microbialita y enterrando a otras comunidades bentónicas. Tienen ciclos de vida cortos y genomas reducidos. Son cianobacterias cocales de los órdenes Synechococcales y Chroococcales que precipitan carbonatos extracelularmente por fotosíntesis, aunque también hay ejemplos de Oscillatoriales y Nostocales planctónicos (**Figura 15**). Son comunes en sistemas de marinos y lacustres, oligotróficas e hipersalinas.

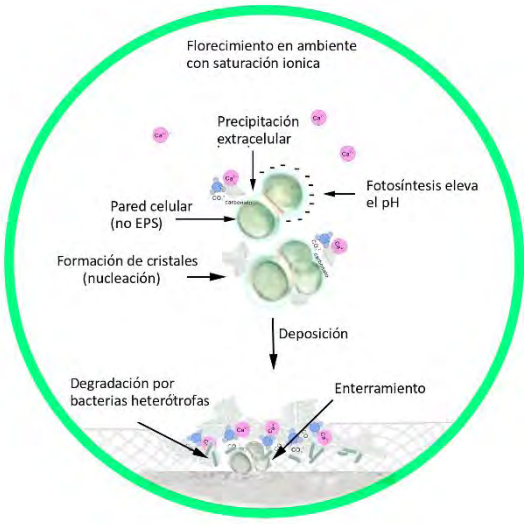


***Figura 15.*** *Los metalitos incluyen a los géneros como Chroococcus, Cyanothece, Microcystis, Nodularia, Nostoc, Oscillatoria, Acaryochloris, Aphanocapsa, Eucapsis, Synechocystis, Cyanobium, Prochlorococcus, Synechococcus*

**Grupo IV. Euendolitos**

Cianobacterias que penetran activamente rocas y sedimento, provocando estructuras fenestrales y túneles en sustratos minerales calcáreos, fosfatados y travertinos. Tienen una actividad fotosintética reducida y presentan metabolismos heterótrofos como fermentación. Facilitan la cementación de microbialitas con la sucesión ecológica de bacterias heterótrofas. Incluye pocos géneros del orden Pleurocapsales y Nostocales (**Figura 16**). Solo se han descrito en microbialitas de sistemas marinos y de cuevas.

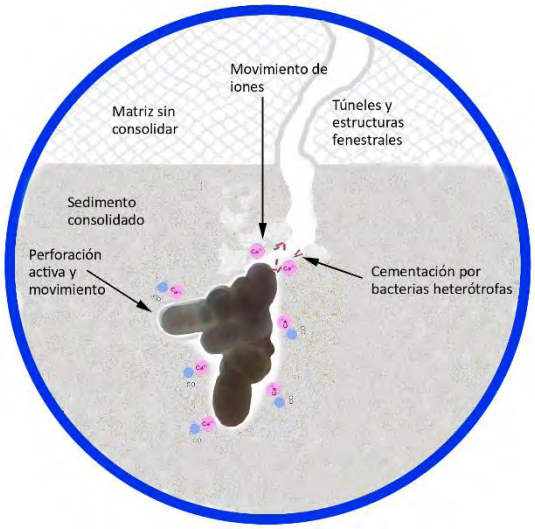


***Figura 16.*** *Los euendolitos incluyen a los géneros como Solentia, Hyella, Cyanosaccus, Hormathonema, Kyrtuthrix, Mastigocoleus.*

**Grupo V. Epilitos**

Cianobacterias sésiles que se insertan con su base en el substrato y forman tapetes homogéneos de colonias circulares o radiales. Adaptados a fijar nitrógeno con heterocitos, por lo que pueden sobrevivir en ambientes extremadamente oligotróficos. Tienen genomas grandes, mecanismos complejos de regulación genética y alto contenido GC. Producen gran cantidad de foto pigmentos por lo que toleran alta radiación UV. Son la base trófica de otros organismos degradadores y heterotróficos, por eso son importantes para el crecimiento y mantenimiento de los tapetes microbianos. Incluyen a todos los Nostocales bentónicos (**Figura 17**). Se encuentran en lagos hipersalinos-alcalinos, lagos oligotróficos y pozas oligotróficas a profundidades no mayores de 15 m.

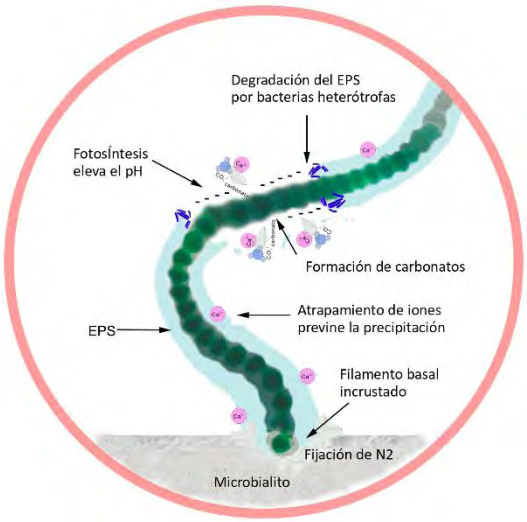


***Figura 17.*** *Los epilitos incluyen a los géneros como Calothrix, Dichothrix, Rivularia, Chakia, Scytonema, Fischerella, Stigonema, Tolypothrix.*

## 6.3 Importancia del estudio de cianobacterias y microbialitas

Conservación: Además de ser un componente fundamental de la diversidad bacteriana, las cianobacterias son un excelente bioindicador de salud en los ecosistemas acuáticos como microbialitas, útiles para el monitoreo y conservación de los ecosistemas en los que se desarrollan. Las microbialitas son sistemas únicos en el mundo que tienen funciones a nivel regional, sirviendo como refugio y guardería de fauna, secuestro de carbono, filtrado y atrapamiento de contaminantes y detritos en la columna de agua, además de contener información genómica y paleo ambiental invaluable.

Biología evolutiva: Al ser comunidades estables temporalmente (miles de años), las microbialitas son un excelente modelo para estudiar interacciones bióticas, construcción de nicho, sucesión biótica y comprobar hipótesis evolutivas (hipótesis de la reina y rey rojo, hipótesis de la reina negra, la hipótesis del mutualismo-parasitismo continuo, entre otros).

Paleo clima y astrobiología: La importancia de su estudio es que las microbialitas son usadas como proxis climáticos para datar los estratos geológicos. También al ser una de las primeras formas de vida son relevantes en el campo de la exobiología, donde se esperarían encontrar indicios de estas estructuras en otros planetas.

Secuestro de carbono: Las poblaciones de microbialitas han estado secuestrando carbono desde hace al menos 3.4 Ga y posiblemente, desde hace 3.7 Ga (Noffke, Gerdes et al. 2001) y en nuestros sitios de estudio desde hace 2 mil a 9.5 mil años. Las microbialitas tienen aplicación potencial como

sumideros y secuestradores de carbono (Zhu and Dittrich 2016). Por lo tanto, la presencia y desarrollo de microbialitas en ecosistemas acuáticos modernos, puede usarse como una estrategia o medida de mitigación de carbono.

# 7. CONCLUSIONES Y PERSPECTIVAS

En esta tesis se lograron estudiar a las cianobacterias de las microbialitas en los sistemas Lago Alchichica, Laguna Bacalar y Cenote Azul.

Se describieron nuevos grupos taxonómicos y se describió su perfil funcional, el cual fue similar entre los sistemas. Esta información ayudó a elaborar una propuesta de grupos litobiónticos para facilitar el estudio de los mecanismos que tiene cada cianobacteria en la formación de microbialitas.

A diferencia de los datos metagenómicos sobre redundancia funcional, es evidente por análisis microscópicos y de cultivo, que las cianobacterias tienen diferentes funciones y adaptaciones, pero este nivel de detalle aun es difícil de encontrar para las bases de datos bioinformáticas, ya que las comunidades microbianas de sistemas organosedimentarios están subestudiadas. Esta clasificación puede aplicarse a otros sistemas organosedimentarios para realizar más estudios comparativos, mismos que son necesarios para complementar la propuesta de grupos litobiónticos formulada en esta tesis.

Este proyecto doctoral aportó información relevante para la conservación de diferentes sistemas que albergan microbialitas, incluyendo el Lago Alchichica, Laguna Bacalar, Cenote Azul y otros sistemas acuáticos de la península de Yucatán, en donde se describen patrones de disbiosis o conservación de cianobacterias en las microbialitas.

Se aportó material microbiológico para futuros estudios fisiológicos y bioquímicos ya que los cultivos aislados fueron puestos a disposición del Laboratorio de Ecología Bacteriana (Instituto de Ecología) y del Laboratorio de Ficología (Facultad de Ciencias), UNAM. Las muestras microscópicas de cultivos aislados se resguardaron en el Herbario Nacional de la UNAM. También se aportó información sobre nuevos grupos taxonómicos y nuevos genes putativos en microbialitas. Los genes putativos descubiertos pueden tener aplicaciones biotecnológicas, en especial en la obtención de productos naturales y metabolitos secundarios como fármacos y antibióticos (Burns, Goh et al. 2004). Las

cianobacterias aisladas en este trabajo tienen potencial para la producción de metabolitos de interés. Debido a su capacidad para formar minerales y precipitar material detrítico también se han sugerido aplicaciones de biorremediación para sistemas acuáticos, en especial para ser usados como posibles sumideros de carbono, mediante el secuestro de $CO_2$ atmosférico (carbono azul) (Jacob-Lopes, Zepka et al. 2014, Zhu and Dittrich 2016, Payandi-Rolland, Roche et al. 2019).

Los cultivos aislados en este trabajo serán útiles para ensayos fisiológicos de precipitación *in vitro*, para experimentos de sucesión ecológica y para corroborar genes nuevos putativos encontrados con nuestras técnicas de metagenómica.

## 8. REFERENCAS


Abed, R. M., S. Golubic, F. Garcia-Pichel, G. F. Camoin and S. Sprachta (2003). "Characterization of microbialite-forming cyanobacteria in a tropical lagoon: Tikehau Atoll, Tuamotu, French Polynesia." Journal of Phycology **39**(5): 862-873.

Águila, B., R. J. Alcántara-Hernández, G. Montejano, R. López-Martínez, L. I. Falcón and I. Becerra-Absalón (2021). "Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization." European Journal of Phycology: 1-16.

Águila, B., A. Yanez-Montalvo, R. Mercado-Juárez, G. Montejano, I. Becerra-Absalón and L. Falcón (2022). "Microbialites show a distinct cyanobacterial phylogenetic structure and functional redundancy in Bacalar lagoon and Cenote Azul sinkhole, Yucatan peninsula, Mexico." FEMS Microbiology Ecology **98**(5): fiac039.

Aitken, J. D. (1967). "Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta." Journal of Sedimentary Research **37**(4): 1163-1178.

Alcántara-Hernández, R. J., P. M. Valdespino-Castillo, C. M. Centeno, J. Alcocer, M. Merino-Ibarra and L. I. Falcón (2017). "Genetic diversity associated with N-cycle pathways in microbialites from Lake Alchichica, Mexico." Aquatic Microbial Ecology **78**(2): 121-133.

Alcocer, J. and A. Lugo (2003). "Effects of El Niño on the dynamics of Lake Alchichica, central Mexico." Geofísica Internacional **42**(3): 523-528.

Armienta, M. A., G. Vilaclara, S. De la Cruz-Reyna, S. Ramos, N. Ceniceros, O. Cruz, A. Aguayo and F. Arcega-Cabrera (2008). "Water chemistry of lakes related to active and inactive Mexican volcanoes." Journal of Volcanology and Geothermal Research **178**(2): 249-258.

Armitage, D. W., K. L. Gallagher, N. D. Youngblut, D. H. Buckley and S. H. Zinder (2012). "Millimeter-scale patterns of phylogenetic and trait diversity in a salt marsh microbial mat." Frontiers in Microbiology **3**: 293.

Arp, G., J. Hofmann and J. Reitner (1998). "Microbial fabric formation in spring mounds (" microbialites") of alkaline salt lakes in the Badain Jaran sand sea, PR China." Palaios **13**(6): 581-592.

Arp, G., A. Reimer and J. Reitner (2003). "Arp G, Reimer A, Reitner J.. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. J Sediment Res 73: 105-127." Journal of Sedimentary Research - J SEDIMENT RES **73**: 105-127.

Arp, G., V. Thiel, A. Reimer, W. Michaelis and J. Reitner (1999). "Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA." Sedimentary Geology **126**(1-4): 159-176.

Awramik, S., L. Margulis and E. Barghoorn (1976). Evolutionary processes in the formation of stromatolites. Developments in Sedimentology, Elsevier. **20:** 149-162.

Awramik, S. M. (1971). "Precambrian columnar stromatolite diversity: reflection of metazoan appearance." Science **174**(4011): 825-827.

Bąbel, M. (2004). "Models for evaporite, selenite and gypsum microbialite deposition in ancient saline basins." Acta Geologica Polonica **54**(2): 219-249.

Baumgartner, L. K., R. P. Reid, C. Dupraz, A. W. Decho, D. Buckley, J. Spear, K. M. Przekop and P. T. Visscher (2006). "Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries." Sedimentary Geology **185**(3-4): 131-145.

Beltrán, Y., C. M. Centeno, F. García-Oliva, P. Legendre and L. I. Falcón (2012). "N2 fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia from different aquatic environments in Mexico." Aquatic microbial ecology **67**(1): 15-24.

Berelson, W., F. Corsetti, C. Pepe-Ranney, D. Hammond, W. Beaumont and J. Spear (2011). "Hot spring siliceous stromatolites from Yellowstone National Park: assessing growth rate and laminae formation." Geobiology **9**(5): 411-424.
Boidi, F. J., E. C. Mlewski, F. J. Gomez and E. Gérard (2020). Characterization of microbialites and microbial mats of the Laguna Negra hypersaline lake (Puna of Catamarca, Argentina). Microbial Ecosystems in Central Andes Extreme Environments, Springer**:** 183-203.
Bouton, A., E. Vennin, A. Pace, R. Bourillot, C. Dupraz, C. Thomazo, A. Brayard, G. Désaubliaux and P. T. Visscher (2016). "External controls on the distribution, fabrics and mineralization of modern microbial mats in a coastal hypersaline lagoon, Cayo Coco (Cuba)." Sedimentology **63**(4): 972-1016.
Breitbart, M., A. Hoare, A. Nitti, J. Siefert, M. Haynes, E. Dinsdale, R. Edwards, V. Souza, F. Rohwer and D. Hollander (2009). "Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico." Environmental Microbiology **11**(1): 16-34.
Bundeleva, I. A., L. S. Shirokova, O. S. Pokrovsky, P. Bénézeth, B. Ménez, E. Gérard and S. Balor (2014). "Experimental modeling of calcium carbonate precipitation by cyanobacterium Gloeocapsa sp." Chemical Geology **374**: 44-60.
Burne, R. V. and L. S. Moore (1987). "Microbialites; organosedimentary deposits of benthic microbial communities." Palaios **2**(3): 241-254.
Burne, R. V., L. S. Moore, A. G. Christy, U. Troitzsch, P. L. King, A. M. Carnerup and P. J. Hamilton (2014). "Stevensite in the modern thrombolites of Lake Clifton, Western Australia: A missing link in microbialite mineralization?" Geology **42**(7): 575-578.
Burns, B. P., F. Goh, M. Allen and B. A. Neilan (2004). "Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia." Environmental Microbiology **6**(10): 1096-1101.
Büttner, S. H., E. W. Isemonger, M. Isaacs, D. van Niekerk, R. E. Sipler and R. A. Dorrington (2021). "Living phosphatic stromatolites in a low-phosphorus environment: Implications for the use of phosphorus as a proxy for phosphate levels in paleo-systems." Geobiology **19**(1): 35-47.
Camoin, G. and P. Gautret (2006). "Microbialites and microbial communities: Biological diversity, biogeochemical functioning, diagenetic processes, tracers of environmental changes." Sedimentary Geology **185**: 127-130.
Campbell, M. A., M. J. Coolen, P. T. Visscher, T. Morris and K. Grice (2021). "Structure and function of Shark Bay microbial communities following tropical cyclone Olwyn: A metatranscriptomic and organic geochemical perspective." Geobiology **19**(6): 642-664.
Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser and M. Bauer (2012). "Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms." The ISME journal **6**(8): 1621-1624.
Carrillo-Araujo, M., N. Taş, R. J. Alcantara-Hernandez, O. Gaona, J. E. Schondube, R. A. Medellin, J. K. Jansson and L. I. Falcon (2015). "Phyllostomid bat microbiome composition is associated to host phylogeny and feeding strategies." Frontiers in microbiology **6**: 447.
Catling, D. C. and K. J. Zahnle (2020). "The archean atmosphere." Science Advances **6**(9): eaax1420.
Centeno, C. M., P. Legendre, Y. Beltrán, R. J. Alcántara-Hernández, U. E. Lidström, M. N. Ashby and L. I. Falcón (2012). "Microbialite genetic diversity and composition relate to environmental variables." FEMS microbiology ecology **82**(3): 724-735.
Centeno, C. M., O. Mejía and L. I. Falcón (2016). "Habitat conditions drive phylogenetic structure of dominant bacterial phyla of microbialite communities from several locations in Mexico." Revista de biologia tropical **64**(3): 1057-1066.
Chacon-Baca, E., A. Santos, A. M. Sarmiento, A. T. Luís, M. Santisteban, J. C. Fortes, J. M. Dávila, J. M. Diaz-Curiel and J. A. Grande (2021). "Acid Mine Drainage as Energizing Microbial Niches for the Formation of Iron Stromatolites: The Tintillo River in Southwest Spain." Astrobiology **21**(4): 443-463.

Chagas, A. A., G. E. Webb, R. V. Burne and G. Southam (2016). "Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy." Earth-Science Reviews **162**: 338-363.
Chan, O. W., D. C. Bugler-Lacap, J. F. Biddle, D. S. Lim, C. P. McKay and S. B. Pointing (2014). "Phylogenetic diversity of a microbialite reef in a cold alkaline freshwater lake." Canadian journal of microbiology **60**(6): 391-398.
Chen, J. and M. Strous (2013). "Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution." Biochimica et Biophysica Acta (BBA) - Bioenergetics **1827**(2): 136-144.
Cockell, C. S. and J. A. Raven (2007). "Ozone and life on the Archaean Earth." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **365**(1856): 1889-1901.
Coshell, L., M. Rosen and K. McNamara (1998). "Hydromagnesite replacement of biomineralized aragonite in a new location of Holocene stromatolites, Lake Walyungup, Western Australia." Sedimentology **45**(6): 1005-1018.
Couradeau, E., K. Benzerara, D. Moreira, E. Gerard, J. Kaźmierczak, R. Tavera and P. López-García (2011). "Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico)." PloS one **6**(12): e28767.
da Silva, D. R., K. L. Mansur and L. F. B. de Almeida (2019). "Distribution and Growth Morphology of the Recent Microbialites: the Case of Lagoa Salgada, Rio de Janeiro-Brazil." Anuário do Instituto de Geociências **42**(1): 439-453.
De Anda, V., I. Zapata-Peñasco, J. Blaz, A. C. Poot-Hernández, B. Contreras-Moreira, M. Gonzalez-Laffitte, N. Gamez-Tamariz, M. Hernandez-Rosales, L. E. Eguiarte and V. Souza (2018). "Understanding the mechanisms behind the response to environmental perturbation in microbial mats: a metagenomic-network based approach." Frontiers in microbiology **9**: 2606.
Decho, A. W. and T. Gutierrez (2017). "Microbial extracellular polymeric substances (EPSs) in ocean systems." Frontiers in microbiology **8**: 922.
Decho, A. W., P. T. Visscher and R. P. Reid (2005). Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Geobiology: Objectives, concepts, perspectives, Elsevier**:** 71-86.
Dittrich, M. and S. Sibler (2010). "Calcium carbonate precipitation by cyanobacterial polysaccharides." Geological Society, London, Special Publications **336**(1): 51-63.
Djokic, T., M. J. Van Kranendonk, K. A. Campbell, M. R. Walter and C. R. Ward (2017). "Earliest signs of life on land preserved in ca. 3.5 Ga hot spring deposits." Nature communications **8**(1): 1-9.
Dodd, M. S., D. Papineau, T. Grenne, J. F. Slack, M. Rittner, F. Pirajno, J. O'Neil and C. T. Little (2017). "Evidence for early life in Earth's oldest hydrothermal vent precipitates." Nature **543**(7643): 60-64.
Dupraz, C. (2004). Microbe-carbonate mineral interactions in modern microbialite formation (open marine and hypersaline environments, Bahamas). The 2nd Swiss Geoscience Meeting, Lausanne.
Dupraz, C., R. P. Reid, O. Braissant, A. W. Decho, R. S. Norman and P. T. Visscher (2009). "Processes of carbonate precipitation in modern microbial mats." Earth-Science Reviews **96**(3): 141-162.
Dupraz, C., R. P. Reid, P. T. Visscher, J. Reitner and V. Thiel (2011). "Microbialites, modern." Encyclopedia of geobiology: 617-635.
Dupraz, C. and P. T. Visscher (2005). "Microbial lithification in marine stromatolites and hypersaline mats." Trends in microbiology **13**(9): 429-438.
Eymard, I., A. Bilmes, M. d. P. Alvarez, R. Feo, G. Hunger, C. Vasconcelos and D. Arizteguí (2019). "Growth morphologies and plausible stressors ruling the formation of Late Pleistocene lacustrine carbonate buildups in the Maquinchao Basin (Argentina)." The Depositional Record **5**(3): 498-514.
Falcón, L. I., E. Escobar-Briones and D. Romero (2002). "Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico." Hydrobiologia **467**(1-3): 71-78.

Farias, M. E., M. Contreras, M. C. Rasuk, D. Kurth, M. R. Flores, D. G. Poire, F. Novoa and P. T. Visscher (2014). "Characterization of bacterial diversity associated with microbial mats, gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile." Extremophiles **18**(2): 311-329.
Flannery, D. T. and M. R. Walter (2012). "Archean tufted microbial mats and the Great Oxidation Event: new insights into an ancient problem." Australian Journal of Earth Sciences **59**(1): 1-11.
Frantz, C., V. Petryshyn and F. Corsetti (2015). "Grain trapping by filamentous cyanobacterial and algal mats: implications for stromatolite microfabrics through time." Geobiology **13**(5): 409-423.
Garcia-Pichel, F., F. A. Al-Horani, J. D. Farmer, R. Ludwig and B. D. Wade (2004). "Balance between microbial calcification and metazoan bioerosion in modern stromatolitic oncolites." Geobiology **2**(1): 49-57.
Gautret, P., G. Camoin, S. Golubic and S. Sprachta (2004). "Biochemical control of calcium carbonate precipitation in modern lagoonal microbialites, Tikehau Atoll, French Polynesia." Journal of Sedimentary Research **74**(4): 462-478.
Gérard, E., S. De Goeyse, M. Hugoni, H. Agogué, L. Richard, V. Milesi, F. Guyot, L. Lecourt, S. Borensztajn and M.-B. Joseph (2018). "Key role of alphaproteobacteria and cyanobacteria in the formation of stromatolites of Lake Dziani Dzaha (Mayotte, Western Indian Ocean)." Frontiers in microbiology **9**: 796.
Gérard, E., B. Ménez, E. Couradeau, D. Moreira, K. Benzerara, R. Tavera and P. López-García (2013). "Specific carbonate–microbe interactions in the modern microbialites of Lake Alchichica (Mexico)." The ISME journal **7**(10): 1997-2009.
Gischler, E., M. A. Gibson and W. Oschmann (2008). "Giant holocene freshwater microbialites, laguna bacalar, quintana roo, Mexico." Sedimentology **55**(5): 1293-1309.
Golubic, S. (1991). "Modern stromatolites: a review." Calcareous algae and stromatolites: 541-561.
Gomez, M. G., C. M. Anderson, J. T. DeJong, D. C. Nelson and X. H. Lau (2014). Stimulating in situ soil bacteria for bio-cementation of sands. Geo-Congress 2014: Geo-characterization and Modeling for Sustainability.
Graham, L. E., J. J. Knack, M. J. Piotrowski, L. W. Wilcox, M. E. Cook, C. H. Wellman, W. Taylor, L. A. Lewis and P. Arancibia-Avila (2014). "Lacustrine N ostoc (N ostocales) and associated microbiome generate a new type of modern clotted microbialite." Journal of phycology **50**(2): 280-291.
Gumsley, A. P., K. R. Chamberlain, W. Bleeker, U. Söderlund, M. O. de Kock, E. R. Larsson and A. Bekker (2017). "Timing and tempo of the Great Oxidation Event." Proceedings of the National Academy of Sciences **114**(8): 1811.
Han, Z., H. Yan, H. Zhao, S. Zhou, M. Han, X. Meng, Y. Zhang, Y. Zhao, B. Sun and C. Yao (2014). "Bio-precipitation of calcite with preferential orientation induced by Synechocystis sp. PCC6803." Geomicrobiology Journal **31**(10): 884-899.
Havemann, S. A. and J. S. Foster (2008). "Comparative characterization of the microbial diversities of an artificial microbialite model and a natural stromatolite." Applied and Environmental Microbiology **74**(23): 7410-7421.
Jacob-Lopes, E., L. Q. Zepka and M. I. Queiroz (2014). "Cyanobacteria and carbon sequestration." Cyanobacteria: An Economic Perspective: 65-71.
Johnson, D., P. A. Beddows, T. Flynn and M. R. Osburn (2018). "Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar, Mexico." Geobiology **16**(3): 319-337.
Johnson, M. E., J. Ledesma-Vázquez, D. H. Backus and M. R. González (2012). "Lagoon microbialites on Isla Angel de la Guarda and associated peninsular shores, Gulf of California (Mexico)." Sedimentary Geology **263**: 76-84.

Kanik, M., M. Munro-Ehrlich, M. C. Fernandes-Martins, D. Payne, K. Gianoulias, L. Keller, A. Kubacki, M. R. Lindsay, B. K. Baxter, M. D. V. Berg, D. R. Colman, E. S. Boyd and H. Atomi (2020). "Unexpected Abundance and Diversity of Phototrophs in Mats from Morphologically Variable Microbialites in Great Salt Lake, Utah." Applied and Environmental Microbiology **86**(10): e00165-00120.
Kaźmierczak, J., T. Fenchel, M. Kühl, S. Kempe, B. Kremer, B. Łącka and K. Małkowski (2015). "CaCO3 Precipitation in Multilayered Cyanobacterial Mats: Clues to Explain the Alternation of Micrite and Sparite Layers in Calcareous Stromatolites." Life **5**(1): 744-769.
Kazmierczak, J. and S. Kempe (2006). "Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga." Naturwissenschaften **93**(3): 119-126.
Kaźmierczak, J., S. Kempe, B. Kremer, P. López-García, D. Moreira and R. Tavera (2011). "Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico." Facies **57**(4): 543-570.
Kazmierczak, J., S. Kempe, P. López-Garcia, R. Tavera, B. Kremer and D. Moreira (2008). Modern and sub-recent carbonate microbialites from the alkaline crater lake Alchichica, Mexico. Geobiology of Stromatolites. International Kalkowsky-Symposium, Abstract Volume and Field Guide to excursions, Gottingen, Germany.
Keim, C. N., H. N. dos Santos, C. S. Santiago, S. Pennafirme, R. Neumann, J. Schnellrath, I. Lima, M. A. Crapez and M. Farina (2020). "Microstructure and mineral composition of Holocene stromatolites from Lagoa Vermelha, a hypersaline lagoon in Brazil: Insights into laminae genesis." Journal of Sedimentary Research **90**(8): 887-905.
Kempe, S., J. Kazmierczak, G. Landmann, T. Konuk, A. Reimer and A. Lipp (1991). "Largest known microbialites discovered in Lake Van, Turkey." Nature **349**(6310): 605-608.
Kipp, M. A. and E. E. Stüeken (2017). "Biomass recycling and Earth's early phosphorus cycle." Science Advances **3**(11): eaao4795.
Knoll, A. H., I. J. Fairchild and K. Swett (1993). "Calcified Microbes in Neoproterozoic Carbonates: Implications for Our Understanding of the Proterozoic/Cambrian Transition." PALAIOS **8**(6): 512-525.
Kopp, R. E., J. L. Kirschvink, I. A. Hilburn and C. Z. Nash (2005). "The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis." Proceedings of the National Academy of Sciences **102**(32): 11131-11136.
Kremer, B., J. Kazmierczak, M. Łukomska-Kowalczyk and S. Kempe (2012). "Calcification and silicification: fossilization potential of cyanobacteria from stromatolites of Niuafo 'ou's Caldera Lakes (Tonga) and implications for the early fossil record." Astrobiology **12**(6): 535-548.
Krumbein, W. E., U. Brehm, G. Gerdes, A. A. Gorbushina, G. Levit and K. A. Palinska (2003). Biofilm, biodictyon, biomat microbialites, oolites, stromatolites geophysiology, global mechanism, parahistology. Fossil and recent biofilms, Springer**:** 1-27.
Last, F. M., W. M. Last and N. M. Halden (2010). "Carbonate microbialites and hardgrounds from Manito Lake, an alkaline, hypersaline lake in the northern Great Plains of Canada." Sedimentary Geology **225**(1-2): 34-49.
Laval, B., S. L. Cady, J. C. Pollack, C. P. McKay, J. S. Bird, J. P. Grotzinger, D. C. Ford and H. R. Bohm (2000). "Modern freshwater microbialite analogues for ancient dendritic reef structures." Nature **407**(6804): 626-629.
Lepland, A., M. A. van Zuilen, G. Arrhenius, M. J. Whitehouse and C. M. Fedo (2005). "Questioning the evidence for Earth's earliest life—Akilia revisited." Geology **33**(1): 77-79.
Lepot, K. (2020). "Signatures of early microbial life from the Archean (4 to 2.5 Ga) eon." Earth-Science Reviews **209**: 103296.
Léveillé, R. J., W. S. Fyfe and F. J. Longstaffe (2000). "Unusual secondary Ca-Mg-carbonate-kerolite deposits in basaltic caves, Kauai, Hawaii." The Journal of Geology **108**(5): 613-621.

Lindsay, M. R., E. C. Dunham and E. S. Boyd (2020). Microbialites of Great Salt Lake. Great Salt Lake Biology, Springer**:** 87-118.
Louyakis, A. S., H. Gourlé, G. Casaburi, R. M. Bonjawo, A. A. Duscher and J. S. Foster (2018). "A year in the life of a thrombolite: comparative metatranscriptomics reveals dynamic metabolic changes over diel and seasonal cycles." Environmental microbiology **20**(2): 842-861.
Mancilla Villa, O. R., A. L. Bautista Olivas, H. M. Ortega Escobar, E. I. Sánchez Bernal, Á. Can Chulim, R. D. Guevara Gutiérrez and Y. M. Ortega Mikolaev (2014). "Hidrogeoquímica de salinas Zapotitlán y los lagos-cráter Alchichica y Atexcac, Puebla." Idesia (Arica) **32**(1): 55-69.
Marshall, C. R. (2006). "Explaining the Cambrian "explosion" of animals." Annu. Rev. Earth Planet. Sci. **34**: 355-384.
Mata, S. A. and D. J. Bottjer (2012). "Microbes and mass extinctions: paleoenvironmental distribution of microbialites during times of biotic crisis." Geobiology **10**(1): 3-24.
McCall, J. (2010). "Lake Bogoria, Kenya: Hot and warm springs, geysers and Holocene stromatolites." Earth-Science Reviews **103**(1-2): 71-79.
Meyer, F., S. Bagchi, S. Chaterji, W. Gerlach, A. Grama, T. Harrison, T. Paczian, W. L. Trimble and A. Wilke (2019). "MG-RAST version 4—lessons learned from a decade of low-budget ultra-high-throughput metagenome analysis." Briefings in bioinformatics **20**(4): 1151-1159.
Michiels, C. C., F. Darchambeau, F. A. E. Roland, C. Morana, M. Llirós, T. García-Armisen, B. Thamdrup, A. V. Borges, D. E. Canfield, P. Servais, J.-P. Descy and S. A. Crowe (2017). "Iron-dependent nitrogen cycling in a ferruginous lake and the nutrient status of Proterozoic oceans." Nature Geoscience **10**(3): 217-221.
Mobberley, J., C. Khodadad, P. Visscher, R. Reid, P. Hagan and J. Foster (2015). "Inner workings of thrombolites: spatial gradients of metabolic activity as revealed by metatranscriptome profiling." Scientific reports **5**(1): 1-15.
Mobberley, J. M., C. L. Khodadad and J. S. Foster (2013). "Metabolic potential of lithifying cyanobacteria-dominated thrombolitic mats." Photosynthesis research **118**(1-2): 125-140.
Mole, D. R., M. L. Fiorentini, N. Thebaud, K. F. Cassidy, T. C. McCuaig, C. L. Kirkland, S. S. Romano, M. P. Doublier, E. A. Belousova and S. J. Barnes (2014). "Archean komatiite volcanism controlled by the evolution of early continents." Proceedings of the National Academy of Sciences **111**(28): 10083-10088.
Moreira, D., R. Tavera, K. Benzerara, F. Skouri-Panet, E. Couradeau, E. Gérard, C. L. Fonta, E. Novelo, Y. Zivanovic and P. López-García (2017). "Description of Gloeomargarita lithophora gen. nov., sp. nov., a thylakoid-bearing basal-branching cyanobacterium with intracellular carbonates, and proposal for Gloeomargaritales ord. nov." International journal of systematic and evolutionary microbiology **67**(3): 653.
Myshrall, K. L., C. Dupraz and P. T. Visscher (2014). Patterns in microbialites throughout geologic time: is the present really the key to the past? Experimental Approaches to Understanding Fossil Organisms, Springer**:** 111-142.
Newell, D. L., J. L. Jensen, C. M. Frantz and M. D. Vanden Berg (2017). "Great Salt Lake (Utah) Microbialite δ13C, δ18O, and δ15N Record Fluctuations in Lake Biogeochemistry Since the Late Pleistocene." Geochemistry, Geophysics, Geosystems **18**(10): 3631-3645.
Noffke, N., G. Gerdes, T. Klenke and W. E. Krumbein (2001). "Microbially induced sedimentary structures: a new category within the classification of primary sedimentary structures." Journal of Sedimentary Research **71**(5): 649-656.
Nübel, U., F. Garcia-Pichel and G. Muyzer (1997). "PCR primers to amplify 16S rRNA genes from cyanobacteria." Applied and environmental microbiology **63**(8): 3327-3332.
Obst, M., J. Dynes, J. Lawrence, G. Swerhone, K. Benzerara, C. Karunakaran, K. Kaznatcheev, T. Tyliszczak and A. Hitchcock (2009). "Precipitation of amorphous CaCO3 (aragonite-like) by

cyanobacteria: a STXM study of the influence of EPS on the nucleation process." Geochimica et Cosmochimica Acta **73**(14): 4180-4198.
Olson, J. M. (2006). "Photosynthesis in the Archean era." Photosynthesis research **88**(2): 109-117.
Olson, K. R. and K. D. Straub (2016). "The role of hydrogen sulfide in evolution and the evolution of hydrogen sulfide in metabolism and signaling." Physiology **31**(1): 60-72.
Parikh, A. and D. Madamwar (2006). "Partial characterization of extracellular polysaccharides from cyanobacteria." Bioresource Technology **97**(15): 1822-1827.
Paterson, D. M., R. J. Aspden, P. T. Visscher, M. Consalvey, M. S. Andres, A. W. Decho, J. Stolz and R. P. Reid (2008). "Light-Dependant Biostabilisation of Sediments by Stromatolite Assemblages." PLOS ONE **3**(9): e3176.
Payandi-Rolland, D., A. Roche, E. Vennin, P. T. Visscher, P. Amiotte-Suchet, C. Thomas and I. A. Bundeleva (2019). "Carbonate Precipitation in Mixed Cyanobacterial Biofilms Forming Freshwater Microbial Tufa." Minerals **9**(7): 409.
Perry, E., A. Paytan, B. Pedersen and G. Velazquez-Oliman (2009). "Groundwater geochemistry of the Yucatan Peninsula, Mexico: constraints on stratigraphy and hydrogeology." Journal of Hydrology **367**(1-2): 27-40.
Perry, E., G. Velazquez-Oliman and L. Marin (2002). "The hydrogeochemistry of the karst aquifer system of the northern Yucatan Peninsula, Mexico." International Geology Review **44**(3): 191-221.
Planavsky, N. and R. N. Ginsburg (2009). "Taphonomy of modern marine Bahamian microbialites." Palaios **24**(1): 5-17.
Planavsky, N. J., D. Asael, A. Hofmann, C. T. Reinhard, S. V. Lalonde, A. Knudsen, X. Wang, F. Ossa Ossa, E. Pecoits and A. J. Smith (2014). "Evidence for oxygenic photosynthesis half a billion years before the Great Oxidation Event." Nature Geoscience **7**(4): 283-286.
Pratt, B. R. (1982). "Stromatolite decline a reconsideration." Geology **10**(10 ): 512-515.
Pruesse, E., J. Peplies and F. O. Glöckner (2012). "SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes." Bioinformatics **28**(14): 1823-1829.
Rasuk, M. C., P. T. Visscher, M. Contreras Leiva and M. E. Farías (2020). "Mats and microbialites from Laguna La Brava." Microbial Ecosystems in Central Andes Extreme Environments: 221-230.
Rego, E. S., V. Busigny, S. V. Lalonde, P. Philippot, A. Bouyon, C. Rossignol, M. Babinski and A. de Cássia Zapparoli (2021). "Anoxygenic photosynthesis linked to Neoarchean iron formations in Carajás (Brazil)." Geobiology **19**(4): 326-341.
Reid, R. P., I. G. Macintyre and R. S. Steneck (1999). "A microbialite/algal ridge fringing reef complex, Highborne Cay, Bahamas." Atoll Research Bulletin.
Reid, R. P., P. T. Visscher, A. W. Decho, J. F. Stolz, B. Bebout, C. Dupraz, I. Macintyre, H. Paerl, J. Pinckney and L. Prufert-Bebout (2000). "The role of microbes in accretion, lamination and early lithification of modern marine stromatolites." Nature **406**(6799): 989-992.
Reitner, J., J. Paul, G. Arp, D. Hause-Reitner, F. Neuweiler and F. Gunkel (1996). "Lake Thetis domal microbialites; a complex framework of calcified biofilms and organomicrites (Cervantes, Western Australia)." Global and regional controls on biogenic sedimentation **1**: 85-89.
Riding, R. (1991). Calcified cyanobacteria. Calcareous algae and stromatolites, Springer**:** 55-87.
Riding, R. (2006). "Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic–Cambrian changes in atmospheric composition." Geobiology **4**(4): 299-316.
Riding, R. (2011). Microbialites, stromatolites, and thrombolites. Encyclopedia of geobiology.
Riding, R. (2012). Calcareous algae and stromatolites.
Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier (1979). "Generic assignments, strain histories and properties of pure cultures of cyanobacteria." Microbiology **111**(1): 1-61.

Roche, A., E. Vennin, I. Bundeleva, A. Bouton, D. Payandi-Rolland, P. Amiotte-Suchet, E. C. Gaucher, H. Courvoisier and P. T. Visscher (2019). "The Role of the Substrate on the Mineralization Potential of Microbial Mats in A Modern Freshwater River (Paris Basin, France)." Minerals **9**(6): 359.
Rose, C., A. Maloof, B. Schoene, R. Ewing, U. Linnemann, M. Hofmann and J. Cottle (2013). "The end-cryogenian glaciation of South Australia." Geoscience Canada: Journal of the Geological Association of Canada/Geoscience Canada: journal de l'Association Géologique du Canada **40**(4): 256-293.
Rousk, J., P. C. Brookes and E. Baath (2009). "Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization." Applied and Environmental Microbiology **75**(6): 1589-1596.
Russell, J., A. Brady, Z. Cardman, G. Slater, D. Lim and J. Biddle (2014). "Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada." Geobiology **12**(3): 250-264.
Russell, M. J., J. K. Ingham, V. Zedef, D. Maktav, F. Sunar, A. J. Hall and A. E. Fallick (1999). "Search for signs of ancient life on Mars: expectations from hydromagnesite microbialites, Salda Lake, Turkey." Journal of the Geological Society **156**(5): 869-888.
Saghaï, A., Y. Zivanovic, D. Moreira, K. Benzerara, P. Bertolino, M. Ragon, R. Tavera, A. I. López-Archilla and P. López-García (2016). "Comparative metagenomics unveils functions and genome features of microbialite-associated communities along a depth gradient." Environmental microbiology **18**(12): 4990-5004.
Saghaï, A., Y. Zivanovic, N. Zeyen, D. Moreira, K. Benzerara, P. Deschamps, P. Bertolino, M. Ragon, R. Tavera and A. I. López-Archilla (2015). "Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites." Frontiers in microbiology **6**: 797.
Samylina, O. S. and L. V. Zaytseva (2019). "Characterization of modern dolomite stromatolites from hypersaline Petukhovskoe Soda Lake, Russia." Lethaia **52**(1): 1-13.
Santos, F., A. Pena, B. Nogales, E. Soria-Soria, M. Á. G. del Cura, J. A. González-Martín and J. Anton (2010). "Bacterial diversity in dry modern freshwater stromatolites from Ruidera Pools Natural Park, Spain." Systematic and Applied Microbiology **33**(4): 209-221.
Sarkar, S., P. G. Eriksson and S. Chakraborty (2004). "Epeiric sea formation on Neoproterozoic supercontinent break-up: a distinctive signature in coastal storm bed amalgamation." Gondwana Research **7**(2): 313-322.
Schneider, D., G. Arp, A. Reimer, J. Reitner and R. Daniel (2013). "Phylogenetic analysis of a microbialite-forming microbial mat from a hypersaline lake of the Kiritimati Atoll, Central Pacific." PloS one **8**(6): e66662.
Schopf, J. W. (2006). "Fossil evidence of Archaean life." Philosophical Transactions of the Royal Society B: Biological Sciences **361**(1470): 869-885.
Schopf, J. W., A. B. Kudryavtsev, A. D. Czaja and A. B. Tripathi (2007). "Evidence of Archean life: stromatolites and microfossils." Precambrian Research **158**(3-4): 141-155.
Schwantes-Cezario, N., M. V. N. d. N. Peres, T. K. Fruet, G. S. F. Nogueira, B. M. Toralles and D. D. S. Cezario (2018). "Crack filling in concrete by addition of Bacillus subtilis spores-Preliminary study." Dyna **85**(205): 132-139.
Sim, M. S., B. Liang, A. P. Petroff, A. Evans, V. Klepac-Ceraj, D. T. Flannery, M. R. Walter and T. Bosak (2012). "Oxygen-dependent morphogenesis of modern clumped photosynthetic mats and implications for the Archean stromatolite record." Geosciences **2**(4): 235-259.
Souza-Egipsy, V., J. Wierzchos, C. Ascaso and K. H. Nealson (2005). "Mg–silica precipitation in fossilization mechanisms of sand tufa endolithic microbial community, Mono Lake (California)." Chemical Geology **217**(1-2): 77-87.

Souza, V., A. Moreno-Letelier, M. Travisano, L. D. Alcaraz, G. Olmedo and L. E. Eguiarte (2018). "The lost world of Cuatro Ciénegas Basin, a relictual bacterial niche in a desert oasis." eLife **7**: e38278.
Souza, V., J. L. Siefert, A. E. Escalante, J. J. Elser and L. E. Eguiarte (2012). "The cuatro ciénegas basin in Coahuila, Mexico: an astrobiological precambrian park." Astrobiology **12**(7): 641-647.
Stüeken, E. E. and R. Buick (2018). "Environmental control on microbial diversification and methane production in the Mesoarchean." Precambrian Research **304**: 64-72.
Stüeken, E. E., D. C. Catling and R. Buick (2012). "Contributions to late Archaean sulphur cycling by life on land." Nature Geoscience **5**(10): 722-725.
Tarhan, L. G., N. J. Planavsky, C. Laumer, J. Stolz and R. Reid (2013). "Microbial mat controls on infaunal abundance and diversity in modern marine microbialites." Geobiology **11**(5): 485-497.
Tavera, R. and J. Komárek (1996). "Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla State, Mexico." Arch. Hydrobiol.(Suppl.)(Algol. Stud.) **117**: 511-538.
Temperton, B. and S. J. Giovannoni (2012). "Metagenomics: microbial diversity through a scratched lens." Current opinion in microbiology **15**(5): 605-612.
Uveges, B. T., M. A. Teece, J. M. Fulton and C. K. Junium (2018). "Environmental controls on pigment distributions in the freshwater microbialites of Fayetteville Green Lake." Organic Geochemistry **125**: 165-176.
Valdespino-Castillo, P. M., B. Águila, J. Torres-Huesca, C. M. Centeno, J. Martínez-Díaz, M. Reyes-Salas, S. Angeles-García, Y. Beltrán, R. J. Alcántara-Hernández and H.-Y. N. Holman (2022). Microbialites: Diversity Hotspots in the Mexican Plateau. Lake Alchichica Limnology, Springer**:** 375-390.
Valdespino-Castillo, P. M., P. Hu, M. Merino-Ibarra, L. M. López-Gómez, D. Cerqueda-García, G.-D. Zayas, T. Pi-Puig, J. A. Lestayo, H.-Y. Holman and L. I. Falcón (2018). "Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba." Frontiers in microbiology **9**: 510.
van Smeerdijk Hood, A. and M. W. Wallace (2015). "Extreme ocean anoxia during the Late Cryogenian recorded in reefal carbonates of Southern Australia." Precambrian Research **261**: 96-111.
Visscher, P. T., S. E. Hoeft, T.-M. L. Surgeon, D. R. Rogers, B. M. Bebout, J. S. Thompson Jr and R. P. Reid (2002). Microelectrode measurements in stromatolites: unraveling the Earth's past?, ACS Publications.
Wang, W., R. Bolhar, M.-F. Zhou and X.-F. Zhao (2018). "Enhanced terrestrial input into Paleoproterozoic to Mesoproterozoic carbonates in the southwestern South China Block during the fragmentation of the Columbia supercontinent." Precambrian Research **313**: 1-17.
Warden, J. G., L. Coshell, M. R. Rosen, D. O. Breecker, K. X. Ruthrof and C. R. Omelon (2019). "The importance of groundwater flow to the formation of modern thrombolitic microbialites." Geobiology **17**(5): 536-550.
Weisburg, W. G., S. M. Barns, D. A. Pelletier and D. J. Lane (1991). "16S ribosomal DNA amplification for phylogenetic study." Journal of bacteriology **173**(2): 697-703.
White III, R. A. (2014). Metagenomic and genomic analyses of modern freshwater microbialites: unmasking a community of complex metabolic potential, University of British Columbia Vancouver, BC.
White III, R. A., A. M. Chan, G. S. Gavelis, B. S. Leander, A. L. Brady, G. F. Slater, D. S. Lim and C. A. Suttle (2016). "Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community." Frontiers in microbiology **6**: 1531.
Wolfe, J. M. and G. P. Fournier (2018). "Horizontal gene transfer constrains the timing of methanogen evolution." Nature ecology & evolution **2**(5): 897-903.
Woo, J. and S. K. Chough (2010). "Growth patterns of the Cambrian microbialite: Phototropism and speciation of Epiphyton." Sedimentary Geology **229**(1): 1-8.
Yanez-Montalvo, A., B. Águila, E. S. Gómez-Acata, Y. Beltrán, P. M. Valdespino-Castillo, C. M. Centeno and L. I. Falcón (2019). Microbialites: What on Earth?, eScholarship, University of California.

Yanez-Montalvo, A., B. Águila, S. Gómez-Acata, M. Mass-Vargas, N. Cabanillas-Terán, A. Vega-Zepeda, H. Bahena, H. Hernández-Arana and L. I. Falcón (2021). "Depth Related Structure and Microbial Composition of Microbialites in a Karst Sinkhole, Cenote Azul, Mexico." Geomicrobiology Journal **38**(3): 237-251.
Yanez-Montalvo, A., S. Gómez-Acata, B. Águila, H. Hernández-Arana and L. I. Falcón (2020). "The microbiome of modern microbialites in Bacalar Lagoon, Mexico." PloS one **15**(3): e0230071.
Yin, B., D. Crowley, G. Sparovek, W. J. De Melo and J. Borneman (2000). "Bacterial functional redundancy along a soil reclamation gradient." Applied and environmental microbiology **66**(10): 4361-4365.
Zamagni, J., A. Košir and M. Mutti (2009). "The first microbialite-coral mounds in the Cenozoic (Uppermost Paleocene) from the Northern Tethys (Slovenia): Environmentally-triggered phase shifts preceding the PETM?" Palaeogeography, Palaeoclimatology, Palaeoecology **274**(1-2): 1-17.
Zhu, T. and M. Dittrich (2016). "Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review." Frontiers in bioengineering and biotechnology **4**: 4.

## 9. ANEXOS

Artículo de divulgación:

1. Microbialites: What on Earth? Yanez-Montalvo A, **Águila B**, Gómez-Acata ES, Beltrán Y, Valdespino-Castillo PM, Centeno CM, Falcón LI (2019). Frontiers for Young Minds. https://doi.org/10.3389/frym.2019.00112.

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2. Falcón LI, Valdespino-Castillo MP, Alcántara-Hernández RJ, Gómez-Acata ES,Yanez-Montalvo A, **Águila B**. Stromatolites in crater-lake Alchichica and Bacalar lagoon. Souza, V., Segura A., Foster, J (Editoras) (2020) Astrobiology and Cuatro Ciénegas Basin as an Analog of Early Earth. Springer. ISSN 2523-7292

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5. Yanez-Montalvo A, **Águila B**, Gómez-Acata S, Maas-Vargas M, Cabanillas N, Vega A, Bahena H, Hernández-Arana H, Falcón L.I. (2020). Depth related structure and microbial composition of microbialites in a karst sinkhole, Cenote Azul, Mexico. Geomicrobiology. https://doi.org/10.1080/01490451.2020.1836086

## 9.1 Microbialites: What on Earth?

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# MICROBIALITES: WHAT ON EARTH?

*Alfredo Yanez-Montalvo[1,2], Bernardo Águila[1], Elizabeth S. Gómez-Acata[1], Yislem Beltrán[3], Patricia M. Valdespino-Castillo[4], Carla M. Centeno[1] and Luisa I. Falcón[1*]*

[1] *Instituto de Ecología, Universidad Nacional Autónoma de México, Mérida, Mexico*

[2] *El Colegio de la Frontera Sur, Chetumal, Mexico*

[3] *Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Puerto Morelos, Mexico*

[4] *Lawrence Berkeley National Laboratory, Berkeley, CA, United States*

YOUNG REVIEWERS:


DANA
AGE: 14


MARÍA JOSE
AGE: 14


MARIANA
AGE: 15

Microbialites are rock-like underwater structures that look like reefs but are made entirely of millions of microbes. These structures are very ancient and can be found in different environments on every continent on Earth. Mexico has many microbialite reefs, in desert valleys, crater-lakes, and coastal lagoons. Science helps us to understand the microbes that build microbialites, to know whether the same kinds of microbes make up microbialites in different regions of the world, and to figure out how the microbes organize into microbialites. Many things are damaging microbialites in Mexico, including poorly planned development, pollution from lack of sewage treatment, too much water usage, and the fertilizers used for agriculture. Policies that regulate development are urgently needed to help save these diverse and ancient microbial reefs.

## WHAT ARE MICROBIALITES?

**MICROBIALITE**

A rock-like structure made by millions of microbes that precipitate carbonate.

**MICROBE**

An organism that is seen under the microscope.

**STROMATOLITE**

Fossilized microbialites, and are the oldest fossils dated so far.

**CYANOBACTERIA**

A phylum of bacteria that can make photosynthesis.

**EXOPOLYMERIC SUBSTANCES (EPS)**

Mucous-like molecules released by microorganisms into their environment, which help the microbes remain close together and communicate with each other.

Do you know what a **microbialite** is? Not many people do. This is because microbialites look like slimy underwater rocks, but they are actually reefs made up of **microbes** (simple, one-celled organisms). Microbialites are fascinating, because these rock-like structures are made by the interaction of millions of microbes that live in certain aquatic environments. The microbes facilitate the precipitation of minerals from the water, to form the microbialite structure [1]. Since microbialites are like rocks, they have remained on earth since extremely ancient times. Fossilized microbialites, known as **stromatolites** (from the Greek *strõma,* meaning bed or layer, and *lithos,* meaning rock), are the oldest evidence of life on Earth, dating back to 3.7 billion years ago [2].

**Cyanobacteria** are one type of microbe found in microbialites, and these bacteria do all kinds of important work. Cyanobacteria build shelters that protect the microbial community from dangerous things in the environment, including protecting them from drying out and from damage by the sun's UV rays. The shelters built by cyanobacteria trap and bind sediments and minerals, which help to grow the microbialites [3, 4]. Cyanobacteria are also involved in producing slimy substances called **exopolymeric substances (EPS)**, which are like mucous. Exopolymeric substances help microbes to stay close to each other and allow the cells to communicate with each other [4]. Cyanobacteria also have pigments that interact with photons from the Sun, allowing them to perform photosynthesis, which leads to the incorporation of carbon from the atmosphere (in the form of $CO_2$) into their cells. Cyanobacteria take up water and produce oxygen during photosynthesis. Since microbialites are so old, they probably participated in oxygenating the early Earth. Gently and slowly, bubble by bubble, microbialites produced oxygen, and by ~2.4 billion years ago, the chemistry of earth's atmosphere had changed enough to support the evolution of other life forms [5]. Cyanobacteria are one of the food sources for other kinds of microbes, helping many different kinds of microbes work together as a unit to form the microbialite community.

## WHERE DO MICROBIALITES LIVE?

There are living microbialites in different aquatic environments all over the globe, including polar, temperate, and tropical locations. In Mexico, there are microbialite communities in different locations, including coastal lagoons, crater-lakes, and desert ponds (Figure 1).

## ARE ALL MICROBIALITES IN MEXICO THE SAME?

In our study, we wanted to understand if microbialites from different locations in Mexico are similar or different, in terms of the microbes

**Figure 1**

Microbialite reef locations in Mexico that were examined in our study: Cuatro Ciénegas basin in Northern México, crater-lake Alchichica in Central México, and Bacalar lagoon in the Yucatán Península.


Figure 1

that make them up. To study this, we explored various sites using catamarans, kayaks, and boats. Once we identified microbialite reefs, we took small samples (~2 g) of microbialites from the first few millimeters of their surface, using clean, microbe-free tools and containers. We stored these samples in coolers and then froze them when we got back to the laboratory. Later, we extracted the DNA from these microbialite samples, using different enzymes, soaps, and organic molecules. To study this DNA, we first amplified it using a technique known as **polymerase chain reaction (PCR)** and analyzed how similar or different the microbial DNA sequences were to each other.

**POLYMERASE CHAIN REACTION (PCR)**

A laboratory technique for obtaining millions of copies of pieces of DNA, so that they can be more easily analyzed.

By comparing certain regions of the microbial DNA, thousands of microbes can be identified from a pea-sized rocky sample!

We found that all the microbialite communities we studied had the same groups of microbes, just in slightly different proportions (Figure 2) [6]. The main microbes in microbialites are Cyanobacteria (which we already discussed), Proteobacteria and Bacteroidetes

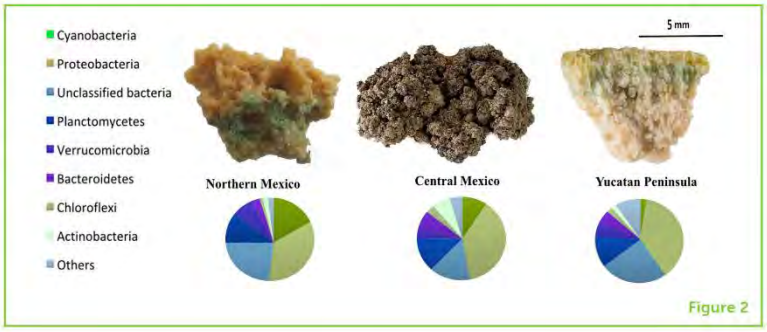


**Figure 2**

Several different kinds of bacteria make up the microbialites found in various regions of Mexico. The types of bacteria are listed on the left, and the pie charts under the pictures of the microbialites show that microbialites from each location are made up of different proportions of these same types of bacteria.

(which are very diverse and can digest many types of substrates, including carbon, nitrogen, and sulfur), Chloroflexi (which can do a type of photosynthesis that does not produce oxygen, but instead produces a substance called sulfide), and Archaea (which can produce and eat methane) (Figure 3).

Proteobacteria are the most diverse bacteria in microbialites, making up 30–40% of the total diversity in these structures, followed by a group of bacteria that we know very little about and are called unclassified bacteria, which make ~20% of the bacteria in microbialites. Other bacteria, including Bacteroidetes, Plactomycetes, Verrucomicrobia, Chloroflexi, and Cyanobacteria comprise the remaining diversity (Figure 2). The microbes in microbialites have been interacting amongst themselves and with the environment for many millions of years, and have helped transform the Earth, making our life on this planet possible through the production of oxygen and other important processes. Microbial communities like the ones that form microbialites help the Earth to function as a unit.

## ARE MICROBIALITES IN MEXICO DOING WELL?

Since microbialites are self-sufficient, they only need clean water, sunlight, and gases from the atmosphere to grow and develop. All the microbialites we have studied in Mexico live in places with clean and clear water, and we have found massive microbialite formations that have been dated to be ~10,000 years of age [7]. Some of the beautiful aquatic environments where microbialites have thrived for thousands of years have warm, clear, tropical waters. This is the case for a coastal lagoon called Bacalar in southern Mexico, which has attracted loads of tourists. But a problem arises when there is no infrastructure to treat the sewage from the growing population, leading to pollution of the lagoon. Microbialites in Bacalar lagoon and in other areas of Mexico are being threatened by human activities. In northern Mexico, the microbialites that thrive in the

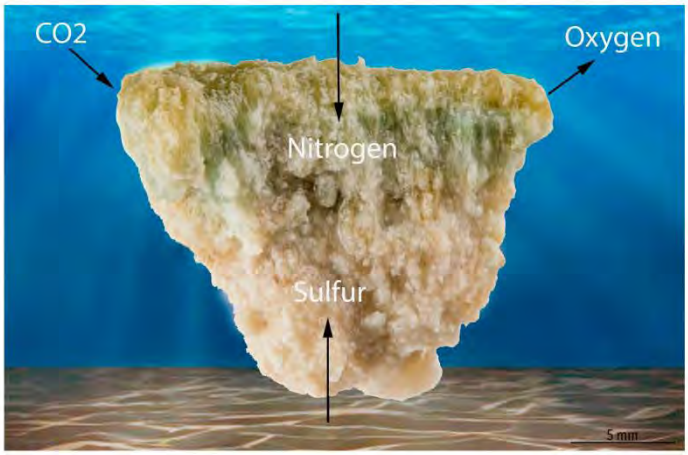


**Figure 3**

Many microbes build microbialites. They do photosynthesis, and capture $CO_2$, producing oxygen and are active in the nitrogen, sulfur and methane cycles.

Cuatro Cienegas valley, which has many ponds in the middle of a desert, are at risk because too much water is being removed from the ponds for agriculture. In central Mexico, microbialites that live in crater-lakes are also at risk, due to both pollution and too much water usage.

Humans are the main problem for the world's biodiversity, but we are also the solution. Microbialites are part of Earth's history and it is wonderful that we can observe these ancient communities today! We have the responsibility to be educated about our environment and to make intelligent choices. Let us use efficient water treatment and technology to save microbialites and other aquatic life! Before going on a holiday, read about the places you will visit and learn about the plants, animals, and bacteria that live there, so that you can enjoy their company without harming them or their environment.

## AUTHOR CONTRIBUTIONS

The authors contributed equally to writing this paper.

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## REFERENCES


1. Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., et al. 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature* 406:989–92. doi: 10.1038/35023158
2. Nutman, A. P., Bennett, V. C., Friend, C. R., Van Kranendonk, M. J., and Chivas, A. R. 2016. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. *Nature* 537:535–8. doi: 10.1038/nature19355
3. Watnick, P., and Kolter, R. 2000. Biofilm, city of microbes. *J. Bacteriol.* 182:2675–9. doi: 10.1128/JB.182.10.2675-2679.2000
4. Flemming, H. C., and Wingender, J. 2010. The biofilm matrix. *Nat. Rev. Microbiol.* 8:623–33. doi: 10.1038/nrmicro2415
5. Johnston, D. T., Wolfe-Simon, F., Pearson, A., and Knoll, A. H. 2009. Anoxygenic photosynthesis modulated proterozoic oxygen and sustained Earth's middle age. *Proc. Natl. Acad. Sci. U.S.A.* 106:16925–9. doi: 10.1073/pnas.0909248106
6. Valdespino-Castillo, P. M., Hu, P., Merino-Ibarra, M., López-Gómez, L. M., Cerqueda-García, D., González-De Zayas, R., et al. 2018. Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. *Front. Microbiol.* 9:510. doi: 10.3389/fmicb.2018.00510
7. Gischler, E., GIbson, M. E., and Oschmann, W. 2008. Giant holocene freshwater microbialites, laguna bacalar, quintana roo, Mexico. *Sedimentology* 55:1293–309. doi: 10.1111/j.1365-3091.2007.00946.x


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## YOUNG REVIEWERS

**DANA, AGE: 14**
My name is Dana. I am 14 years old and I live in Guanajuato, Mexico. I have been dancing ballet and jazz since I was 2 years. I love science, specially astronomy. I would like to study it in the future. I also would like to be an engineer or a lawyer.

**MARÍA JOSE, AGE: 14**
My name is María Jose, my friends call me Majo. I am 14 years old, I live in Guanajuato, Mexico. I like ballet, I have been practice it since I was 3 years old. I like science and I want to be a doctor.

**MARIANA, AGE: 15**
My Name is Mariana, I am 15 years old and I live in Guanajuato, Mexico. My favorite sport is squash, I have been playing it since I was 6 years old. My favorite subjects are History and Chemistry. In the future I would like to become a Scientist.

## AUTHORS

**ALFREDO YANEZ-MONTALVO**
I am Ph.D. student in Ecology and Sustainable Development. I am passionate about the study of microbial ecology and the understanding of how this microbial diversity is affected by natural and human activities. I love poetry, sports, and Bacalar lagoon. Every day we can learn from people and nature.

**BERNARDO ÁGUILA SALGADO**
I am Ph.D. student, a curious and geeky person. I am in love with arts, technology and popular science, specially natural science topics, such as biology, geology, astronomy, and environmental sciences. I am very interested in microorganisms that have an important role in the function of ecosystems. I am also aware of the environmental problems we may face in a future and I would like to contribute in some way to the solution.

**ELIZABETH S. GÓMEZ-ACATA**
I am a microbiologist working as a post-doctoral researcher at Universidad Nacional Autonoma de Mexico. I am fascinated with microbial life. I love studying microorganisms in any environment because they are very important for life on the planet. I also like to investigate biotechnological applications of microorganisms for taking care of the environment. I enjoy looking at them on the microscope and studying them with molecular techniques. In my free time, I enjoy traveling, photography, and cross-stitch embroidery. I love my family and enjoy life.

**YISLEM BELTRÁN**
I am a post-doctoral fellow at UNAM in Mexico. I am interested in microbial diversity and to understand patterns that characterize the presence of bacterial communities in different environments, as well as their response to environmental changes. What I like about this research is that I have been able to travel and know

beautiful places, but also, I can combine field and molecular work and learn about computer sciences.

**PATRICIA M. VALDESPINO-CASTILLO**

I am a Ph.D. in Marine Science and Limnology. I am fascinated by the microbes of the environment, they are the most ancient and the most powerful mini-machines of life. Science, music and outdoor activities are very important for me; therefore I want to try any possible combination of these. I love being illuminated by curious young minds.

**CARLA M. CENTENO**

Since I was a little girl I wanted to be a biologist, because I was interested in understanding natural phenomena. When I learned about microorganisms I was fascinated by their shape, their tricky simplicity, the time they spend on our planet (millions of years) and their diversity. I love laboratory work and I really enjoy teaching. I have two small children to whom I teach respect and love toward all life forms.

**LUISA I. FALCÓN**

I am a microbial ecologist at UNAM in Mexico, fascinated with the diversity of bacteria and archaea in the environment. I love how microbes interact with each other to complement their metabolic capabilities, allowing for communities and ecosystems to exist. *falcon@ecologia.unam.mx

## 9.2 Microbialites: Diversity hotspots in the Mexican Plateau.

## Chapter 22

# Microbialites: Diversity Hotspots in the Mexican Plateau

Patricia M. Valdespino-Castillo[1], Bernardo Águila[2], Jesús Torres-Huesca[3], Carla M. Centeno[4], Margarita Reyes-Salas[5], Sonia Angeles-García[5], Yislem Beltrán[6], Rocío J. Alcántara-Hernández[7], Hoi-Ying N. Holman[1] and Luisa I. Falcón[8]

1 Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory. One Cyclotron Rd, Berkeley, 94720, CA, USA. pmvaldespino@lbl.gov, hyholman@lbl.gov

2 Programa de Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México. Av. Universidad 3000, Alcaldía Coyoacán, 04510, Ciudad de México. Mexico. basbajo12@gmail.com

3 Programa de Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México. Av. Universidad 3000, Alcaldía Coyoacán, 04510, Ciudad de México. Mexico. jesustohu.unam@gmail.com

4 Baden Powell School. Av. Juárez 162 San mateo Tecoloapan, Atizapán de Zaragoza 52920, Edo. México. centenocarla@hotmail.com

5 Electronic Microscopy and Microanalysis Laboratory & LANGEM. Instituto de Geología. Universidad Nacional Autónoma de México. Ciudad Universitaria. Av. Universidad 3000, Alcaldía Coyoacán, 04510, Ciudad de México, México. adelars@hotmail.com, ags@unam.mx

6 DGAPA/UNAM Posdoctoral Stage at the Instituto de Ciencias Del Mar y Limnología, Unidad Académica de Sistemas Arrecifales, Universidad Nacional Autónoma de México, Puerto Morelos, Quintana Roo, México. Av. Niños Héroes S/N. Domicilio conocido C.P. 77580,. Puerto Morelos, Q. Roo, México. yislem@gmail.com

7 Instituto de Geología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Av. Universidad 3000, Alcaldía Coyoacán, 04510, Ciudad de México, México. rocio.alcantara.h@gmail.com

8 Laboratorio de Ecología Bacteriana, Instituto de Ecología, Campus Yucatán, Universidad Nacional Autónoma de México, 97357. Carretera Mérida-Tetiz Km. 4.5, Municipio de Ucú, Yucatán, México. falcon@ecologia.unam.mx

**Abstract**

The white rocks delineating the littoral of Lake Alchichica have called the attention of locals, foreigners, explorers and curious scientists over the years. More recently, science has revealed that these mineral constructions are hotspots of previously hidden microbial diversity. The metabolism of diverse microbial assemblages mediates the formation of massive biomineral structures of hydromagnesite, calcite and aragonite, reaching more than 40 m in depth. The high taxonomical diversity resembles a high metabolic diversity, reported here by the potential of microbialite prokaryotic communities for biogeochemical cycling. Today, hundreds of the microbialite prokaryotes have no identified matches in the present microbial taxonomic databases. This fact has called the attention of scientists worldwide, and Lake Alchichica has become a model system for microbial ecology, evolution, biogeochemistry and astrobiology studies. Microbialites of Lake Alchichica provide a unique environment for the development of many other endemic species. The conservation of these microbial communities is challenging in the panorama of global change and will require coordinated efforts at all levels of the society.

**Keywords:** Biomineralization, microbial metabolic diversity, soda lake, biominerals, microbialite history, stromatolites, Lake Alchichica

## 22.1 Overview

Microbialites have been called “texcales” in Mexico since pre-Hispanic times. In the native Náhuatl language, this word translates to "rocks". Similarly to corals, a thin living surface in submerged microbialites mediates the precipitation, cementation, and accretion of sediment, minerals, and organic matter, forming massive mineral rock-like structures. The difference between them is that in microbialites, biomineral formation is mediated mainly by prokaryotes. Today, in Lake Alchichica ("the place of bitter water" for locals), some of these massive structures have emerged because the lake's water

level has been decreasing in the last decades and living microbialites develop along the lake periphery and up to a depth of 30 meters. As a result, Lake Alchichica offers a very particular landscape to visitors, where a ring of white stones extends along the litoral (Fig. 22.1). These white constructions turn brown-green as they get closer to the water. The dark color is evidence of complex benthic microbial communities' presence and activity that are engineering mineral sedimentary fabrics. Hence these organo-sedimentary structures are the result of interactions between benthic microorganisms and their environment. Microbialites have also been called tufa, stromatolites, or bacterial reefs.

The singular panorama offered by the microbialites of this turquoise color lake has fascinated travelers over the years. Besides travelers, scientists have been attracted to these “uncommon” bioconstructions. While the formation of the sedimentary structures was long attributed to "blue-green algae", today we know that diverse microbial consortia populate microbialites (Kaźmierczak *et al.*, 2008, Couradeau *et al.*, 2011, Centeno et al. 2012, Saghaï *et al.*, 2015). These numerous microbes are actively doing photosynthesis and fixing nitrogen (Falcón et al. 2002, 2020, Beltrán et al., 2012) and are models to understand microbially mediated mineral formation (e.g., Valdespino-Castillo et al. 2018). These benthic structures allow the establishment and development of many other species. This chapter explores these organo-sedimentary structures in detail, the geochemical features that explain their presence and their role as biodiversity hotspots.

**22.2 Research History**

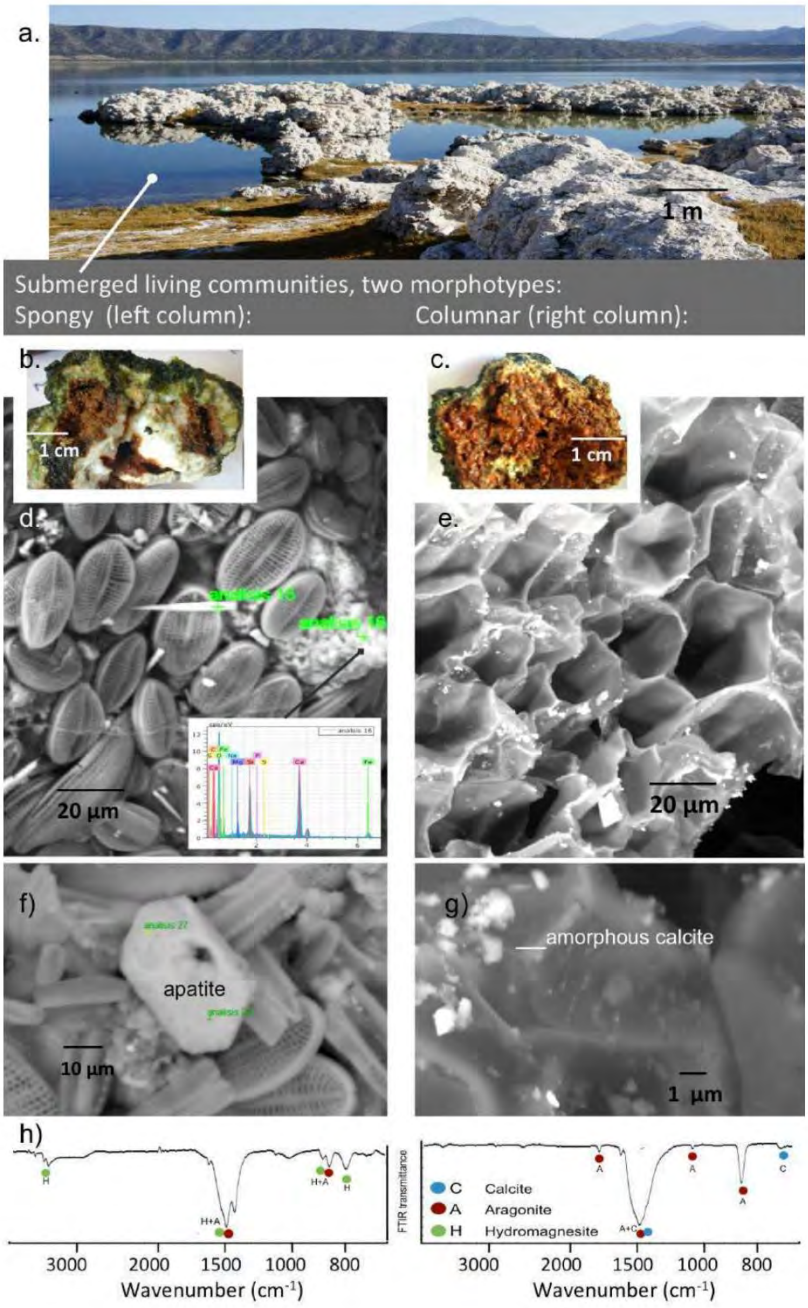
The geochemistry of the lake and other biological compartments was studied long before the availability of technical advancements to study the biological component within microbialites. While geochemical features of Lake Alchichica are summarized in chapter 5 (Hydrochemistry of Lake Alchichica and its drainage basin) three features of the lake are crucial to explaining the presence of microbialites: a) clear oligotrophic waters allowing light penetration, b) availability of major anions including bicarbonate ($HCO_3^-$) and sulfate ($SO_4^{-2}$) and major cations such as $Na^+$, $Ca^{+2}$ and $Mg^{+2}$, and; c) relatively high alkalinity contributing to chemical species availability and thermodynamically favoring biomineralization; these features have been documented

largely in Chapter 5 (Hydrochemistry of Lake Alchichica and its drainage basin). The geochemical survey of volcanic lakes of Mexico published by Armienta et al. (1991) and other studies (see chapters 5, Hydrochemistry of Lake Alchichica and its drainage basin, and 7, Physicochemical characteristics) also account for the hyposaline character of the lake´s water, depicting a high Mg:Ca ratio when compared to lakes worldwide.

Lake Alchichica held scientists' attraction in the '70s and '80s; limnologists, geologists, and protistologists often studied the lake to hypothesize about life in the past. The microbial communities related to microbialites were not studied until the '90s. As a Ph.D. student advised by J. Komarek, R.L. Tavera published a fine description of the cyanobacterial components populating the mineral bioconstructions (Tavera and Komarek 1996). The reader should consider that the Nostocales cyanobacterial cells studied by these authors populated the surface of the microbialites. Unlike many other smaller microbes, Nostocales cyanobacteria are ~10 by 50 μm, and therefore, their details were accessible under a regular benchtop optical microscope. Besides describing the cyanobacterial components and their taxonomy, these authors pointed out the coexistence of two different microbialites morphotypes within the lake. One was called spongy because of its round microstructure, and the second one, columnar, because of its vertically disposed microstructure (Fig. 22.1). Kaźmierczak et al. (2011) provided a comprehensive examination (e.g., age, structure, and mineralogy) of Lake Alchichica's microbialites and morphological and molecular analysis of the living cyanobacterial mats. Further, Couradeau et al. (2012) reported a unicellular cyanobacterium associated to carbonate mineral precipitations in Lake Alchichica's microbialites which is proposed to form amorphous calcium-magnesium-strontium-barium carbonate inclusions intracellularly. This cyanobacterium is proposed to be *Gloeomargarita lithophora* (Moreira et al. 2017), and further studies are needed to understand its phylogeny and natural history.

Over the years, microbial systematics studies have provided direct evidence of high microbial diversity in these assemblages. Like ocean reefs, microbialites contribute to the formation of micro-habitats along the littoral zone, populated by mollusks, arthropods, protists, helminths, fishes, amphibians, and other organisms (see chapters 10, the littoral benthic community, 11, bacterioplankton, and 12, phytoplankton). This aquatic diversity has led to the growing recognition of the lakes of the Central Highlands

of Mexico as biodiversity hotspots, areas that are extremely important for biodiversity conservation (Reid, 1998). Many prokaryotes in Lake Alchichica microbialites have no parallel in the current databases. Likewise, eukaryotic organisms related to Alchichica microbialites exhibit a high endemism (as seen in chapters 16, Alchichica axolotl, 20, Diversification and endemisms, and 21, Conservation actions). Diversity indices of Shannon and Chao (6.1 and 6.3; 2026 and 2271 for spongy and columnar types, respectively; Centeno et al. 2012) indicate a high degree of prokaryotic diversity in these assemblages. DNA sequences and taxonomic assignment of Bacteria and Archaea in Alchichica microbialites can be found in GenBank under NCBI BioProject PRJNA418176 (BioSamples: SAMN08017101 and SAMN08017098). Pyrosequences were deposited in the Sequence Read Archive (SRA, NCBI), under BioProject SRA040061. Information regarding the most abundant prokaryotic phyla can be found in Centeno et al. (2012). By comparing microbial composition from five different Mexican sites, Centeno et al. reported that the most abundant phyla for all these locations was Proteobacteria (~ 31% to 42%; where Alpha > Beta > Gamma > Deltaproteobacteria), followed by Cyanobacteria (overall range 1-24%). Notably, cyanobacterial abundance in Alchichica was the highest. Microbial phyla with medium abundance in the Mexican microbialite survey were Planctomycetes (5–11%), Verrucomicrobia (3–8%), Bacteroidetes (4–8%), Acidobacteria (1–2.4%), Chloroflexi (1–2.4%) and Firmicutes (1–3%). Phyla with low abundance included Actinobacteria, Nitrospira, Chlamydiae, Spirochaetes, Chlorobi, Fusobacteria, and Gemmatimonadetes. The challenges of extracting total DNA from these complex mineral mixtures have been discussed in Gómez-Acata et al. (2019).


a.
1 m
Submerged living communities, two morphotypes:
Spongy (left column):
Columnar (right column):
b.
1 cm
c.
1 cm
d.
20 µm
e.
20 µm
f)
apatite
10 µm
g)
amorphous calcite
1 µm
h)
H
H+A
H+A
H
3000
2000
1500
1000
800
Wavenumber (cm-1)
FTIR transmittance
C Calcite
A Aragonite
H Hydromagnesite
A+C
A
A
C
3000
2000
1500
1000
800
Wavenumber (cm-1)

6

**Fig. 22.1** Lake Alchichica microbialites: left = spongy, right = columnar morphotypes. From top to bottom a) field view, a cross-section of spongy (b) and columnar (c) morphotypes. SEM-EDS micrographs and elemental analyses of d) spongy, showing amorphous calcite formations often surrounded by benthic diatoms, and e) columnar microbialites depicting a hexagonal-based aragonite structure. At higher magnification, f) fluorohydroxyapatite crystals were detected in the spongy morphotype, and g) amorphous precipitations of 1 μm over honeycomb-like aragonite structure in the columnar morphotype. (h) SR-FTIR spectral signals of main carbonates of spongy and columnar microbialites: aragonite, calcite, and hydromagnesite (for details, see Valdespino-Castillo et al. 2018).

### 22.3 Microbialite Microbiology: Microbialites are Active Communities

As a M.S. student, LI Falcón provided the first physiological evidence that these microbial consortia were metabolically active, performing $N_2$ fixation in rates that averaged 3 μM $N_2$ $m_2$ $h^{-1}$, with higher rates in the spongy compared to the columnar forms. The evidence of activity (Falcón et al. 2002) broke skepticism and was a first step for the research she has led on the microbialites of Lake Alchichica. $N_2$ fixation was further related to specific microbes (*nifH*), mostly within cyanobacterial Nostocales (Beltrán et al. 2012). Basal-heterocyst cyanobacteria seem to be a key component of this process in Alchichica microbialites.

The microbial components of microbialites were first described by Couradeau et al. (2011) and Centeno et al. (2012) using next-generation sequencing methods. These studies showed a diverse community, which varied along a depth gradient up to 14 m (Couradeau et al. 2011). Centeno et al. (2012 and 2016) suggested that the microbial composition of Alchichica microbialites was taxonomically similar to microbialites from other regions of Mexico but intriguingly richer in cyanobacteria. The cross-system comparison included microbialites from coastal and inland karstic locations: Bacalar, Muyil, and Cuatro Cienegas Basin. Microbialite communities among systems shared taxonomic features, and the main prokaryote groups were generally Proteobacteria, Cyanobacteria, Planctomycetes, and Verrucomicrobia. Archaea seemed to be less abundant in the microbialites studied, whereas the main phyla were Euryarchaeota and Crenarchaeota. Centeno et al. (2012) also identified conductivity, pH, and nitrate

availability as the ambient environmental factors significantly related to microbial communities' differences.

### 22.4 Microbialites in Deep Waters

As explained in Chapter 5 (Hydrochemistry of Lake Alchichica and its drainage basin), Lake Alchichica is one of Mexico's deepest lakes ($z_{max}$= 60 m). While the lake's shallow environment harbors at least two visually recognizable types of microbialites, these comprise a continuum that extends to depths of ~ 40 m. The microbial composition and the characteristics of these microbialites constitute an intense subject of research. Couradeu et al. (2011) reported a shift in the composition of Cyanobacteria with depth, where Pleurocapsales increased in abundance at 14m. A similar pattern was reported by Águila (2018) and Águila et al., (2021). Furthermore, deeper microbialites harbor a larger diversity of Actinobacteria and Firmicutes than surface structures (Couradeau et al., 2011).

### 22.5 Cyanobacteria: Main Components of Microbialites

In Alchichica microbialites, cyanobacterial taxa showed a depth gradient distribution pattern (Kaźmierczak et al. 2011, Saghaï et al. 2015, Águila et al., 2021). Dominant cyanobacterial taxa of different depths are shown in Figure 22.2. These authors suggested that filamentous Synechococcales (*Haloleptolyngbya* sp., *Leptolyngbya* sp., *Nodosilinea* sp. and *Oculatella* sp.) with Nostocales (*Rivularia* sp. and *Calothrix* sp.) were major components for microbialites developing at a depth of 3 m. *Nodularia* sp. was only present in winter but was not a central component of microbialites. Unicellular Synechococcales (*Acaryochloris* sp., *Cyanobium* sp. and *Prochlorococcus* sp.) strongly associated to deep-water (20 and 30 m) microbialites. Most importantly, Chroococcales (*Entophysalis* sp.) and Pleurocapsales (*Chroococcidium* sp. and *Myxosarcina* sp.) were common in all microbialites; their abundance increased at a depth of 20 m. The study of *Entophysalis* sp., *Chroococcidium* sp., and Myxosarcina sp. has provided significant insights into the depth-related pigments diversity and photosynthetic activity in spongy microbialites. Pleurocapsales have also been described for microbialites of Lake Pavilion in Canada (Russell et al. 2014), Highborne Cay in the Bahamas (Mobberley et al., 2013), being dominant in some dome-shaped microbialites of Lake Dziani-Dzaha in

Mayotte (Gérard et al. 2018). The endolithic ecology of Pleurocapsales probably depends on low light conditions; nonetheless, the difficulty of culture isolation has made this group of cyanobacteria historically understudied (Brito et al. 2017). Since their role in mineral precipitation and photosynthesis in Alchichica microbialites appears to vary with depth, more research is needed to untangle the specific roles of the different cyanobacterial components of these structures (Águila et al. 2021).

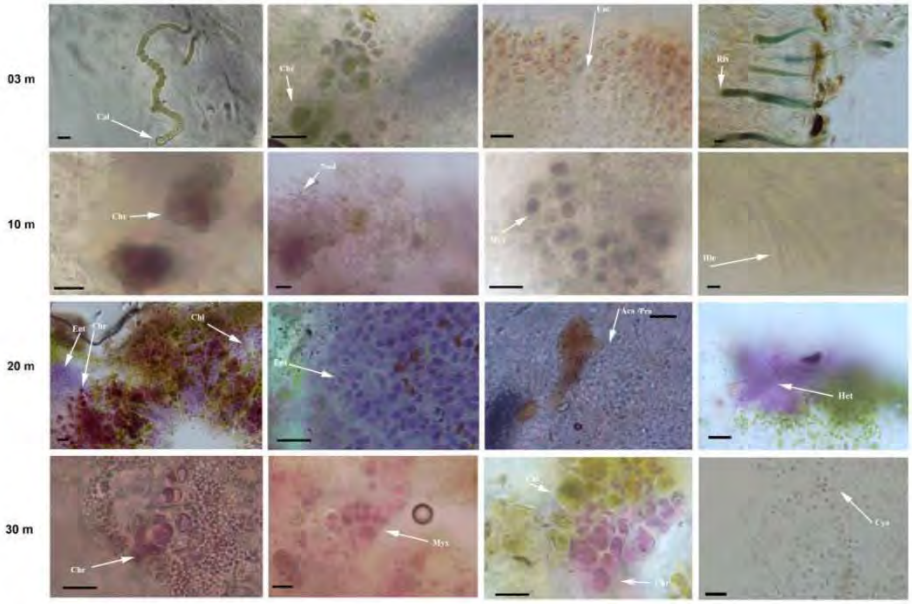



**Fig. 22.2** Optical microscopy of cyanobacteria in microbialites. For 3m: *Calothrix* (Cal) filaments were scarcely found, *Chroococcidium* showed a green morphotype (Chr), *Entophysalis* (Ent) showed a red morphotype, and *Rivularia* (Riv) colonies were found abundantly in the exterior of microbialites. For 10m: *Chroococcidium* exhibited a violet morphotype, *Nodosilinea* (Nod) was abundant in the exterior of microbialites, *Myxosarcina* (Myx), and *Haloleptolyngbya* (Hal) were also present. For 20m: a consortium of *Entophysalis* with purple morphotype, *Chroococcidium*, and a Chlorophyte (probably *Cladophora*) were common, there were also cells of *Acaryochloris* or *Prochlorococcus* (Aca/Pro) and filaments of *Heteroleibleinia* (Het). For

30 m: *Chroococcidium* and *Myxosarcina* were found abundantly, as well cells of *Cyanobium* (Cya) Scale Bar = 10 μm.

**22.6 Biomineral Structures**

Lake Alchichica is a maar alkaline and saline system with a relatively recent history (see chapter 5, hydrochemistry). To understand their fossilized biosignatures, Kaźmierczak et al. (2011) studied microbialites mineral composition, texture, and isotopic composition. They used U-Th radiometric dating on both stromatolite-types. Their results suggested the formation of the different microbialite morphotypes might be related to distinct stages of lake hydrogeology, which could explain the difference in mineral composition in both morphotypes, where the spongy types were composed mainly of hydromagnesite with an admixture of huntite and calcite, and dating to ~ 2800 years B.P. Meanwhile, the columnar-types were composed of aragonite and an admixture of Mg-calcite, dating to ~ 1100 years B.P.

Structural microbialite surface minerals were primary carbonates, mainly exhibiting micrometric-scale micric, amorphous precipitations (Fig. 22.1b,c). As reported in Valdespino-Castillo et al. (2018), microarchitectural observations showed that benthic diatoms (and silicification potentially) might play a role in building micrometric environments in the surface of the microbialites (Fig. 22.1b,d), which needed further clarification. A regular honeycomb-like structure of hexagonal caveats was observed at the columnar type's surface, where aragonite was the main mineral found. Other minerals were also formed, for example, apatites (Fig. 22.1c) as detected by SEM-EDS microscopy-spectroscopy (Fig. 22.1d).

A novel bioimaging strategy was developed and applied to characterize living cells and biogeochemical systems at microscale (Holman et al. 2010; Probst et al., 2013, 2014; Valdespino et al. 2018). Synchrotron infrared spectromicroscopy was used to characterize minerals and biomass of live microbialites from various locations. Infrared spectral features of the carbonates reported in Valdespino-Castillo et al. (2018) included generalized hydromagnesite signals, aragonite, and calcite (Fig. 2h), but also spatially segregated calcite with traces of strontium and siderite.

Microbialites from Lake Alchichica have inspired many geo-microbiologists to investigate how aquatic biological diversity is structured and maintained (Coradeau et

al. 2011, Centeno et al. 2016). By using novel strategies of microscopy and chemical imaging techniques, Gérard et al. (2013) linked the potential formation of aragonite to Pleurocapsales cyanobacteria in microbialites from the depths of 20 m. Kaźmierczak et al. (2011) and Valdespino-Castillo et al. (2018) reported that calcite nucleation on surface layers of microbialites is spatially related cyanobacterial filaments. Deeper analyses of the microbialites open new questions and challenges to the study of these communities.

**22.7 Biogeochemical Cycles**

The potential role of nitrogen and phosphorus as the nutrients limiting the productivity of lake Alchichica has been documented in different studies (e.g., Ramírez-Olvera et al. 2009; see chapter 7, physicochemical characteristics). Therefore, the role of microbes in nitrogen and phosphorus biogeochemical cycling has been extensively explored in Lake Alchichica. Different studies reveal that microbialites harbor a significant genetic diversity potential for N and P remineralization (Valdespino-Castillo et al. 2014, 2017; Alcántara-Hernández et al. 2017). Recent studies document the genetic potential of microbialites associated with sulfur cycling (Torres-Huesca, in preparation). A general scheme of the main biogeochemical transformations in microbialites as detailed by the bacterial genes explored is shown in Figure 22.4

***22.7.1 Nitrogen Cycling***

The work of Alcantara-Hernández et al. (2017) analyzed the diversity associated with N cycling by coupling genomic characterization and expression patterns of nitrogenase (*nifH*), ammonia monooxygenase (amoA), nitrite oxidoreductase (*nxrA, nxrB*), hydrazine oxidoreductase (*hzo*), and nitrite (*nirS* and *nirK*) and nitrous oxide (nosZ) reductases. Alcantara-Hernández et al. (2017) showed that the genetic potential for $N_2$ fixation, ammonia oxidation, anammox, and denitrification was present in the microbialites of Alchichica. The taxonomic affiliation of the amplicons is shown in Figure 22.3 for each step of the nitrogen cycle. However, compared to the transcriptomic signal, the main metabolism expressed was $N_2$ fixation linked to Nostocales cyanobacteria.

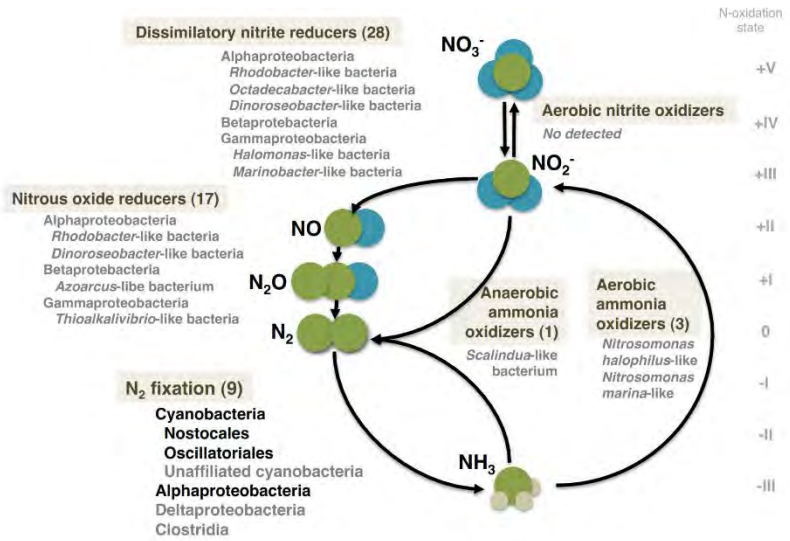



**Fig. 22.3** N-cycling genetic markers and their taxonomical affiliation detected in microbialites of Lake Alchichica (Alcántara-Hernández et al. 2017).

### *22.7.2 Phosphorus Cycling*

Dissolved organic phosphorus (DOP) is usually the most abundant and largely uncharacterized fraction of phosphorus in aquatic environments (Kolowith et al. 2001; Dyhrman et al. 2007). To make phosphorus available from this fraction, microbes harbor diverse strategies such as using pH-dependent (acid or alkaline) phosphatases to release orthophosphate or polyphosphate chains from organic matter phosphate esters (Ruttenberg and Dyhrman 2005). Phytate or phytic acid is another molecule that may be abundant in aquatic environments DOP due to plant debris runoff (Suzumura and Kamatani 1995). Some microbes can release orthophosphate from phytate using phytases. A diverse set of beta-propeller phytase (BPP) markers were detected in microbialites and bacterioplankton of Lake Alchichica. In their studies, Valdespino-

Castillo et al. (2014, 2017) found the pool of alkaline phosphatases (AP) *phoA* and *phoD*, as well as beta-propeller phytases (3-phytase, BPP) was higher in microbialites than in bacterioplankton. This P remineralization potential was taxonomically related to Alpha-, Beta-, and Gamma-proteobacteria. Compared with other AP surveys, this taxonomic affiliation is similar among systems exhibiting contrasting environmental characteristics (e.g., trophic state, phosphorus condition, the concentration of $Zn^{+2}$, $Mg^{+2}$, and $Ca^{+2}$, metal cofactors of these type of enzymes) (Valdespino-Castillo et al. 2014). The diversity of BPP was taxonomically related to phyla alpha- and beta-proteobacteria and Bacteroidetes, and Flavobacteriales. Beta-propeller phytases found in this alkaline lake were similar to phytases found in forest soils, glaciers, lake sediments, and fish gut microbiota (Huang et al. 2009).

Alkaline phosphatase phoX was the marker among DOP utilization enzymes that showed the highest ratio of unique sequences/total sequences (Valdespino-Castillo et al. 2014). Gene dynamics showed that the lake's hydrologic cycles (summer stratification-winter circulation) could impact the lake's DOP utilization potential. The highest DOP utilization diversity in microbialites together with this result may account for the resilience of microbialites. The bacterioplankton, instead, exhibited a faster response to environmental change and less diversity (Valdespino-Castillo et al. 2014, 2017). Together, these results outline divergent biogeochemical strategies between microbialites and bacterioplankton to face environmental change, which should be further studied, particularly to understand microbialite formation and history.

### *22.7.3 Sulfur Cycling*

The circulation-stratification hydrological cycle leads to different sulfur (S) redox environments related to the season and the lake compartments. Nevertheless, the microbial mats of microbialite surface most likely offer millimetric redox gradients relevant for sulfur transformations. Torres-Huesca (2018) studied some of the genetic markers related to the sulfur cycle in littoral microbialites and at different depths, taking samples up to hypolimnion from the stratified water column with anoxic conditions and reducing redox conditions. The author focused on the characterization of sulfate-reducing bacteria through 16S rRNA analysis using Next-generation sequencing (NGS) and the diversity of the functional gene *dsrA*. A low abundance of potential sulfate-

reducing bacteria (~ 1%) was found relative to the total prokaryotic community, probably because the microbial mat samples in the Alchichica microbialites were thin (0.5 - 1 mm from the surface) and not optimal for anaerobic metabolisms (Muyzer and Stams, 2008).

Similarly to these results, deltaproteobacteria's contribution as sulfate reducers accounted for less than 0.01% of the relative abundance in the littoral microbialite samples (Valdespino-Castillo et al. 2018). In the metagenomics study of Alchichica's microbialites, conducted by Saghaï et al. (2016), sulfate reduction gene signals exhibited very low abundance. In his study, Torres-Huesca (2018) showed genetic diversity of *dsrA* genes was affiliated mainly with Deltaproteobacteria within the *Syntrophaceae*, *Desulfobacteraceae*, *Desulfobulbaceae*, and *Desulfovibrionaceae* families, characteristic groups in saline-alkaline microbial mats and other microbialites (Foti *et al.*, 2007; Sorokin *et al.*, 2011; Casaburi *et al.*, 2016). Ongoing analyses show that sulfur oxidation's potential might be related to gammaproteobacteria Chromatiales in Lake Alchichica (Torres-Huesca, 2018), their exploration has been expanded to lake's different compartments: sediments, water column, and microbialites. Previous work by Santillan-Manjarrez (2018); Torres-Huesca (2018); showed the potential of sulfate reduction and sulfur oxidation in these microbialites.

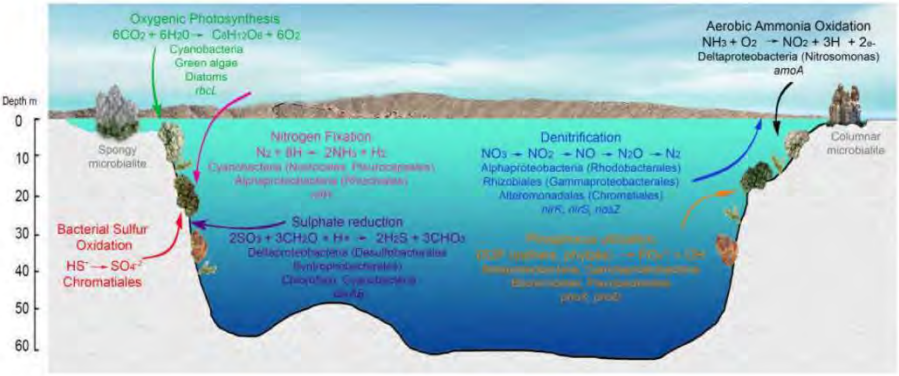



**Fig. 22.4** A general scheme of the role of microbialites microbes in biogeochemical transformations is shown. Biogeochemical pathways equations are simplified and

shown along with their prokaryotic signals in microbialites, including taxonomic affiliation and genes. For example, Alphaproteobacteria and Cyanobacteria are main participants in carbon and nitrogen fixation. Alpha- Beta- and Gamma-proteobacteria are main participants in organic phosphorus utilization (contributing to orthophosphate availability). Additionally, Deltaproteobacteria, Chloroflexi, and Cyanobacteria are relevant taxa in sulfate reduction, and finally, Gammaproteobacteria in sulfur oxidation.

### *22.7.4 Trace Metal-Microbe Interactions*

Mineral matrices of living microbialites accumulate organic matter and trace metals from the surrounding environments. This enrichment has been attributed to the binding of cations to EPS in the surface of microbial mats and the redistribution into sulfide-rich laminae upon degradation of EPS during early diagenesis (see Bruggmann et al. 2020). A cross-comparison of different microbialite locations, including those from lake Alchichica revealed specific relationships of microbes and trace metals (Valdespino-Castillo et al. 2018). The microbialites studied showed higher concentrations of trace elements than other microbial carbonates (Kamber and Webb 2007). The results reported in Valdespino-Castillo et al. (2018) revealed a strong correlation pattern of microbial taxa with transition elements such as cadmium, cobalt, chromium, and copper. Chromium and copper, known to be toxic for many microorganisms, showed positive correlations with some bacterial groups. In detail, Alphaproteobacterial families Sphingomonadacea and Rhodobacteraceae (e.g., *Rubellimicrobium*) were strongly and positively correlated to Cr concentration in the mineral matrix. Specific homeostasis proteins were linked to bacterial strategies to deal with trace metals. For example, Proteobacteria harbored more than half of the proteins annotated for copper homeostasis (NCBI Protein database, Jan 2018), and more than 80% of the proteins were associated with copper resistance. Copper homeostasis genes (e.g., protein *cutC* and copper transporter *cupA*) have been identified in Lake Pavilion's freshwater microbialites (White et al., 2015). Alphaproteobacteria also harbor 10.7 and 40% (respectively) of the total annotated bacterial *cutC* and *cupA* genes (NCBI Protein database, Jan 2018). In contrast, cadmium and cobalt were significantly but inversely related (lower microbial abundance correlates with higher metal content) to the distribution of dominant microbialite organisms, overall supporting their toxic effect.

Specific microbe-trace metal correlations showed the highest scores for Cyanobacteria (Oscillatoriales and Synechococcales), Bacteroidetes (Cytophagales and Flavobacteriales), Alphaproteobacteria (Rhodobacterales, Rhizobiales, and Sphingomonadales), Gammaproteobacteria (Pasteurellales, Aeromonadales, and Enterobacteriales), Firmicutes (Lactobacillales and Clostridiales).

**22.8 Biodiversity Conservation Challenges**

The research of Alchichica microbialites has just started. Their unique microbial composition, their vast metabolic potential, and the formation of microhabitats favoring other species settlement such as *Ambystoma taylori,* an endemic amphibian species of the lake, leads to the recognition of microbialites as biodiversity hotspots, which is of utmost importance to setting conservation priorities and implementing strategies to preserve the Alchichica crater lake biodiversity. Microbialites represent ancient micro-ecosystems of high microbial diversity and constitute excellent study systems to understand microbial evolution and elemental cycling.

The high diversity found in microbialites might be a key factor to their success from thousands to millions of years. The increasing research of these structures has been providing valuable information to Astrobiology, Geoscience, Ecology, and Biogeochemistry. However, these structures are threatened because of regional water use associated with intensive agricultural practices. Puebla and Veracruz's local governments hold a major responsibility in this natural laboratory's fate. A micromolar increase in the nutrient content, regional temperature increase, and drought-related climate patterns are potential drivers of drastic changes. We have already started to increase academia-society efforts to bring awareness to the society about the presence and the significance of these peculiar communities, for example, by celebrating the National Day of Stromatolites in Mexico (July 5th, https://youtu.be/ac5aev2XMVs) and publishing science materials for a broader audience (Valdespino et al. 2015 and 2019; Falcón et al. 2020; Yanez-Montalvo et al. 2020), besides press releases and informal talks. The conservation of Lake Alchichica and the Axalapascos offers a challenging panorama involving climate change and increasing water use. Scientists, local government, and society must work together in the conservation of Alchichica biodiversity. By overcoming the challenges at this biodiversity hotspot, we can set up a

model system for sustainability and conservation throughout the Central Mexican Highlands.

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**References**

Águila B (2018) Caracterización de cianobacterias en microbialitas del lago cráter Alchichica en un gradiente de profundidad. MS Dissertation thesis. Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, UNAM, Ciudad de México, Mexico

Águila B, Alcántara-Hernández RJ, Montejano G, López-Martínez R, Falcón LI, Becerra-Absalón I (2021) Cyanobacteria in microbialites of Alchichica Crater Lake: a polyphasic characterization, Europ J Phycol DOI: 10.1080/09670262.2020.1853815

Alcántara-Hernández RJ, Valdespino-Castillo PM, Centeno CM, Alcocer J, Merino-Ibarra M, Falcón LI (2017) Genetic diversity associated with N-cycle pathways in microbialites from Lake Alchichica, Mexico. Aquat Microb Ecol 78:121–133

Armienta MA, Vilaclara G, De la Cruz-Reyna S, Ramos S, Ceniceros N, Cruz O, Aguayo A, Arcega-Cabrera F (2008) Water chemistry of lakes related to active and inactive Mexican volcanoes. J Volcanol Geotherm Res 178(2): 249–258

Beltrán Y, Centeno CM, García-Oliva F, Legendre P, Falcón LI (2012) N2 fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia from different aquatic environments in Mexico. Aquat Microb Ecol 67(1): 15–24

Bruggmann S, Rodler AS, Klaebe RM, Goderis S, Frei R (2020) Chromium isotope systematics in modern and ancient microbialites. Minerals 10(10), 928. https://doi.org/10.3390/min10100928

Casaburi G, Duscher AA, Reid RP, Foster JS (2016) Characterization of the stromatolite microbiome from Little Darby Island, The Bahamas using predictive and whole shotgun metagenomic analysis. Environ Microbiol 18(5): 1452-1469

Centeno CM, Legendre P, Beltrán Y, Alcántara-Hernández RJ, Lidstromm UE, Ashby MN, Falcón LI (2012) Microbialite genetic diversity and composition related to environmental variables. FEMS Microbiol Ecol 82: 7 24–735

Centeno CM, Mejía O, Falcón LI (2016) Habitat conditions drive phylogenetic structure of dominant bacterial phyla of microbialite communities from several locations in Mexico. Rev Biol Trop 64(3): 1057-1066

Couradeau E, Benzerara K, Moreira D, Gerard E, Kaźmierczak J, Tavera R, López-García P (2011) Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). PloS one 6(12), e28767

Couradeau E, Benzerara K, Gérard E, Moreira D, Bernard S, Brown Jr GE, López-García P (2012) An early-branching microbialite cyanobacterium forms intracellular carbonates. Science 336(6080): 459-462

Dyhrman ST, Ammerman JW, Van Mooy BAS (2007) Microbes and the marine phosphorus cycle. Oceanography 20: 110–116

Falcón LI, Escobar-Briones E, Romero D (2002) Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico. Hydrobiologia 467(1): 71–78

Falcón LI, Valdespino-Castillo PM, Alcántara-Hernández RJ, Gómez-Acata ES, Yanez-Montalvo A, Águila B (2020) Stromatolites in Crater-Lake Alchichica and Bacalar Lagoon. In: Souza V., Segura A., Foster J. (eds) Astrobiology and Cuatro Ciénegas Basin as an Analog of Early Earth. Cuatro Ciénegas Basin: An Endangered Hyperdiverse Oasis. Springer, Cham doi:https://doi.org/10.1007/978-3-030-46087-7_9

Foti M, Sorokin DY, Lomans B, Mussman M, Zacharova E E, Pimenov NV, Muyzer G (2007) Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. Appl Environ Microbiol 73(7), 2093-2100.

Gérard E, Ménez B, Couradeau E, Moreira D, Benzerara K, Tavera R, López-García P (2013) Specific carbonate–microbe interactions in the modern microbialites of Lake Alchichica (Mexico). ISME J 7(10):1997

Gómez-Acata ES, Centeno CM, Falcón LI (2019) Methods for extracting'omes from microbialites. J Microbiol Methods 160: 1–10

Holman HYN, Bechtel HA, Hao Z, Martin MC (2010) Synchrotron IR spectromicroscopy: chemistry of living cells. Anal Chem. 2010, 82, 21, 8757–8765. https://doi.org/10.1021/ac100991d

Huang H, Shi P, Wang Y, Luo H, Shao N, Wang G, Yang P, Yao B (2009) Diversity of beta-propeller phytase genes in the intestinal contents of grass carp provides insight into the release of major phosphorus from phytate in nature. Appl Environ Microbiol 75(6): 1508–1516.

Kaźmierczak J, Kempe S, Kremer B, López-García P, Moreira D, Tavera R (2011) Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico. Facies 57(4): 543–570.

Kolowith LC, Ingall ED, Benner R (2001) Composition and cycling of marine organic phosphorus. Limnol Oceanogr 46: 309–320.

Mobberley JM, Khodadad CLM, Visscher PT, Reid RP, Hagan P, Foster JS (2015) Inner workings of thrombolites: spatial gradients of metabolic activity as revealed by metatranscriptome profiling. Sci Rep 5: 12601

Moreira D, Tavera R, Benzerara K, Skouri-Panet F, Couradeau E, Gérard E, Loussert Fonta C, Novelo E, Zivanovic Y, López-García P (2017) Description of *Gloeomargarita lithophora* gen. nov., sp. nov., a thylakoid-bearing, basal-branching cyanobacterium with intracellular carbonates, and proposal for Gloeomargaritales ord. nov. Int J Syst Evol Microbiol 67: 653-658

Muyzer G, Stams AJ (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nature Rev Microbiol 6(6): 441-454

Probst AJ, Holman HYN, DeSantis TZ, Andersen GL, Birarda G, Bechtel HA, Piceno YM, Sonnleitner M, Venkateswaran K, Moissl-Eichinger C. (2013) Tackling the minority: sulfate-reducing bacteria in an archaea-dominated subsurface biofilm. The ISME J 7(3): 635–651 https://doi.org/10.1038/ismej.2012.133

Probst AJ, Birarda G, Holman HYN, DeSantis TZ, Wanner G, Andersen GL, Moissl-Eichinger C (2014) Coupling genetic and chemical microbiome profiling reveals heterogeneity of archaeome and bacteriome in subsurface biofilms that are dominated by the same archaeal species. Plos One, 9(6) e99801

Ramírez-Olvera MA, Alcocer J, Merino-Ibarra M, Lugo A (2009) Nutrient limitation in a tropical saline lake: a microcosm experiment. Hydrobiologia, 626(1): 5-13.

Russell JA, Brady AL, Cardman Z, Slater GF, Lim DSS, Biddle J F (2014) Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada. Geobiology 12(3): 250-264.

Ruttenberg KC, Dyhrman ST (2005) Temporal and spatial variability of dissolved organic and inorganic phosphorus, and metrics of phosphorus bioavailability in an upwelling-dominated coastal system. J Geophys Res Oceans 110(C10)

Saghaï A, Zivanovic Y, Zeyen N, Moreira D, Benzerara K, Deschamps P, Bertolino P, Ragon M, Tavera R, López-Archilla AI, López-García P (2015) Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. Front Microbiol 6: 797

Santillan-Manjarrez J (2018) Caracterización del componente bacteriano en los sedimentos superficiales del Lago cráter Alchichica, México. MS Dissertation thesis. UNAM. México. 88 p

Sorokin DY, Kuenen JG, Muyzer G (2011) The microbial sulfur cycle at extremely haloalkaline conditions of soda lakes. Front Microbiol 2(44): 1-16

Suzumura M, Kamatani A (1995) Origin and distribution of inositol hexaphosphate in estuarine and coastal sediments. Limnol Oceanogr 40: 1254–1261

Tavera R, Komárek J (1996) Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla State, Mexico. Arch Hydrobiol 117: 511–538

Torres-Huesca J (2018). Detección y caracterización molecular de las bacterias sulfato reductoras en microbialitas de un lago cráter alcalino de México. MS Dissertation thesis. UNAM. México. 59 p

Valdespino-Castillo PM (2015) Identification of the differential role of bacterial communities in the P cycle: microbialites and bacterioplankton from Alchichica lake as study models. PhD Dissertation. PCML, Universidad Nacional Autónoma de México, DF, Mexico.

Valdespino-Castillo PM, Alcántara-Hernández RJ, Alcocer J, Macek M, Merino-Ibarra M, Oseguera LA, Castillo FS, Cruz A, Gaona O, Falcón LI (2015) Diversidad genética de fosfatasas alcalinas para la remineralización de fósforo orgánico disuelto en comunidades microbianas de un lago sódico. In: Alcocer J., M. Merino-Ibarra, E. Escobar-Briones. (Ed). Tendencias de investigación en Limnología tropical: Perspectivas universitarias en Latinoamérica: (191-201). Asociación Mexicana de Limnología, A.C. e Instituto de Ciencias del Mar y Limnología, UNAM. México. ISBN 978-607-02-7199-1.

Valdespino-Castillo PM, Alcántara-Hernández RJ, Alcocer J, Merino-Ibarra M, Macek M, Falcón LI (2014) Alkaline phosphatases in microbialites and bacterioplankton from Alchichica soda lake, Mexico. FEMS Microbiol Ecol 90(2): 504–519

Valdespino-Castillo PM, Alcántara-Hernández RJ, Merino-Ibarra M, Alcocer J, Macek M, Moreno-Guillén OA, Falcón LI (2017) Phylotype dynamics of bacterial P utilization genes in microbialites and bacterioplankton of a monomictic endorheic lake. Microb Ecol 73(2): 296–309

Valdespino-Castillo PM, Hu P, Merino-Ibarra M, López-Gómez LM, Cerqueda-García D, González-De Zayas R, Pi-Puig T, Lestayo JA, Holman HY, Falcón LI (2018) Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. Front Microbiol 9: 510

Valdespino P M, Alcántara-Hernández R, Falcón LI (2019) Capítulo 6. Los texcales. In: Alcocer J. (Editor). 2019. Lago Alchichica: Una joya de biodiversidad. Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Iztacala y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. 244 pp. ISBN: 978-607-30-2278-1

Yanez-Montalvo A, Águila B, Gómez-Acata ES, Beltrán Y, Valdespino-Castillo PM, Centeno CM, Falcón L I (2020). Microbialites: what on earth? Frontiers for Young Minds 7(112): 59-66.

## 9.3 Stromatolites in crater-lake Alchichica and Bacalar lagoon.

# Chapter 6
# The Importance of the Rare Biosphere for Astrobiological Studies and the Diversification and Resilience of Life on Earth

**Jazmín Sánchez-Pérez, Juan Diego Villar, Nohely Alvarez-López, Bernardo Águila, Jhoselinne Buenrostro, Luis J. Chino-Palomo, Marisol Navarro-Miranda, Julián Felipe Cifuentes, Ana G. Cruz-Cruz, Benjamín Vega-Baray, Mariette Viladomat, Maria Kalambokidis, Luis E. Eguiarte, and Valeria Souza**

**Abstract** Let's travel in our mind eye to either early Earth or Mars: life had already evolved, but it was most probably patchy and hard to find; it was likely rare. We can even imagine the same process in exoplanets or moons, life starting as an organic oddity that eventually starts to evolve by Darwinian evolution, consuming resources to grow and reproduce. Under that scenario, we can speculate that after the last universal common ancestor, LUCA, diversified locally, life kept on diversifying in

J. Sánchez-Pérez · B. Águila · M. Navarro-Miranda · M. Viladomat · M. Kalambokidis · L. E. Eguiarte · V. Souza (✉)
Instituto de Ecología, Universidad Nacional Autónoma de México, UNAM, Mexico City, Mexico
e-mail: souza@unam.mx

J. D. Villar · J. F. Cifuentes
Pontificia Universidad Javeriana, Bogotá, DC, Colombia

N. Alvarez-López
Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, UNAM, Morelia, Michoacán, Mexico

J. Buenrostro · A. G. Cruz-Cruz
Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, UNAM, Tlalnepantla, Estado de México, Mexico

L. J. Chino-Palomo
Facultad de Medicina, Universidad Nacional Autónoma de México, UNAM, Mexico City, Mexico

B. Vega-Baray
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, UNAM, Mexico City, Mexico



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a multitude of non-abundant lineages that were locally adapted and perhaps may explain why we see such early radiation in the tree of life. Therefore, life started as a rare event and the rare biosphere, that represent most of the taxonomic and functional diversity in actual microbial communities, probably represents the way communities have organized themselves since the beginning of the conquest of the Earth. In this chapter, we will explain what it means to have a low abundance in a community, how we study rarity, and why it is relevant for astrobiology.

## 6.1 What Does It Means to Be Rare and Its Importance in Microbial Communities?

For most of the last century, the understanding of microbial diversity was still based solely on the identification of phenotypic and morphometric characteristics through the use of microscopy by culture-dependent experimentation (Lynch and Neufeld 2015). In recent years, sequencing of the 16S rRNA gene has been broadly used for the study of microbial communities. The first extensive work that incorporated these new methods took place in the North Atlantic Ocean in the early 2000s (Sogin et al. 2006). This study revealed that most microbial communities had a heterogeneous and asymmetric distribution of species, where relatively few dominant species coexist with a high number of low-abundant species (Sogin et al. 2006; Nemergut et al. 2011). It was therefore believed that niches with a wide range of microorganisms at low frequencies were rare, due to the low performance of these species in different environments. Today, nearly 15 years later, shotgun metagenomics has confirmed that in most communities, the existence of the rare biosphere is a general feature of microbial communities. This technique not only seeks to characterize species, but it also estimates the magnitude of the functional potential of the community, in particular showing us the potential genomes of non-cultivable and undescribed microorganisms (Scholz et al. 2012).

Due to the initial low coverage of the sample, in the first studies of microbial communities, the phylotypes with extremely small populations were found only in specific niches, such as in marine sponge parenchyma (Taylor et al. 2012), or in amphibian skin (Walke et al. 2014), among others. One group (Gobet et al. 2012) identified low-abundance bacterial taxa in coastal sands in response to nutrient stress. Likewise, another group (Bowen et al. 2009) found a large composition of low-abundance species within marsh sediments, which were highly distinct from samples of the nearby water column. As a result, it was erroneously assumed that these species were not well adapted and therefore not important for the function and structure of these communities. However, through further studies, this vision changed.

First, let's specify that there are different definitions and approaches to measuring rarity. Though rarity concepts were initially applied to plants and animals, they are nonetheless easily extrapolated to microorganisms. The first type of rarity

is defined by an organism with low abundance, which describes species that are found at a low frequency despite the size of the distribution area. Similarly, species can also be considered rare if it is only found in very specific niches; many of the existing examples include extremophilic microorganisms. Finally, there are organisms that are delimited by the geographical area in which they live, being almost endemic to a site.

The first kind of rarity we will explore is the low abundance. There are different ways in which a species can become low in abundance, such as through stochastic mechanisms that cause an abrupt change in the population (e.g., being uncompetitive in a new environment) or the result of a recent migration. In contrast, low abundance can also be a life strategy for many species either because that way they escape predators or because they use low abundance resources that may be provided by the rest of the community. For this reason, having low abundance allows for the survival of organisms in a trade-off mechanism, and it is very possible that rarity confers an adaptive advantage. This effect has been seen at the genotypic level with a phenomenon called frequency-dependent negative selection, where a genotype fitness is inversely proportional to its frequency in the population. This mechanism causes a genotype to initially increase its frequency when they are rare and reduce it when they become common. This process of succession of genotypes prevents one genotype from becoming fixed, thus maintaining diversity at different levels, as seen in mitochondria (Kazancıoğlu and Arnqvist 2014), clonal populations (Weeks and Hoffmann 2008), and among different cell strains (Minter et al. 2015). Moreover, predator and parasite dynamics can also modify the frequency of a bacterial population (Dybdahl and Lively 1998), where rare organisms are less predated compared to common ones (Winter et al. 2010).

The large proportion of rare species found in diverse ecosystems (Galand et al. 2009; Sogin et al. 2006; Lynch and Neufeld 2015) has led to further study the role of these organisms in microbial communities, as well as their ecological relevance. This rare biosphere, defined as all the organisms with a low frequency, is a relevant aspect of the microbial communities, as it includes the greatest amount of diversity within the community (Locey and Lennon 2016; Elshahed et al. 2008) and, in some cases, the largest proportion of the community (Sogin et al. 2006; Lynch and Neufeld 2015). Although, it is possible that the rarity of some species is a byproduct of processes like local extinction or low competitiveness in the community, many rare taxa could remain rare in the community over long periods (De Anda et al. 2018). This constancy indicates that they may play an important role in the community. If these organisms are not a stochastic product of migration or extinction and are preserved in microbial communities, then: What role do they play within the community?

One of the possible roles of the rare biosphere may be the contribution of diversity contained as a stability factor. Diversity is associated with the resilience of a system and functional capacity; so species perform functions in a given environmental condition. This aspect may be counterintuitive, given the high functional redundancy expected (Rousk et al. 2009). With so many

organisms sharing functions, the rare biosphere does not seem necessary to maintain them; however, species with functions considered irrelevant could become important in another environmental condition, either by adding a new trait to the community or participating in new interspecific interactions (Fetzer et al. 2015). The rare biosphere could, therefore, function as a seed bank. By preserving diversity, different metabolic functions can be conserved over time, becoming beneficial when different environmental conditions arise. This is why the community, through its seed bank, can react and effectively cope with different environmental fluctuations.

Furthermore, rare organisms seem to have a different role in the maintenance of communities compared to abundant species, since they react to different stress factors (Zhang et al. 2018). For example, one of the most important roles reported is the response to pollution by degradation of organic compounds (Zhang et al. 2018; Aanderud et al. 2015; Wang et al. 2017). The degradation of organic compounds is carried out through a network of complex metabolic pathways that are shared and coupled along with several taxa (Fuentes et al. 2014). Many of these functions are associated with the rare biosphere since experimental studies have reported that the capacity to degrade pollutants and toxins is reduced when rare microorganisms are removed from wastewater (Hernandez-Raquet et al. 2013).

Additionaly, rare biosphere, with its large repertoire of functions, seems to be part of the complex metabolic pathways associated with biogeochemical cycles. By providing new substrates, these cycles play a key role in the assembly of communities. Low-abundant species of green and purple sulfur bacteria appear to be crucial for the absorption of carbon and nitrogen in aquatic communities (Musat et al. 2008). Likewise, there are several examples associated with different nutrient cycles, such as the use of alternative carbon sources as a nutritional substrate (Hernandez-Raquet et al. 2013; Mallon et al. 2015; Matos et al. 2005; Franklin et al. 2001) and in the different phases of the nitrogen cycle, specifically in nitrification (Griffiths et al. 2004), nitrogen uptake (Musat et al. 2008), and denitrification (Philippot et al. 2013).

Another role of the rare biosphere in a micorbial community is against invasive species (Vivant et al. 2013). As a large part of the diversity, low-abundant organisms manage to occupy many niches within the community. Therefore, the limited availability of niches and resources succeeds in suppressing the invasion of new organisms into the community (van Elsas et al. 2012). The establishment of an invasive species is dependent on the amount of resources not consumed by local species, as well as the rate at which both consume the existing nutrients: in other words, who is consuming nutrients faster (Tilman 1999). Thus, when a niche is not occupied by the native species, the invasion by an exogenous species can be favored (Pedrós-Alió 2012). In this way, the greater the diversity and the greater the number of organisms that consume different things, the less likely it is that there will be excess resources to enable the entry and establishment of an invasive species.

## 6.2 Dynamism in the Community: Succession Between Common and Rare

Currently, metagenomic analyses have shown the presence of thousands of new species in all the explored ecosystems, which are in low abundance in the community (Pedrós-Alió 2012). This ability to describe the different species that inhabit a particular community made it possible to compare their presence and abundance in different locations. On some occasions, members of the abundant species in one community may be part of the rare biosphere in another. In this case, certain species may be abundant in a particular environment, but when dispersed to other communities, they become rare (Lynch and Neufeld 2015). The succession between abundance and rarity can then be determined by environmental conditions and biotic interactions.

The rare biosphere can respond to environmental fluctuations in two ways: (1) the "conditionally rare", whose frequency is determined by certain environmental factors, so when there is a favorable change in environmental conditions, its population frequency increases (Coveley et al. 2015; Sogin et al. 2006); and (2) the "functionally rare", who after a favorable environmental fluctuation, does not increase their growth rate but rather increase their transcriptional activity (Hausmann et al. 2019). Whatever their response is (increase in number or increase in activity), this low-abundant genetic diversity becomes crucial to maintaining ecosystem functions during stressful conditions (Shade et al. 2012). Therefore, understanding the process of succession and exchange between rare and common species would provide a great deal of information about the dynamics of the communities, including how they are structured, their response to environmental fluctuations, and the resilience of the community provided by the seed bank of low-abundance organisms that are preserved.

## 6.3 Who Came First? The Common or the Rare?

It is possible that the origin of rarity began shortly after the origin of life, the extraordinary event that took place on ancient Earth, from which emerged the last universal common ancestor (LUCA). Due to the abrupt changes that occurred during the Archean (Pester et al. 2010), an early diversification by adaptive events likely transpired on early Earth. Considering the impossibility of finding chemical fossils as old as LUCA, no direct evidence has been found to corroborate this diversification event, so, logic has to take the place of evidence to fill the void in data.

Following such logic, the idea that rare species have a key function in regulation, survival, biogeochemical coupling, and community assemblages across all ecosystems may offer indirect evidence for the role that rarity may have had in the early events of life's evolution. These key-stone roles of the rare biosphere support the

hypothesis that species rarity has an important role in the temporal stability of the long-term community assemblage (De Anda et al. 2018; Pepe-Ranney et al. 2012).

To elucidate the origin of the rare biosphere in the structuring of primitive communities, analogous communities must be studied. Thus, the study of microbial mats and stromatolites is an excellent approach to recapitulate the assembly of these ancestral niches. These complex communities are perhaps the oldest niches of which there is fossil evidence, showing evidence of life from more than 3.7 billion years ago (Brasier et al. 2002; Knoll et al. 2016).

Modern stromatolites and microbial mats contain finely structured communities of specialized organisms organized by micro-regions and functions. In the outermost layers of these structures live photosynthetic organisms, such as microalgae, cyanobacteria, and diatoms, while in inner layers live anoxygenic photosynthetic bacteria, sulfate-reducing bacteria, and methanogenic archaea. While in the deepest layers, there are a large variety of heterotrophic and anaerobic microorganisms (Wong et al. 2015). Stromatolites and microbial mats coexist with a great amount of diversity represented by the rare biosphere, and this seems to have been vital in giving them stability for millions of years, as they coped with great environmental changes. In this way, they probably worked as diversity hot spots and seed banks during catastrophic events for life (De Anda et al. 2018; Reeder and Knight 2009). Further studies are needed to determine the role of rare species in ancestral models, such as microbialites or microbial mats, to determine if rarity is the product of biotic/abiotic interactions or if rarity is the main determinant that gives stability to communities over long periods of time (Ruiz-González et al. 2019).

## 6.4 Rare Biosphere and Extremophiles: The Importance of Ancestral Niches for Astrobiological Studies

Today, through technological innovations, life has been found in environments previously considered uninhabitable (Singh et al. 2019). The microorganisms that inhabit these extreme environments are adapted to them, surviving conditions such as extreme temperatures (Galand et al. 2009; Goordial et al. 2016; Russell and Fukunaga 1990), high salt concentrations (Ventosa et al. 2015), low nutrient availability (Plümper et al. 2017; Drummond et al. 2015), high atmospheric pressure, or abnormal pH (Gaboyer et al. 2019; Florentino et al. 2016), among others. For astrobiology, these adaptations are particularly interesting, as they extend the possibilities of detecting life elsewhere in the cosmos.

The first evidence of life on Earth was found in the traces of isotopically light carbon in 4.1 billion-year-old rocks (Tashiro et al. 2017; Dodd et al. 2017); soon thereafter, fossils of microbial communities in stromatolites were discovered. In the case of astrobiology, the ancestral niches built by microbial communities that form stromatolites and microbial mats are a study model of interest (Merino et al. 2019; Clements 1936), especially because these communities are currently found in

extreme conditions where life can develop slowly, without the fast-growing algae shading the sun from the microbial phototrophs that are part of such communities. It is, therefore, possible to imagine that ancestral species were likely more similar to the communities of current extremophilic microorganisms, as similar environmental conditions existed on ancient Earth (Rampelotto 2013).

Since the only experiment of life that we know is on Earth, we assume that at the beginning of life, the organisms were associated to environmental conditions as high concentrations of salts, highly variable temperatures, high incidence of UV rays, and a lack of easily accessible nutritional/energy sources (Anbar and Knoll 2002). These conditions led organisms to specialize toward different carbon sources to survive, which subsequently caused a peak in diversification in the highly oligotrophic conditions of early life (Souza et al. 2012). For this reason, it is important to understand the modification and superposition of niches associated with historical, biotic, and abiotic factors, as well as the interactions that determine the abundance of a species in a given habitat.

With this in mind, there is a principle called resource allocation that is based on the costs of staying alive; any organism is limited by its resources, so its survival depends on how it uses resources for its maintenance cycle, growth, and reproduction (Sánchez-Silva et al. 2005). This principle is closely related to the adaptations acquired by extremophiles. Among these examples are thermophiles with specialized membrane lipids that make them more compact and resistant to denaturation at high temperatures (Stetter 1999), as well as psychrophiles who avoid cell freezing by utilizing a greater amount of unbranched fatty acids, an antifreeze intracellular fluid called DMC, and adaptations in metabolic regulation (D'Amico et al. 2006). Acidophiles, on the other hand, employ sulfate as an electron acceptor, as is the case in acid mine drainage and hydrothermal vents, maintaining homeostasis by ion pumping to regulate internal cell pressure with respect to their external environment (Mosier et al. 2013). Similarly, halophiles are osmotically protected with organic solutes, constantly keeping their cell membrane hydrated (Oren 2002). Extremophile studies have been carried out in various extreme environments on Earth, such as in Alaska, Antarctica, hydrothermal vents, hot springs, the ocean floor and its associated faults, and hypersaline lakes in Australia, among other model ecosystems (Galand et al. 2009; Goordial et al. 2016; Plümper et al. 2017; Salas et al. 2015). However, all that is known about the physiology of these extremophilic organisms is from a few cultivated strains; the role of the diverse rare biosphere in these extreme ecosystems largely remains unknown.

The interest of astrobiology in the rare biosphere is centered on the fact that many of the celestial bodies of which their environmental conditions have been described (e.g., the moon, Mars, Titan, and numerous asteroids) have extreme environments potentially habitable by organisms similar to those found among the rare terrestrial biosphere. Mars is particularly interesting as there is evidence that a few billion years ago its environmental conditions were very similar to those of early Earth (Zhang et al. 1993). So far, although the missions to Mars have focused more on chemical description rather than the search for life, the abiotic components discovered, such as the presence of surface solid water, underground liquid, and

methane (a simple molecule that on Earth is the product of methanogenesis of Archaea), support the idea of an ancestral or recent existence of extraterrestrial microbial life (Vago et al. 2017). Another candidate for this type of life is Titan, Saturn's largest moon, which has a dense and nitrogen-rich atmosphere. Titan's atmosphere is similar to Earth's in terms of its prevalence of nitrogen, and beneath the atmosphere, the moon likely has methane, ethane, and other hydrocarbons in the liquid state – icy and frozen on the surface but potentially warm in the layers closest to internal magmatic systems. This points to the presence of both atmospheric and surface components that could provide sufficient raw material for biosynthesis, enabling the possibility of an independent and persistent origin of life on Titan (Clarke and Ferris, 1997). On the contrary, even on planets without an atmosphere or with chemically unfavorable atmospheres for life, it is hypothesized that we could still discover microorganisms inhabiting their inner layers.

For the aforementioned reasons, among the most important discoveries for astrobiology in recent times are those that describe microbial life forms hidden in the depths of the Earth that thrive in conditions independent of those in the surface biosphere. According to Belilla and coauthors (2019), new organisms are found every year in environments that were previously thought to be uninhabitable: such as water systems within gold mines with estimated ages of tens of millions of years (Lin et al. 2006); hydrothermal vents deep in the sea (Sogin et al. 2006); lagoons with high temperatures, acidity, or alkalinity (Ward et al. 2006); hypersaline and oligotrophic lagoons (Souza et al. 2012); and even in places in particular the meteoric alkaline water that emanates from a well intersecting quartzite fractures kilometers underground (Moser et al. 2005). In such unique systems, it seems that finally the limits of life have been reached (Belilla et al. 2019) – environments where only organisms with unique abilities can inhabit, isolated and independent of Earth's biosphere and sunlight suggest the possibility of finding homologous isolated biospheres on other planets, proliferating despite hostile surface conditions.

However, access to samples and avoiding contamination represent some of the bigger challenges for the study of these microorganisms (Lin et al. 2006). In addition, being so adapted or specialized to a specific environment with unique metabolisms and interactions makes traditional isolation and culture methods impossible (Al-Awadhi et al. 2013; Zapka et al. 2017). It was not until the development of sequencing technologies and the emergence of metagenomic analysis methodologies that it was possible to study the rare biosphere in greater depth (Lynch and Neufeld 2015).

## 6.5 Cuatro Ciénegas, a Model for the Study of Rare Biosphere and Astrobiology

The extraordinary oasis in the Cuatro Ciénegas Basin (CCB) is particularly diverse (De Anda et al. 2018; Taboada et al. 2018), not only because each site that has been explored is unique with a large beta diversity (Souza et al. 2006), but also because

most of its microbial diversity is part of the rare biosphere, giving each microbial mat, stromatolite, sediment sample, water sample, or soil sample a unique signature (Lee et al. 2017; Souza et al. 2018). The reason behind such a high diversity is its ancestry and unique geological history, making CCB a true lost world that survived different mass extinction events throughout Earth's history. Yet, today in the Anthropocene, CCB risks destruction, as the wetlands are being drained for local agricultural use (Souza et al. 2018).

This "lost world" characteristic is not merely a metaphor; it is of great astrobiological relevance. The ancestral lineages survived in these extremely oligotrophic conditions, which allowed ancient microbes to co-evolve along with their community, resulting in an extraordinary codependency. For this reason, they survived: they could grow and reproduce given the "extended genotype" of the community. It is, therefore, possible that this feature can explain the resilience of life on Earth, helping us explore age-old questions: Does life exist on other planets or the moon? Perhaps, in other planetary systems? If so, how did it survive and diversify? It is highly probable that the survival of the fittest is a universal rule; however, the fittest, in this case, is the one that can coexist and collaborate, not the one that outcompetes the neighbor. May this be a lesson of survival for us all.

## 6.6 Conclusions

New sequencing technologies, such as the sequencing of highly variable regions of the 16S rRNA gene and shotgun metagenomics, have been crucial in understanding the ecology of microbial communities today. The use of these tools has made it increasingly possible to demonstrate the presence of rare species in communities, identify them, and understand their role within the community. These molecular tools are the reasons why the growth and contributions of research in this topic have led to a rethinking of the first hypotheses about microbial communities and their rare biosphere.

This rare fraction includes a large number of taxa, often exceeding those that dominate the community, maintaining a consistent composition despite its low abundance. There are, however, taxa that are unique to a site, providing a new collection of lineages. This rarity exists mainly due to the particular qualities of the habitat to which it belongs, as is the case with many extremophiles, or even the exclusivity of the biogeographic region where they are found, as is the case of several microorganisms in Cuatro Ciénegas. Regardless of the type of rarity being studied, there is still much to learn about the role of these organisms in microbial communities, as well as the evolutionary and ecological mechanisms that lead a species to be considered rare.

From a functional point of view, the role and importance of the rare biosphere in ecological niches warrant investigation. From the advantages for a species to remain at a low frequency to speculating on the reason why a community maintains its rare biosphere, there are many interesting avenues to explore further.

Thanks to the large amount of diversity found in a set of rare species, one of their most important roles is to function as a seed bank. Together, this pool of species conserves functions that may be necessary during an environmental change, ensuring the survival of the community. This, in turn, can increase the frequency of non-abundant species, making them part of the common biosphere, which creates a dynamic exchange between the abundant and rare. On the other hand, there are some occasions where the rare biosphere holds particular functions required in low abundance, which may play a key role in the regulation, assembly, and survival of the community. This dynamic exchange between rare and abundant, along with the presence of indispensable functions for community assembly and conservation, add a further degree of complexity to the interactions of the community, giving rise to new questions about the structure of microbial communities.

The diversity and ability to survive in extreme conditions make the rare biosphere a good study model for astrobiology. Through its role as a seed bank of metabolic functions, the rare biosphere offers functions of vital importance, such as the degradation of hydrocarbons or the fixation of other carbon sources. In this way, metabolic diversity enables their survival in extreme conditions, such as at high or low temperatures, high levels of salinity, or nutrient shortages. Additionally, these types of conditions are those that most closely resemble a primitive environment on Earth or the environments of other planets. Because of this, the rare biosphere is an excellent study model for investigating the origins of life on Earth, as well as the possibility of life emerging elsewhere.

One hypothesis about the first forms of life after LUCA, is that life could continue due to the rapid diversification that filled the many available niches. It is possible that at the beginning of this diversification, there were no abundant species; rather, there was a large diversity of functions and a low population abundance. In this way, it is parsimonious to suggest that at the beginning, there were generalist organisms that performed different functions, that rapidly evolve into the current diversity we see today. However, given that there is a diversity of functions and most species are specialists, the possibility of their survival alone is lower, even when considering that there were likely low levels of competition. The rare biosphere can then be closely linked to life in a community, with high levels of cooperation. In this way, specialists were able to cover all types of nutrient cycling and be able to survive together as a community, despite abrupt environmental changes suffered by Earth throughout its various geological ages. Thus, evolving communities in stromatolites or microbial mats may have been the key to the survival of life. Similarly, microbes that did not live in communities could have survived because they took refuge in places where their ancestral niches were conserved, as can be observed in hydrothermal vents, deep rocks, blocks of permafrost, and more. These microorganisms are extremophiles, which due to the specificity of the habitat in which they are found are considered rare.

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## References

Aanderud ZT, Jones SE, Fierer N, Lennon JT (2015) Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. Front Microbiol 6:24

Al-Awadhi H, Dashti N, Khanafer M, Al-Mailem D, Ali N, Radwan S (2013) Bias problems in culture-independent analysis of environmental bacterial communities: a representative study on hydrocarbonoclastic bacteria. Springerplus 2(1):369

Anbar AD, Knoll AH (2002) Proterozoic Ocean chemistry and evolution: a bioinorganic bridge? Science 297:1137–1142

Belilla J, Moreira D, Jardillier L, Reboul G, Benzerara K, López-García JM, Bertolino P, López-Archilla AI, López-García P (2019) Hyperdiverse archaea near life limits at the polyextreme geothermal Dallol area. Nat Ecol Evol 3:1552–1561

Bowen JL, Crump BC, Deegan LA, Hobbie JE (2009) Salt marsh sediment bacteria: their distribution and response to external nutrient inputs. ISME J 3:924–934

Brasier MD, Green OR, Jephcoat AP, Kleppe AK, Van Kranendonk MJ, Lindsay JF, Steele A, Grassineau NV (2002) Questioning the evidence for Earth's oldest fossils. Nature 416(6876):76–81

Clarke DW, Ferris JP (1997) Chemical evolution on Titan: comparisons to the prebiotic earth. Orig Life Evol Biosph 27:225–248

Clements FE (1936) Nature and structure of the climax. J Ecol 24:252

Coveley S, Elshahed MS, Youssef NH (2015) Response of the rare biosphere to environmental stressors in a highly diverse ecosystem (Zodletone spring, OK, USA). Peer J 2015

D'Amico S, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7(4):385–389

De Anda V, Zapata-Peñasco I, Blaz J, Poot-Hernandez AC, Contreras-Moreira B, Hernandez Rosales M, Eguiarte LE, Souza V (2018) Understanding the mechanisms behind the response to environmental perturbation in microbial mats: a metagenomic-network based approach. Front Microbiol 9:2606

Dodd MS, Papineau D, Grenne T, Slack JF, Rittner M, Pirajno F, O'Neil J, Little CT (2017) Evidence for early life in Earth's oldest hydrothermal vent precipitates. Nature 543:60–64

Drummond JBR, Pufahl PK, Porto CG, Carvalho M (2015) Neoproterozoic peritidal phosphorite from the Sete Lagoas formation (Brazil) and the Precambrian phosphorus cycle. Sedimentology 62:1978–2008

Dybdahl MF, Lively CM (1998) Host-parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. Evolution 52(4):1057–1066

Elshahed MS, Youssef NH, Spain AM, Sheik C, Najar FZ, Sukharnikov LO, Roe BA, Davis JP, Schloss PD, Bailey VL, Krumholz LR (2008) Novelty and uniqueness patterns of rare members of the soil biosphere. Appl Environ Microbiol 74:5422–5428

Fetzer I, Johst K, Schäwe R, Banitz T, Harms H, Chatzinotas A (2015) The extent of functional redundancy changes as species' roles shift in different environments. Proc Natl Acad Sci U S A 112(48):14888–14893

Florentino AP, Weijma J, Stams AJM, Sánchez-Andrea I (2016) Ecophysiology and application of acidophilic sulfur-reducing microorganisms. In: Rampelotto PH (ed) Biotechnology of extremophiles. Springer, Cham, pp 141–175

Franklin RB, Garland JL, Bolster CH, Mills AL (2001) Impact of dilution on microbial community structure and functional potential: comparison of numerical simulations and batch culture experiments. Appl Environ Microbiol 67:702–712

Fuentes S, Méndez V, Aguila P, Seeger M (2014) Bioremediation of petroleum hydrocarbons: catabolic genes, microbial communities, and applications. Appl Microbiol Biotechnol 98:4781–4794

Gaboyer F, Burgaud G, Edgcomb V (2019) The deep subseafloor and biosignatures. In: Cavalazzi B, Westall F (eds) Biosignatures for Astrobiology. Springer, Cham, pp. 87–109

Galand PE, Casamayor EO, Kirchman DL, Lovejoy C (2009) Ecology of the rare microbial biosphere of the Arctic Ocean. Proc Natl Acad Sci U S A 106:22427–22432

Gobet A, Böer SI, Huse SM, Van Beusekom JE, Quince C, Sogin ML, Boetius A, Ramette A (2012) Diversity and dynamics of rare and of resident bacterial populations in coastal sands. ISME J 6(3):542–553

Goordial J, Davila A, Lacelle D, Pollard W, Marinova MM, Greer CW, DiRuggiero J, McKay CP, Whyte LG (2016) Nearing the cold-arid limits of microbial life in permafrost of an upper dry valley, Antarctica. ISME J 10(7):1613–1624

Griffiths BS, Kuan HL, Ritz K, Glover LA, McCaig AE, Fenwick C (2004) The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. Microb Ecol 47(1):104–113

Hausmann B, Pelikan C, Rattei T et al (2019) Long-term transcriptional activity at zero growth of a cosmopolitan rare biosphere member. MBio 10

Hernandez-Raquet G, Durand E, Braun F, Cravo-Laureau C, Godon JJ (2013) Impact of microbial diversity depletion on xenobiotic degradation by sewage-activated sludge. Environ Microbiol Rep 5:588–594

Kazancıoğlu E, Arnqvist G (2014) The maintenance of mitochondrial genetic variation by negative frequency-dependent selection. Ecol Lett 17:22–27. https://doi.org/10.1111/ele.12195

Knoll AH, Bergmann KD, Strauss JV (2016) Life: the first two billion years. Philos Trans R Soc B Biol Sci 371(1707):20150493

Lee ZM, Poret-Peterson AT, Siefert JL, Kaul D, Moustafa A, Allen AE, Dupont CL, Eguiarte LE, Souza V, Elser JJ (2017) Nutrient stoichiometry shapes microbial community structure in an evaporitic shallow pond. Front Microbiol 8:949

Lin LH, Wang PL, Rumble D, Lippmann-Pipke J, Boice E, Pratt LM, Lollar BS, Brodie EL, Hazen TC, Andersen GL, DeSantis TZ (2006) Long-term sustainability of a high-energy, low-diversity crustal biome. Science 314(5798):479–482

Locey KJ, Lennon JT (2016) Scaling laws predict global microbial diversity. Proc Natl Acad Sci U S A 113:5970–5975

Lynch MDJ, Neufeld JD (2015) Ecology and exploration of the rare biosphere. Nat Rev Microbiol 13:217–229

Mallon CA, Poly F, Le Roux X, Marring I, van Elsas JD, Salles JF (2015) Resource pulses can alleviate the biodiversity-invasion relationship in soil microbial communities. Ecology 96:915–926

Matos A, Kerkhof L, Garland JL (2005) Effects of microbial community diversity on the survival of Pseudomonas aeruginosa in the wheat rhizosphere. Microb Ecol 49:257–264

Merino N, Aronson HS, Bojanova DP, Feyhl-Buska J, Wong ML, Zhang S, Giovannelli D (2019) Living at the extremes: extremophiles and the limits of life in a planetary context. Front Microbiol 10:780

Minter EJA, Watts PC, Lowe CD, Brockhurst MA (2015) Negative frequency-dependent selection is intensified at higher population densities in protist populations. Biol Lett 11:20150192

Moser DP, Gihring TM, Brockman FJ et al (2005) Desulfotomaculum and Methanobacterium spp. dominate a 4- to 5-kilometer-deep fault. Appl Environ Microbiol 71:8773–8783

Mosier AC, Justice NB, Bowen BP et al (2013) Metabolites associated with adaptation of microorganisms to an acidophilic, metal-rich environment identified by stable-isotope-enabled metabolomics. MBio 4:e00484-12

Musat N, Halm H, Winterholler B, Hoppe P, Peduzzi S, Hillion F, Horreard F, Amann R, Jørgensen BB, Kuypers MM (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. Proc. Natl. Acad. Sci USA 105:17861–17866

Nemergut DR, Costello EK, Hamady M, Lozupone C, Jiang L, Schmidt SK, Fierer N, Townsend AR, Cleveland CC, Stanish L, Knight R (2011) Global patterns in the biogeography of bacterial taxa. Environ Microbiol 13:135–144

Oren A (2002) Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. J Ind Microbiol Biotechnol 28(1):56–63

Pedrós-Alió C (2012) The rare bacterial biosphere. Annu Rev Mar Sci 4:449–466

Pepe-Ranney C, Berelson WM, Corsetti FA, Treants M, Spear JR (2012) Cyanobacterial construction of hot spring siliceous stromatolites in Yellowstone National Park. Environ Microbiol 14(5):1182–1197

Pester M, Bittner N, Deevong P, Wagner M, Loy A (2010) A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland. ISME J 4(12):1591

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Plümper O, King HE, Geisler T, Liu Y, Pabst S, Savov IP, Rost D, Zack T (2017) Subduction zone forearc serpentinites as incubators for deep microbial life. Proc Natl Acad Sci U S A 114:4324–4329

Rampelotto PH (2013) Extremophiles and extreme environments. Life 3:482–485

Reeder J, Knight R (2009) The "rare biosphere": a reality check. Nat Methods 6:636–637

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol 75:1589–1596

Ruiz-González C, Logares R, Sebastián M, Mestre M, Rodríguez-Martínez R, Galí M, Sala MM, Acinas SG, Duarte CM, Gasol JM (2019) Higher contribution of globally rare bacterial taxa reflects environmental transitions across the surface ocean. Mol Ecol 28:1930–1945

Russell NJ, Fukunaga N (1990) A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. FEMS Microbiol Lett 75:171–182

Salas EC, Bhartia R, Anderson L et al (2015) In situ detection of microbial life in the deep biosphere in igneous ocean crust. Front Microbiol 6:1260

Sánchez-Silva M, Daniels M, Lleras G, Patino D (2005) A transport network reliability model for the efficient assignment of resources. Transp Res Part B Methodol 39:47–63

Scholz MB, Lo C-C, Chain PSG (2012) Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. Curr Opin Biotechnol 23:9–15

Shade A, Peter H, Allison SD, Baho D, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JB, Matulich KL (2012) Fundamentals of microbial community resistance and resilience. Front Microbiol 3:417

Singh P, Jain K, Desai C, Tiwari O, Madamwar D (2019) Microbial community dynamics of extremophiles/extreme environment. In: Das S, Dash HR, Microbial diversity in the genomic era. Academic Press, London, pp 323–332

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere.". Proc Natl Acad Sci 103:12115–12120

Souza V, Espinosa-Asuar L, Escalante AE, Eguiarte LE, Farmer J, Forney L, Lloret L, Rodríguez-Martínez JM, Soberón X, Dirzo R, Elser JJ (2006) An endangered oasis of aquatic microbial biodiversity in the Chihuahuan desert. Proc Natl Acad Sci U S A 103:6565–6570

Souza V, Siefert JL, Escalante AE, Elser JJ, Eguiarte LE (2012) The Cuatro Ciénegas basin in Coahuila, Mexico: an astrobiological Precambrian park. Astrobiology 12(7):641–647

Souza V, Moreno-Letelier A, Travisano M, Alcaraz LD, Olmedo G, Eguiarte LE (2018) The lost world of Cuatro Cienegas Basin, a relictual bacterial niche in a desert oasis. elife 7:e38278

Stetter KO (1999) Extremophiles and their adaptation to hot environments. FEBS Lett 452:22–25

Taboada B, Isa P, Gutiérrez-Escolano AL, Del Ángel RM, Ludert JE, Vázquez N, Tapia-Palacios MA, Chávez P, Garrido E, Espinosa AC, Eguiarte LE (2018) The geographic structure of viruses in the Cuatro Ciénegas Basin, a unique oasis in northern Mexico, reveals a highly diverse population on a small geographic scale. Appl Environ Microbiol 84(11):e00645–e00618

Tashiro T, Ishida A, Hori M, Igisu M, Koike M, Méjean P, Takahata N, Sano Y, Komiya T (2017) Early trace of life from 3.95 Ga sedimentary rocks in Labrador, Canada. Nature 549(7673):516–518

Taylor MW, Tsai P, Simister RL, Deines P, Botte E, Ericson G, Schmitt S, Webster NS (2012) 'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments. ISME J 7(2):438

Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80:1455–1474

Vago JL, Westall F, Coates AJ, Jaumann R, Korablev O, Ciarletti V, Mitrofanov I, Josset JL, De Sanctis MC, Bibring JP, Rull F (2017) Habitability on early Mars and the search for biosignatures with the ExoMars Rover. Astrobiology 17(6–7):471–510

van Elsas JD, Chiurazzi M, Mallon CA, Elhottovā D, Krištůfek V, Salles JF (2012) Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc Natl Acad Sci U S A 109:1159–1164

Ventosa A, de la Haba RR, Sánchez-Porro C, Papke RT (2015) Microbial diversity of hypersaline environments: a metagenomic approach. Curr Opin Microbiol 25:80–87

Vivant AL, Garmyn D, Maron PA, Nowak V, Piveteau P (2013) Microbial diversity and structure are drivers of the biological barrier effect against *Listeria monocytogenes* in soil. PLoS One 8(10):e76991

Walke JB, Becker MH, Loftus SC, House LL, Cormier G, Jensen RV, Belden LK (2014) Amphibian skin may select for rare environmental microbes. ISME J 8(11):2207–2217

Wang Y, Hatt JK, Tsementzi D, Rodriguez-R LM, Ruiz-Pérez CA, Weigand MR, Kizer H, Maresca G, Krishnan R, Poretsky R, Spain JC (2017) Quantifying the importance of the rare biosphere for microbial community response to organic pollutants in a freshwater ecosystem. Appl Environ Microbiol 83(8):e03321-16

Ward DM, Bateson MM, Ferris MJ, Kühl M, Wieland A, Koeppel A, Cohan FM (2006) Cyanobacterial ecotypes in the microbial mat community of mushroom spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. Philos T R Soc B 361(1475):1997–2008

Weeks AR, Hoffmann AA (2008) Frequency-dependent selection maintains clonal diversity in an asexual organism. Proc Natl Acad Sci U S A 105:17872–17877

Winter C, Bouvier T, Weinbauer MG, Thingstad TF (2010) Trade-offs between competition and defense specialists among unicellular planktonic organisms: the "killing the winner" hypothesis revisited. Microbiol Mol Biol Rev 74:42–57

Wong HL, Smith D-L, Visscher PT, Burns BP (2015) Niche differentiation of bacterial communities at a millimeter scale in Shark Bay microbial mats. Sci Rep 5:15607

Zapka C, Leff J, Henley J, Tittl J, De Nardo E, Butler M, Griggs R, Fierer N, Edmonds-Wilson S (2017) Comparison of standard culture-based method to culture-independent method for evaluation of hygiene effects on the hand microbiome. MBio 8(2):e00093–e00017

Zhang MHG, Luhmann JG, Bougher SW, Nagy AF (1993) The ancient oxygen exosphere of Mars: implications for atmosphere evolution. J Geophys Res Planets 98:10915–10923

Zhang Y, Wu G, Jiang H, Yang J, She W, Khan I (2018) Abundant and rare microbial biospheres respond differently to environmental and spatial factors in Tibetan Hot Springs. Front Microbiol 9:2096

## 9.4 The microbiome of microbialites in Bacalar lagoon.

PLOS ONE

RESEARCH ARTICLE

# The microbiome of modern microbialites in Bacalar Lagoon, Mexico

**Alfredo Yanez-Montalvo[1,2], Selene Gómez-Acata[1], Bernardo Águila[1], Héctor Hernández-Arana[2], Luisa I. Falcón[1]***

**1** UNAM, Instituto de Ecología, Parque Científico y Tecnológico de Yucatán, Sierra Papacal, Yucatán, México, **2** El Colegio de la Frontera Sur Unidad Chetumal, Chetumal, Quintana Roo, Mexico

* falcon@ecologia.unam.mx



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## Abstract

Microbialites are highly diverse microbial communities that represent modern examples of the oldest life forms, stromatolites (dated >3.7 Ga). Bacalar Lagoon, in Mexico, harbors the largest freshwater microbialite occurrences of the world; yet diverse anthropogenic activities are changing the oligotrophic conditions of the lagoon. The objective of this work was to perform a spatial exploration of the microbialites of Bacalar Lagoon, analyze their prokaryote diversity, following a high throughput sequencing approach of the V4 region of the 16S rDNA, and correlate to the environmental parameters that influence the structure of these communities. The results indicate the presence of microbialites throughout the periphery of the lagoon. The microbiome of the microbialites is composed primarily of Proteobacteria (40–80%), Cyanobacteria (1–11%), Bacteroidetes (7–8%), Chloroflexi (8–14%), Firmicutes (1–23%), Planctomycetes (1–8%), and Verrucomicrobia (1–4%). Phylogenetic distance analyses suggests two distinct groups of microbialites associated with regions in the lagoon that have differences in their environmental parameters, including soluble reactive silicate (in the north), bicarbonates and available forms of nitrogen (ammonium, nitrates and nitrites) (in the south). These microbialite groups had differences in their microbiome composition associated to strong anthropogenic pressure on water quality (agriculture, landfill leachate, lack of water treatment infrastructure and intensive tourism), which were related to a loss of microbial diversity.

## Introduction

Bacteria and Archaea (prokaryotes) represent the most diverse and abundant organisms on the planet [1]. They are involved in maintaining and controlling biogeochemical cycling of the fundamental elements of life (H, C, N, O, S and P) [2]. Understanding the multiple ecological and evolutionary processes that are related to the distribution and structure of prokaryote diversity at the local and global scales is a main interest of microbial ecology [3–4]. The formation of biogeographic distribution patterns in prokaryotes is determined by environmental heterogeneity (ecological factor) and dispersion (historical factor) [5]. At the local scale, factors that include pH, habitat heterogeneity, system productivity, and more recently, human

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alteration of the habitat, are contributing to shape prokaryote diversity and structure [6–8]. Industrial activities which modify land use including agriculture, mining and wastewater discharges cause direct changes in the structure of microbial communities [9–10]. Studies based on environmental DNA sequencing suggest that prokaryotes are biological monitors of anthropogenic environmental change [11–12].

Knowledge of the factors that define communities, including the interactions that shape community structure and dynamics, within a certain environmental matrix, are fundamental to understand shifts related to habitat transformation [13]. Ecological analysis based on the spatial distribution of diversity (α, β, γ) [14] is the basis to defining the emergent properties of communities [15], and a relevant tool to monitor ecosystem function [16–18].

Microbialites are diverse microbial communities that precipitate carbonates, silicates and sulfate minerals, through the interaction of their metabolisms with the environment [19–23]. Fossil microbialites (stromatolites) have been dated in ~3.5–3.7 Ga years [24–26] and represent the oldest evidence of life on Earth. Microbialites are present in modern aquatic environments, both freshwater and marine. Microbialites can be found in saline marine environments such as the Hamelin Pool of Shark Bay (Western Australia), Cayo Cocos (Cuba) and in Highborne Cay (Bahamas); in lacustrine environments including Pavilion Lake and Clinton Creek (Canada), Lake Tanganyika (Africa), Lake Salda Golu (Turkey), Cuatro Cienegas and Lake Alchichica (Mexico), Ruidera Pools (Spain) and Great Salt Lake (GSL) (United States) [13, 22–23, 27, 83], among others.

The genetic composition of microbialites has been studied with different approaches, and Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes and Chloroflexi are their main constituents [22, 27]. Moreover microbialites from Pavilion lake have a high abundance of Proteobacteria (Alphaproteobacteria and Deltaproteobacteria) and Acidobacteria, principally photoheterotrophic *Rhodobacter*, *Rhodomicorbium*, *Phodopseudomonas* and *Rhodospirrillum*, heterotrophic *Sphingomonas*, nitrogen-fixing *Bradyrhizobium* and *Rhizobium*, dissimilatory sulfate reducing *Desulfobacterium* and *Desulfovibrio*, heterotrophic *Myxococcus*, Cyanobacteria such as *Anabaena*, *Lyngbya*, *Nostoc* and *Oscillatoria* [22]. While microbialites from hypersaline Storr's lake (Bahamas) have high abundance of Chloroflexi, Deltaproteobacteria and Spirochaetes [28]. Microbialites from Great Salt Lake are dominated by Alteromonadales, Oceanospirillales, Flavobacterales, Cytophagales, Chlorococcales and Chromatiales, with archaeal represented by *Halorubrum* sp., Halobacterales and Haloferacales [13]. In Mexico, there are several environments that harbor microbialites which share similar genetic composition at the phylum level, although each microbialite is different at the species level. We now know that microbialites in Mexico show differences in their genetic composition related to geographic region and that conductivity, concentration of nitrate and temperature are among the variables that structure their composition [8].

Microbialites constitute complex communities in which all pathways needed for biomass formation and recycling are present. Nitrogen fixation associated to heterocystous cyanobacteria, which can couple this pathway with oxygenic photosynthesis, is a fundamental metabolism in microbialites [29–30]. Cyanobacteria are fundamental microbialite builders, through the coupling of photosynthesis, nitrogen fixation and Extracellular Polymeric Substance (EPS) matrix synthesis [31–32]. Aerobic and anaerobic heterotrophic bacteria are associated with the cyanobacterial biofilm and contribute to biomass cycling [22, 33]; further, the role of sulfur-bacteria has been related to mineral precipitation in microbialites [34–36].

Bacalar Lagoon has been documented as the largest freshwater microbialite ecosystem in the world [37–38]. Several authors have studied Bacalar microbialites [23, 30, 37–39], but have focused on specific areas of the lagoon. Bacalar microbialites have been described as actively fixing $N_2$ during the daytime [30], and harbor a vast diversity of cyanobacteria and sulfur

bacteria [38–39]. In this study we wanted to answer if habitat transformation of Bacalar Lagoon influences microbialite community structure and composition. We characterized the microbiome of Bacalar microbialites throughout the lagoon and analyzed if there are structuring effects on their prokaryote composition related to environmental variables, following a next-gen sequencing approach of the V4 hypervariable region of the 16S rDNA gene.

## Materials and methods

### Study site

Bacalar is a karstic and freshwater lagoon located in the southeast of Quintana Roo, Mexico in the Yucatan peninsula (Fig 1, S1 Table). The lagoon is a geological fault due to its orientation and shape. Bacalar Lagoon, has been considered an oligotrophic system due to the low concentration of nitrogen (N) and phosphorus (P), and is part of the Transverse Coastal Corridor, a complex water system, where a series of karst freshwater lakes, lagoons and estuaries are connected through underground water flows [40]. Temperature and pH range between 28–31˚C and 7.7–8.2, respectively [27, 39, 41]. Hydrogeochemistry is characterized by higher concentration of calcium ($Ca^{2+}$) [37] and sulfate ($SO_4^{2-}$), compared to other karstic lagoons in the south of the Yucatan peninsula [39]. Bicarbonate concentration ($HCO_3^-$) in southern Bacalar Lagoon, is higher than marine levels, due to the presence of five sinkholes (locally known as "cenotes") that are sites of groundwater intrusion to the lagoon [37–38, 41]. Bacalar Lagoon has a north-south and south-north water circulation pattern, that converges towards the middle of the lagoon, and flows towards the Bay of Chetumal to the East [38].

### Sample collection

Microbialites were collected in 15 sites along the western shore of Bacalar Lagoon along a north-south gradient. Cores of approximately 2.5 cm in diameter were sampled in duplicates from individual microbialite heads, and three to five individuals per site were sampled (Fig 1, S1 Table). Samples were taken with gloves and sterile material to avoid cross-site contamination. Collection was carried out during the spring of 2018. Samples were stored at 4˚C during transport to the laboratory where they remained frozen at -70˚C until processed. All microbialite samples were carried under collector permit PPF/DGOPA-113/14 awarded by SEMARNAT, Mexico. Field studies did not involve endangered or protected species.

Three water samples (500 mL) were taken at each sampling point using Nalgene bottles, previously washed with 15% HCl, and were filtered (0.22 μm Millipore membrane) *in situ* and stored at 4˚C for dissolved nutrients analysis. *In situ* conductivity, pH and temperature were measured using a YSI Professional handheld (YSI model Pro 30) and pH-meter (Hanna HI 9146).

The degree of tourist visitation per site was assessed during the sampling with interviews to locals. A high level represents sites that have tourism throughout the year; medium represents sites that only have tourists during holiday seasons; low represent sites that are seldom visited by tourists.

### Nutrient analysis and statistical analysis of environmental variables

Nutrient measurements were done with colorimetric methods using a UV-visible spectrophotometer (SHIMADZU, Model UV-1700). Ammonium, NOx (nitrites and nitrates), soluble reactive silicate (SRSi) and soluble reactive phosphorus (SRP), were analyzed [42–43]. All analyses were performed in triplicate in the Chemistry Laboratory at ECOSUR, Chetumal, Mexico.

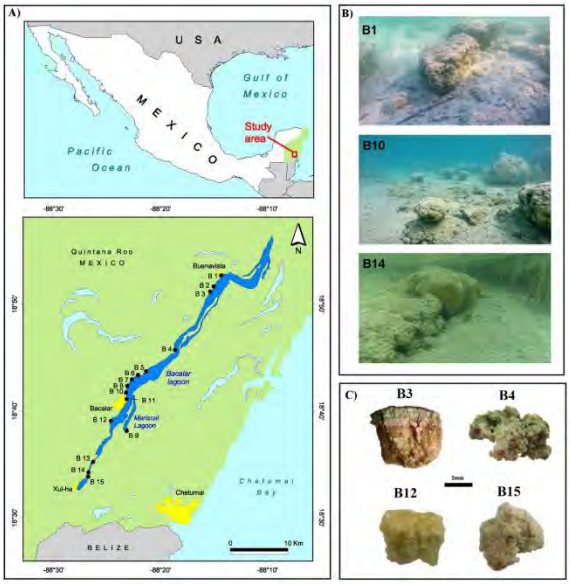


**Fig 1.** a) Location of Bacalar Lagoon, Mexico and microbialite sampling sites for this study; b) Example of morphology of microbialites from Bacalar Lagoon; c) cross-section of microbialites from Bacalar Lagoon, indicating sampling sites.

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Principal Component Analysis (PCA) was used to describe the relationship between the chemical variables measured in the water with each sampling location. The compiled data set representing the environmental variables analyzed in this study was transformed into a "site x variable" matrix. Euclidean distance and ordinations were plotted with FactoMineR and factoextra in Rstudio [44].

## Biogeochemical analysis

The biogeochemical analyzes of the microbialite fabric were carried out with different methodologies. For total carbon and nitrogen we used a soil analyzer (Thermo Scientific Flash 2000). Barnard's calcimeter method [45] was used for inorganic carbon analysis by the determination of calcium carbonates. Total phosphorus was measured through solubilization by acid digestion ($HNO_3/HClO_4$). Available phosphorus was determined with the Olsen method [46]. Organic matter and organic carbon were determined with Walkley and Black method [47]. Determinations were done in the Soils and Plants Analysis Laboratory, ECOSUR, San Cristóbal.

## X-Ray Diffraction (XRD)

For XRD analyses samples were cold dried (10˚C), homogenized with a pestle and agate mortar and sieved through a mesh < 75μm. The measurement was made in the angular interval 2θ from 5˚ to 80˚ in step scanner with a "step scan" of 0.003˚ and an integration time of 40 sec per step, using double-side aluminum holders (unoriented fractions). Each diffractogram was

obtained in a diffractometer (Empyrean) equipped with a Ni filter, a monochromator, a thin tube focus copper and PIXcel3D detector. The diffraction patterns were analyzed with the HighScore software (version 4.5) with reference patterns from the ICDDPDF-2 and ICSD databases. All determinations were done in the X-Ray Diffraction Laboratory, Institute of Geology, UNAM.

## Total DNA extraction and 16S rDNA amplification

DNA extractions of microbialite samples (0.25 g) were done in triplicate using the DNeasy PowerSoil® Kit (Qiagen) following the manufacturer's instructions. Amplifications of the 16S rDNA V4 region were done following an established protocol [48]. Each sample was amplified in three independent PCR reactions. PCR conditions were: 98˚C for 30 s followed by 35 cycles of 95˚C for 30 s, 52˚C for 40 s, and 72˚C for 90 s, and a final elongation step of 12 min at 72˚C, then kept at 4˚C. PCR products were pooled and purified with Ampliclean carboxyl-coated magnetic beads (NimaGen, NDL). The purified amplicon library was quantified with a QUBIT fluorometer (Promega, USA). The amplicon library with 20 ng/μl sample was sequenced on an Illumina MiSeq 2 x 300 platform (Yale Center for Genome Analysis, CT, USA).

## Analysis of Illumina 16S rDNA V4 sequences

The 16S rDNA V4 sequences of 90 samples of microbialites collected throughout Bacalar Lagoon, were deposited in the GenBank under BioProject PRJNA 550210. In addition, the data used during the analyses are available in the Open Science Framework: https://osf.io/zme9y/. Sequences were denoised, chimera and singletons were removed, then sequences were assigned into ASVs (Amplicon Sequence Variants) in QIIME2 (v.2018.6) [49] and truncated at position 200 with DADA2 [50] using the plugin *qiime dada2 denoised-paired*. ASVs representing less than 0.01% of the sequences across the dataset were eliminated. Taxonomy was resolved using the SILVA database (release 132–99% OTUs, 515–806 region), with the *feature-classifier classify-consensus-vsearch* (v2.9.0) plugin [51]. Mitochondrial and chloroplast sequences were filtered out from the feature table before rarefaction. Rarefaction was done at 10,000 ASVs per sample, resulting in the removal of 14 samples that had less than 9,000 sequences. The total dataset includes 90 samples for 15 sites.

After QIIME analyses, all sequence data were analyzed using multivariate correlational and ordination methods in the R statistical environment (version 3.6.2), for this, we used Phyloseq R [52]. We considered using the R markdown document that contains the complete commands for the analysis which is available here: https://github.com/YanezAlfredo/The-microbiome-microbialites-in-Bacalar-Lagoon-Mexico.git. The weighted Unifrac matrix was used to calculate the dissimilarity between the groups (*D*). The associations between environment and prokaryote community structure from different sites are shown using a constrained multidimensional scaling by Canonical Analysis of Principal Coordinates (CAP) based on weighted unifrac distance dissimilarity [53]. The differences between regions in the lagoon was analyzed using the PERMANOVA approach [54], implemented in "vegan" as the ADONIS function using R package.

The ASV table was used to construct the biological matrix of genetic diversity based on 16S rDNA taxonomy. The alpha diversity indices such as species and Shannon index were calculated with the R package "vegan" [https://cran.r-project.org, https://github.com/vegandevs/vegan]. Wilcoxon tests were used to test for group differences in microbial diversity. A Venn diagram was created to compare the North-Center and South-Center regions obtained by unifrac weighted analysis, using the DrawVenn tool available online (http://bioinformatics.psb.

ugent.be/webtools/Venn/).The total sum of-squares of the community composition matrix was partitioned into additive components of species (ASVs) to obtain their contributions to beta diversity (SCBD) and the local contributions of individual sampling units to beta diversity (LCBD) [55]. Following Legendre and De Cáceres [56], we first transformed (Hellinger) the species abundance per site matrix and then we calculated multiple-site β-diversity indices (betapart) [57]; LCBD and SCBD indices were ran in adespatial [58], ade4 [59] and with beta.div functions in "vegan" [56].

## Results

### The physicochemical environment surrounding microbialites in Bacalar Lagoon

The survey conducted in Bacalar Lagoon suggested an overall north-south gradient defined by higher conductivity and SRSi in the north, while the southern region had higher values of bicarbonate and available forms of nitrogen (ammonium, NOx), with similar values of sulfate and calcium throughout the lagoon (S1 Table). In the PCA, two general gradients were observed in Bacalar Lagoon. A north-south gradient based on PC1, where the following correlations were made: SRSi to $HCO_3^-$–NOx ($NO_3^-$ + $NO^-_2$) with correlation coefficient values of -0.52 to 0.49, 0.52 respectively. The second gradient is interpreted on PC2, from the central zone towards the north with variables such as Ca-$NH_4^+$-$SO_4^{2-}$ to Conductivity-SRP, correlation coefficient values of -0.55, -0.042, -0.34 to 0.42, 0.45, respectively. None of the variables represented a strong component to explain the ordination (Fig 2).

Nonetheless, certain variables separated these regions, including bicarbonates, NOx ($NO_3^-$ + $NO^-_2$) and ammonium in the center-southern sampling sites (B 12–15) which increase near urban areas (Fig 2). Likewise, an analysis of previous research in the lagoon showed that concentrations of nitrates and ammonium increased two orders of magnitude between 2008 and 2018 in the southern sampling sites [8, 30, 60, **this study**] (Table 1).

### Microbialite mineral and biogeochemical composition

Bacalar Lagoon microbialites were composed mainly of calcite ($CaCO_3$) (~97%) and other minerals (3%) such as quartz ($SiO_2$), siderite ($FeCO_3$), kieserite ($MgSO_4$) and ternadite ($Na_2SO_4$) (S2 Table). Regarding the biogeochemical characteristics of microbialites, we observed that no regional differences existed. All structures had similar values with respect to organic matter (om), nitrogen and carbon (S3 Table). The C:N ratio suggested a productive community.

### Microbialite genetic composition (16S rDNA V4)

A total of 4,167,392 reads were obtained for the 16S rDNA V4 hypervariable region. The mean number of sequences per site was 40,071. To include samples from all sites we defined a rarefaction at 10,000 sequences per subsample per site. All the microbialites were fully characterized at this sampling coverage.

The prokaryote genetic composition at the phylum level indicates that 99.5% of all reads were assigned to Bacteria (Fig 3) and 0.5% to Archaea (S1 Fig). The main bacterial phyla showed great heterogeneity among sites: Proteobacteria (40–80%) was the most abundant, where class Gammaproteobacteria had the largest abundance at certain sites, (5–79%), followed by Alphaproteobacteria (14–25%) and Deltaproteobacteria (1–10%); Chloroflexi (7.6–14%); Cyanobacteria (1–11%); Firmicutes (1–23%); Bacteroidetes (7–8%); Planctomycetes (1–

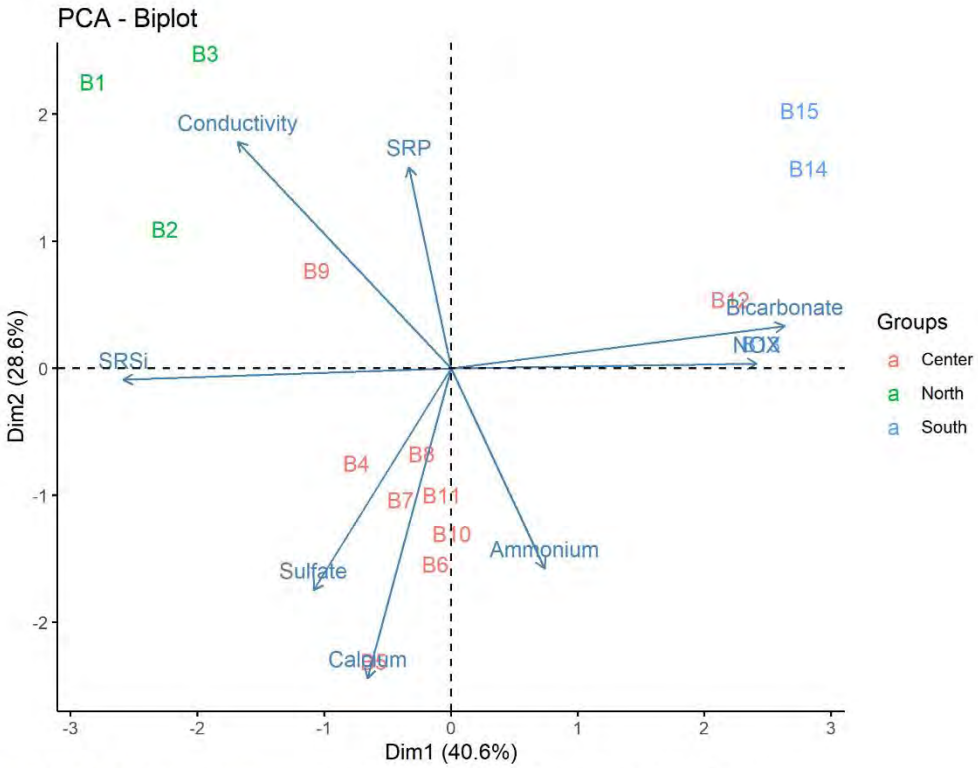


**Fig 2. Spatial ordination (PCA) of environmental variables associated to microbialite sampling sites in Bacalar Lagoon.**

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8%) and Verrucomicrobia (1–4%). Phyla with low abundances in all sites included Acidobacteria, Actinobacteria, Nitrospira, Chlamydiae, Spirochaetes, and Gemmatimonadetes.

The UniFrac weighted distance matrix separated Bacalar microbialites in two phylogenetically differentiated microbial communities. This result allowed us to classify the 15 sampling sites into two regions (S2 Fig). The first region was defined as North-Center and included sites

**Table 1. Available nutrient concentrations (nitrate, ammonia and soluble reactive phosphorus) in Bacalar Lagoon.**

| Region | Year | $NO_3^-$ | $NH_4^+$ | SRP | Reference |
|---|---|---|---|---|---|
| **South** | 2008 | 0 | 0.036 | BLD | Beltrán et al., 2012 |
| **South** | 2009 | 0.15 | 0.11 | BLD | Centeno et al., 2012 |
| **South** | 2016 | 1.94 | 0.15 | BLD | Tobón-Velázquez et al., 2018 |
| **South** | 2018 | 1.42 | 0.12 | 0.07 | This study |
| **North** | 2018 | 0.38 | 0.06 | 0.08 | This study |

The concentration of nutrients are presented in mg/l. BLD, below the limit of detection.

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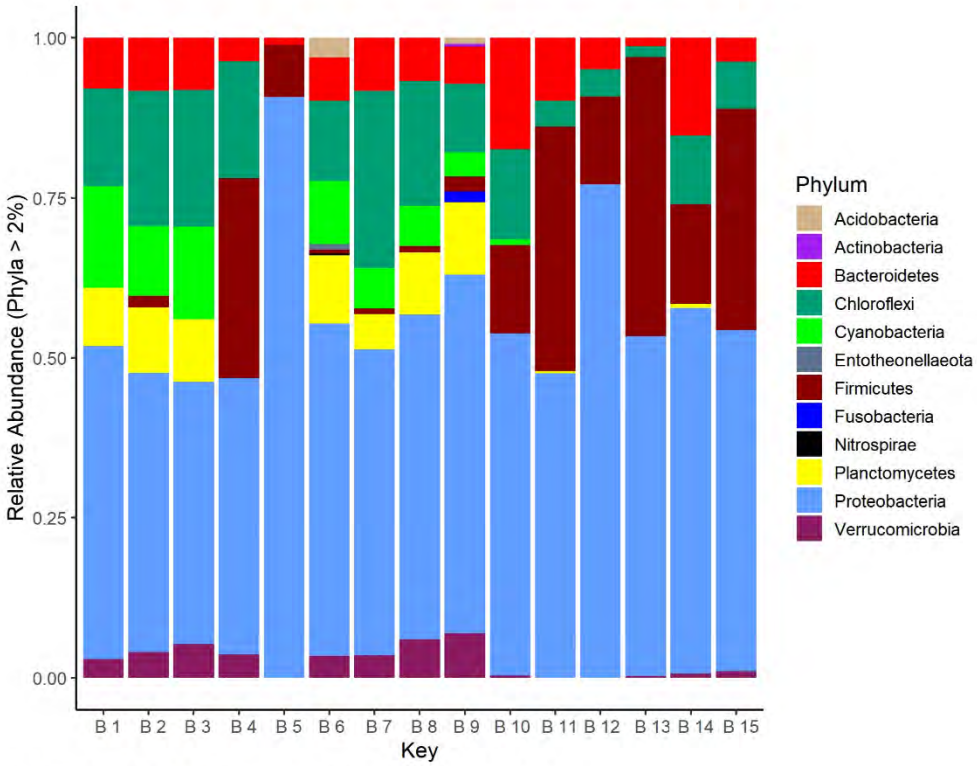


**Fig 3. Microbialite bacterial genetic composition (16S rDNA V4) at the phylum level.**

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B 1–3 and B 6–9, which represented 80% of the global microbial diversity and were very similar between them (D = 0.82). The second region was defined as South-Center with sites B 4–5 and B 10–15. Overall, the CAP of the genetic diversity matrix and environmental dataset, suggested that the factors that correlate in the South-Center region of Bacalar Lagoon with microbialite diversity are the concentrations of available forms of N ($NO_3^-$ and $NH_4^+$, respectively) (Fig 4). PERMANOVA analysis also indicated that the differences between regions in Bacalar Lagoon were significant ($p<0.05$).

A Mann-Whitney-Wilcoxon test was conducted to compare the richness and diversity indices between the North-Center and South-Center regions. Several diversity indices demonstrated that the microbiome diversity of the North-Center was significantly greater than that observed in the South-Center region ($p < 0.01$) (Fig 5). The Shannon index indicated that the sampling sites North-Center of the town of Bacalar had a greater bacterial diversity ($H' = 5.7$), and the sites to the South-Center had 42% less diversity ($H' = 3.3$).

The following groups defined microbialite bacterial diversity within the North-Center region in 66% of the relative abundance: Alphaproteobacteria (25%), Chloroflexi (14%),

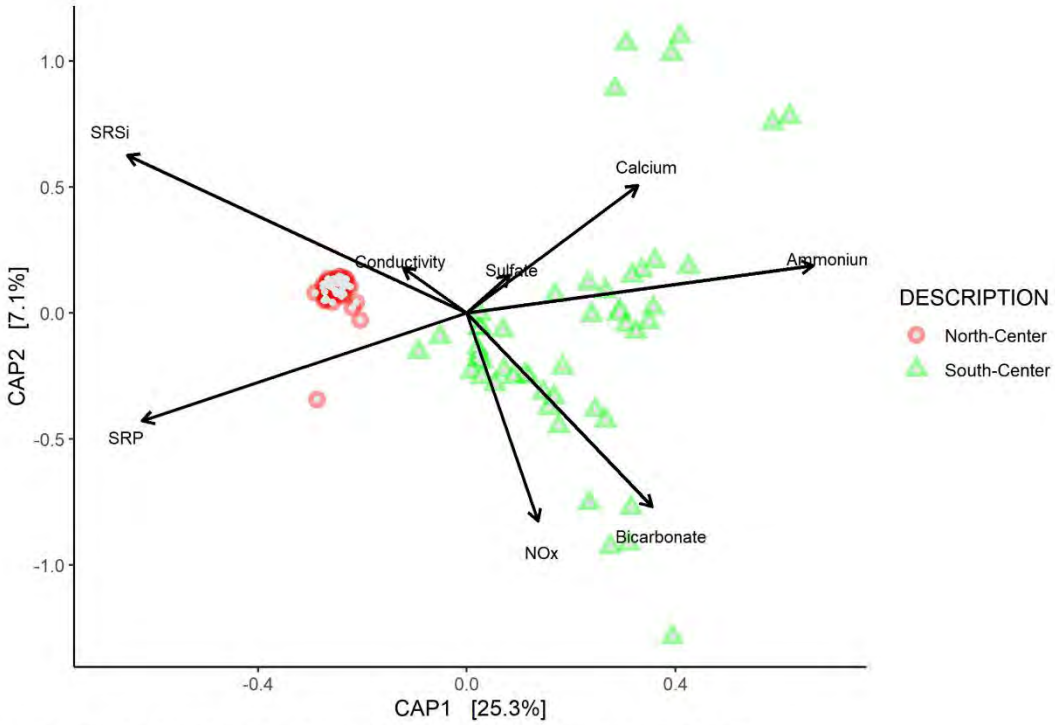


**Fig 4. Constrained Analysis of Principal coordinates (CAP) based on Weighted-Unifrac and environmental variables.**

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Deltaproteobacteria (10%), Cyanobacteria (11%), Bacteroidetes (8%) Planctomycetes (8%) and Verrucomicrobia (4%). On the other hand, the microbialites that develop in the South-Center-region of Bacalar Lagoon, showed less abundance of bacterial groups, while 50% of the bacterial diversity was shared with their North-Center counterparts. Changes in composition between microbialites of both regions was characterized by a decrease in Alphaproteobacteria (14%), Chloroflexi (7.6%), Cyanobacteria (1%) and Deltaproteobacteria (1%) in the south. Bacteria that make up to 64% of the total diversity in microbialites were represented by Gammaproteobacteria (41%) and Firmicutes (23%) (Fig 3, Fig 6A–6J). Cyanobacteria, which are fundamental components of microbialites, shared 50% of their diversity between regions, with an average abundance of 10% for the North-Center and 1% for the South-Center. Shared cyanobacteria among all sites included Nostocales (*Calothrix*, *Rivularia*, *Scytonema*, *Nostoc*, *Mastigocladopsis*), Chroococcales (*Chroococcidiopsis*), Oscillatoriales (*Aliterella*, *Lyngbya*, *Leptolyngbya*, *Phormidium*). Cyanobacteria in the northern region had 16 exclusive species including *Calothrix*, *Geitlerinema*, *Gloeomargarita*, *Leptolyngbya*, *Nostoc*, *Oscillatoria* and *Scytonema*, among others, while the south did not show exclusive species (Fig 6A). Archaea have been reported as regular components of microbialites, yet their contribution is not fully understood. Archaea represented 0.5% and 0.2% of the total diversity in north and south

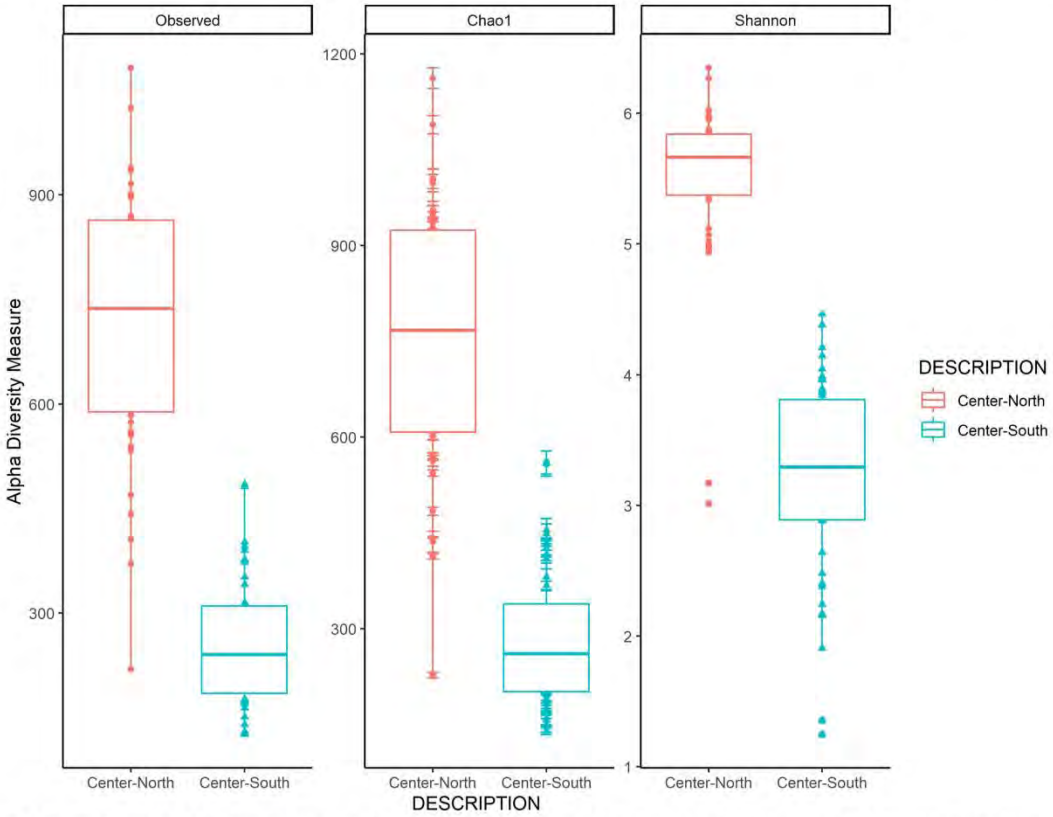


**Fig 5. Observed counts and alpha diversity measured by the Chao1 and Shannon indices in the microbialites of Bacalar Lagoon: North-Center (sites B 1–3 and B 6–9) and South-Center (sites B 4–5 and B 10–15).**

https://doi.org/10.1371/journal.pone.0230071.g005

microbialites, respectively. Six phyla (Altiarchaeota, Asgardaeota, Diapherotrites, Euryarchaeota, Nanoarchaeaeota and Thaumarchaeota) were identified in this study. Again, the microbialites in the North-Center had the greatest diversity, where Heimdallarchaeia, Woesearchaeia and Nitrososphaeria were the most abundant (Fig 6J).

To elucidate why these significant changes in community structure were occurring and which taxa were associated with variations at each site, we used the LCBD and SCBD metrics, as proposed by Legendre and De Cáceres [56]. The highest and most significant differences in LCBDs were found at sites B 5, B 12 and B 13 ($p<0.05$). The SCBD showed that *Pseudomonas*, *Aeromonas*, *Stenotrophomonas*, *Acinetobacter*, *Bacillus*, *Chryseobacterium*, *Achromobacter*, *Brevundimonas* and *Bacillus* were bacterial genera that contributed mostly to community structure substitution.

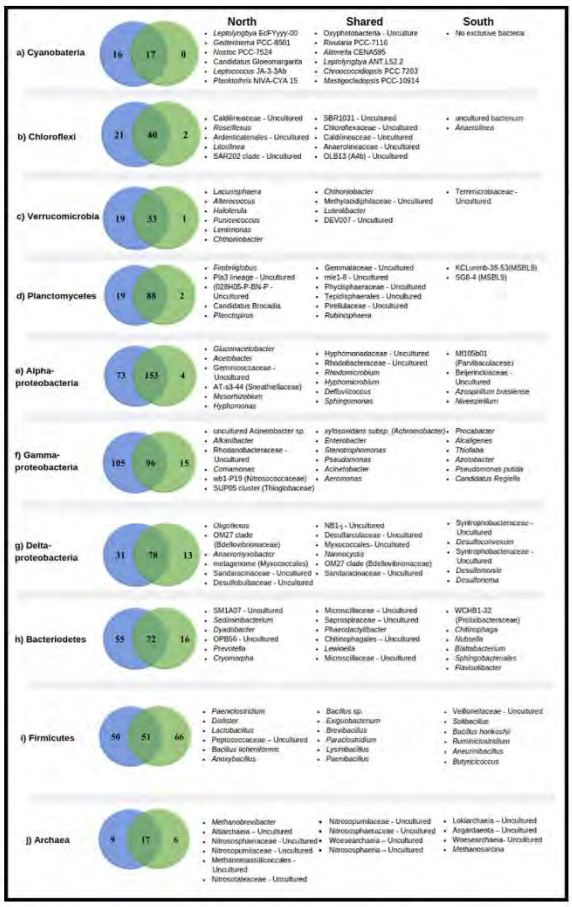


**Fig 6. Venn diagram of main prokaryotes.** (a) Cyanobacteria, (b) Chloroflexi, (c) Verrucomicrobia, (d) Planctomycetes, (e) Alphaproteobacteria, (f) Gammaproteobacteria, (g) Deltaproteobacteria, (h) Bacteroidetes, (i) Firmicutes, (j) Archaea.

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## Discussion

The Yucatan peninsula is an emerged carbonated continental platform. The northern region emerged during the Paleogene and Neogene, while the south began to rise in the Oligocene [61]. There are no rivers in the Yucatan peninsula and karst features including channels and sinkholes (cenotes) are common [62]. In particular, the southeastern Yucatan peninsula is located in an evaporite region [63], that has a high ecological connectivity and is the area where the greatest number of sites with microbialites are reported: Chetumal Bay [64], Muyil (Sian Ka´an) and Bacalar Lagoon [8, 30].

The hydrogeochemical dynamics of Bacalar Lagoon are considered unique, with a high rate of constant exchange between the surface and groundwater flows [40]. The concentration of bicarbonate-NOx and the values of conductivity-SRSi were variables that defined a gradient in Bacalar Lagoon. There are sites around the world that host microbialites with hydrogeochemical characteristics similar to those of Bacalar Lagoon (carbonate saturation), such as Pavillon Lake in Canada; Great Salt Lake in the United States; Satonda in Indonesia [27]. The north zone of tBacalar is characterized by higher electrolytic conductivity, due to the connectivity with other lagoons such as Chile Verde, Salada and the Bay of Chetumal [40, 65–66]. The south of Bacalar, has higher bicarbonate concentration than the North and has higher concentrations of NOx [30, 60]. Sulfates are homogeneous throughout the lagoon, as described by Johnson et al., [39] and Beltrán et al., [30]. While, Sánchez et al., [66] reported that southern Quintana Roo has high rates of infiltration of nutrients—such as nitrates- and there is a high risk of contamination of the aquifer by human activities, such as agriculture.

Economic development and population growth are direct threats to freshwater ecosystems [67–68]. Nitrogen is often the limiting nutrient in aquatic marine environments, and P, in karst regions, is extremely low due to interactions with carbonate [69]. The concentration of ammonium ions and NOx is increasing in Bacalar Lagoon, especially near the city and south of the lagoon [8, 39, 60, this study]. The increasing presence of available forms nitrogen is one of the main causes of water quality change in freshwater bodies [70]. We are observing a change in the natural oligotrophic conditions of Bacalar Lagoon. Other sites with increased eutrophication have shown that the productivity of the system alters the interactions of microbialites with eukaryotes, favoring competition the organisms such as algae, bivalves and diatoms [71].

## Biogeochemical and mineralogical and characterization of Bacalar Lagoon microbialites

Microbialite are spatially distributed throughout Bacalar Lagoon. Bacalar Lagoon's hydrogeochemical dynamics make it different from other sites with microbialites in seawater and continental environments. All the microbialites analyzed in Bacalar Lagoon share mineral composition ($CaCO_3$, ~97%). Valdespino et al., [23] reported a similar mineralogy for the microbialites of Bacalar Lagoon and Cuatro Cienegas Basin, which are water bodies of karstic origin. Bacalar Lagoon, which is located in the evaporative hydrogeochemical region [62], presents carbonate dissolution processes of the subterranean water tunnels that reach the lagoon and the walls (carbonate rock) of the cenotes within the lagoon, favoring the saturation of bicarbonates [37, 72]. The development of larger microbialites in the south of Bacalar might be associated to bicarbonate saturation. Chagas et al., [27] also report for lacustrine systems with microbialites such as Lake Pavilion, Lake Van, Cuatro Cienegas Basin, Alchichica and Clifton, that calcite minerals and aragonite are the main minerals in microbialites.

Depending on the chemistry of the water and the bacterial community, microbialites present a diverse range of minerals, although generally they have been reported in greater percentage aragonite, hydromagnesite, gypsum and calcite [23, 73]. Cyanobacteria such as Pleurocapsales and Chroococcales and Alphaproteobacteria are associated with the formation of aragonite in microbialites from Lake Alchichica (Mexico), a Mg-rich hyperalkaline crater lake (pH 8.9), while in Cuatro Cienegas and Bacalar, a S-rich karstic system, filamentous cyanobacteria and Sulfate Reducing Bacteria (SRB) favor calcite precipitation [39]. The hydrogeochemical conditions in Bacalar Lagoon favor the presence of bacterial groups (including cyanobacteria and S-cycling bacteria) that are involved in carbonate precipitation processes [20, 22, 74] and SRB reduce sulfates to sulfides with a consequent oxidation of organic carbon

to bicarbonates. They contribute to a state of saturation, which occurs within the EPS matrix (associated mainly to cyanobacterial activity), precipitated by cyanobacteria in an alkaline pH, where calcium ions finally precipitate as $CaCO_3$ [33, 75]. We report the same groups of SRB (Desulfovibrionales, Desulfobacteraceae, Syntrophobacteraceae, Desulfobulbaceae and *Desulfomonile*), distributed in all Bacalar Lagoon sites and reported by Johnson et al., [39]. All of these SRB have larger abundances in microbialites of the North-Center region.

In addition, Bacalar Lagoon microbialites have been described for their interactions with organisms such as gastropods (*Pomacea flagellata*), bivalves (*Mytilopsis sallei*), nematodes and mangroves [38, 76]. Johnson et al., [39] reported the presence of Cyanobacteria and Rhizobiales, a nitrogen-fixing Alphaproteobacteria, in the microbialites associated with mangroves in their study sites in southern Bacalar Lagoon.

## Bacterial community structure of the microbialites of Bacalar Lagoon

This study proposes the presence of two phylogenetically differentiated communities in Bacalar Lagoon microbialites. Generally, studies mention that population differences occur in biogeographic patterns with the differentiation of niches at large geographic scales [77]. However, within ecosystems, biogeographic regionalization is possible due to the presence of gradients that induce changes in biological communities [3]. Currently, anthropological activities can be considered a selective force, either physically (implementation of infrastructure) or by chemical alteration, which includes eutrophication of water bodies related to nutrient availability [12].

The microbialite sites that represent the South-Center region of this study are located near the city of Bacalar, and to the south of the lagoon. These sites are associated to urban development in the shoreline of the lagoon which lack infrastructure for domestic water treatment, have leaking septic systems, agriculture and intense tourist activity, that are causing trophic affectation in the system [68, 78–80]. Alterations in water quality was related to changes in the structure of the microbiome of microbialites between the North-Center and the South-Center regions Bacalar Lagoon. Recently, the work of Lindsay et al., [13] reported that in Great Salt Lake (GSL), USA, the bacterial community of microbialites responded to anthropogenic perturbation of the system related to construction of a railroad causeway. These authors demonstrated that microbialites in less disturbed areas of GSL have a greater abundance of cyanobacteria and diatoms compared to the almost total absence of these organisms in the microbialites where disturbance exists. Therefore, the monitoring of the community diversity of the microbialites, could be a strategy to know how bacterial groups react to the processes of alteration of the environment [81], either before a physical affectation or through the chemical changes of the water as in the case of Bacalar Lagoon.

Microbialites in the world maintain, regardless of their geographical region, a similar composition at the phylum level [8, 23]. Actinobacteria, Bacteroidetes, Cyanobacteria and Proteobacteria [8, 37–39], are common components of microbialites. Bacalar Lagoon microbialites in the North-Center region have a high diversity ($H' = 5.7$) (Fig 5), which contributes to understand that oligotrophy is not a limiting factor in the development of complex communities [81]. The decrease of almost half of the bacterial diversity in the South-Center region is associated to dominance of specific microbes of the Gammaproteobacteria and Firmicutes groups.

Cyanobacteria were most abundant in the North-Center region. Considering that the South-Center region of Bacalar Lagoon is suffering an increase of nutrients due to anthropogenic activities, our results coincide with other works where it is reported that cyanobacteria are more diverse in oligotrophic waters than in eutrophic waters [82]. Cyanobacteria, a

phylum that is relevant in EPS formation and has been considered to form nucleation sites for carbonate precipitation [83–84], showed a greater abundance and diversity in the North-Center microbialites of Bacalar Lagoon. The North-Center region also presented a higher diversity of Planctomycetes and Verrucomicrobia (~8.4% and ~3.8%, respectively), both forming part of a taxonomic super phylum called PVC [85–86], described with a relative abundance between 7–12% in different microbialites of the world [8, 86]. Recently, the presence of these bacteria was correlated in places where calcite crystals predominated [23]. Chloroflexi, an anoxygenic phototrophic phylum, which participates in the "alkaline machinery" which in combination with oxygenic photosynthesis by cyanobacteria and sulfate reduction, promote the precipitation of carbonated minerals [20, 36], was also more abundant in the North-Center region. This would suggest that loss of cyanobacterial, PVC and chloroflexi diversity could affect microbialite growth and maintenance in the South-Center region of Bacalar.

Further, the microbiome of microbialites in the South-Center region presented a high abundance of Firmicutes (~23.3%). This group occupies between 0–2% of relative abundance in other microbialites of the world [87], and is thus, not common in healthy microbialite fabrics. Firmicutes generally have low percentages in oligotrophic water conditions and their abundance may suggest an environmental pollution processes [82], as reported for Gonghu Bay, China, where one of the causes of increased nutrients was domestic wastewater [88]. The class (eg. Bacilli) of the Firmicutes are used as indicators of fecal pollution in freshwater and their main sources are untreated domestic waters [89], as may be happening in the South-Center region of our study.

## Changes in the bacterial community of microbialites in Bacalar Lagoon

It is important to define the factors that are causing the environmental disturbance of a system, especially if it is due to human activities [90]. We used the LCBD-SCBD metrics and a CAP to associate the environmental variants of home site (niche) and the association with their bacterial community (dispersion) [91]. Legendre and De Cáceres [56] mention that high values of LCBD indicate the degree of ecological singularity of each sampling site. From this perspective, sites with high values of LCBD may contain unusual species or are sites that respond to human disturbance [92]. In both cases, the use of beta-diversity metrics can be a starting point for decision-making in conservation or ecological restoration scenarios [93]. In this study, the highest values of LCBD were related to sites in the South-Center region. The sites B5, B 12 and 13, obtained the highest LCBD values. Site B5 is a particular case of microbialite growth that has a strong correlation in the CAP to ammonium. Further research is needed to identify the sources of ammonium to this specific area in Bacalar Lagoon that shows an increase in domestic and tourist developments.

The Mexican Caribbean is an area whose economy depends mainly on tourism related to its natural resources [60, 94]. Particularly within Quintana Roo, places like Cancun and Playa del Carmen that have intense tourist activity, show affectations to the water quality of the underground aquifer systems and cenotes [95–96]. Currently, Bacalar presents an increase in tourist occupation. According to the Mexican Government office of statistics (INEGI-SecTur, 2019) Bacalar Lagoon received approximately 90,000 tourists in 2018, which was twice the amount of tourist visitation in 2017 (45,000). This phenomenon is likely to continue and the infrastructure to accommodate these visitors is not available. Tobón-Velázquez et al., [60] mentioned that the lack of infrastructure regulation from the government could result in the degradation of the water quality of Bacalar Lagoon, hence affecting the microbialites.

A direct correlation is reported between the most visited sites for tourists and the lowest prokaryote diversity. In addition, the sites with the lowest diversity that are located at the South-Center region of the lagoon, are the same sites that have been historically used for tourism. These results postulate that the changes in the microbiome of microbialites along Bacalar Lagoon are probably associated to a greater extent, with poor water quality due to high concentrations of ammonium and NOx [80, 97].

## Disturbance in oligotrophic water conditions affect the structure of microbiome in the microbialites of Bacalar Lagoon

Environmental problems in aquatic ecosystems related to nutrient enrichment are observed in different parts of the world [79, 98]. In particular, in karstic environments (such as the Yucatan peninsula), where groundwater is flowing through fractures, and complex cave systems interconnect water bodies, such as lagoons and coastal environments [99]. Groundwater discharge has been identified as an important source of nutrients in many aquatic ecosystems of the peninsula [100]. It should be noted that all human activity in the peninsula (settlement, intensive fertilizer farming practices, deforestation, tourism, lack of wastewater treatment) has a direct impact on nearby water bodies [101–102], and affects the structure of mangrove communities, coral reefs, sea grasses [103], and microbialite diversity.

Understanding the changes in the structure of microbial communities is crucial, as this information may provide insights of the system and later be used as bioindicators for assessing environmental problems [104]. Currently, values for available SRP in Bacalar Lagoon remain close the detection limit [30, 60], but the different forms of available forms of nitrogen ($NH_4^+$, $NO_3^-$) are alarmingly increasing [8, 60] (Table 1). After an environmental disturbance, the possibility of a community of returning to its previous state will depend on its genetic and physiological diversity [105], yet so far, no research has demonstrated that microbialite communities can recover in the short term.

A hypothesis that rises from this study is that microbialites in Bacalar Lagoon have the same phylogenetic origin, yet disturbances in water quality detected in the South-Center region are causing loss of biodiversity. Another possible explanation is that high concentrations of carbonate present in the South-Center region, promote larger and faster microbialite growth, which is associated to a different community structure, differing from their North-Center counterparts. So far, we do not have elements to prove any of these open questions, but we do know that microbialites have fundamental biological constituents, where Cyanobacteria and bacteria associated to S-cycling are the main contributors to microbialite formation and growth. We still need to understand the dynamics of the communities that form microbialites, while trying to document their transformations in fragile habitats, like the tropical lagoon that is represented in this study. The increase in available forms of nitrogen is preoccupying to say the least since our research shows this is associated to lack of water treatment and planned agriculture in the region. How much can the native communities, represented in this study by microbialites, deal with the rate of change that human activities cause in the environment?

## Supporting information

**S1 Table. Physicochemical variables describing the water column where microbialites develop in Bacalar Lagoon, Mexico.**
(DOCX)

**S2 Table. Mineral composition of Bacalar Lagoon microbialites.**
(DOCX)

**S3 Table. Biogeochemical characterization of microbialites in Bacalar Lagoon.**
(DOCX)

**S1 Fig. PCoA showing weighted and unweighted Unifrac distributions of microbial diversity in Bacalar Lagoon microbialites.** North-Center (red), South-Center (blue).
(TIF)

**S2 Fig. Class level diversity of Archaea from Bacalar microbialites defined with the V4 hypervariable region of the 16S rDNA.**
(TIFF)

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## Author Contributions

**Conceptualization:** Alfredo Yanez-Montalvo, Selene Gómez-Acata, Héctor Hernández-Arana, Luisa I. Falcón.

**Data curation:** Alfredo Yanez-Montalvo, Selene Gómez-Acata, Bernardo Águila, Luisa I. Falcón.

**Formal analysis:** Alfredo Yanez-Montalvo, Selene Gómez-Acata, Bernardo Águila, Luisa I. Falcón.

**Funding acquisition:** Luisa I. Falcón.

**Investigation:** Alfredo Yanez-Montalvo, Héctor Hernández-Arana, Luisa I. Falcón.

**Methodology:** Alfredo Yanez-Montalvo, Luisa I. Falcón.

**Project administration:** Luisa I. Falcón.

**Resources:** Alfredo Yanez-Montalvo, Luisa I. Falcón.

**Software:** Alfredo Yanez-Montalvo, Luisa I. Falcón.

**Supervision:** Alfredo Yanez-Montalvo, Héctor Hernández-Arana, Luisa I. Falcón.

**Validation:** Alfredo Yanez-Montalvo, Luisa I. Falcón.

**Visualization:** Alfredo Yanez-Montalvo, Luisa I. Falcón.

**Writing – original draft:** Alfredo Yanez-Montalvo, Selene Gómez-Acata, Bernardo Águila, Héctor Hernández-Arana, Luisa I. Falcón.

**Writing – review & editing:** Alfredo Yanez-Montalvo, Selene Gómez-Acata, Bernardo Águila, Héctor Hernández-Arana, Luisa I. Falcón.

## References


1. Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc. Natl Acad. Sci. USA. 2006; 103: 626–631. https://doi.org/10.1073/pnas.0507535103 PMID: 16407148
2. Falkowski PG, Fenchel T, Delong EF. The microbial engines that drive Earth's biogeochemical cycles. Science. 2008; 320(5879): 1034–1039. https://doi.org/10.1126/science.1153213 PMID: 18497287
3. Lozupone CA, Knight R. Global patterns in bacterial diversity. Proc. Natl Acad. Sci USA. 2007; 104: 11436–11440. https://doi.org/10.1073/pnas.0611525104 PMID: 17592124
4. Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Microbiol. 2012; 10(7): 497–506. https://doi.org/10.1038/nrmicro2795 PMID: 22580365
5. Fierer N. Microbial biogeography: patterns in microbial diversity across space and time. In: Accessing Uncultivated Microorganisms: from the Environment to Organisms and Genomes and Back, Zengler K, editor. ASM Press, Washington, DC. 2008. pp. 95–115. https://doi.org/10.1128/9781555815509.ch6
6. Van der Gucht K, Cottenie K, Muylaert K, Vloemans N, Cousin S, Declerck S, et al. The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc Natl Acad Sci USA. 2007; 104(51): 20404–20409. https://doi.org/10.1073/pnas.0707200104 PMID: 18077371
7. Gibbons SM, Gilbert JA. Microbial diversity-exploration of natural ecosystems and microbiomes. Curr Opin Genet Dev. 2015; 35: 66–72. https://doi.org/10.1016/j.gde.2015.10.003 PMID: 26598941
8. Centeno CM, Legendre P, Beltrán Y, Alcántara-Hernández RJ, Lidström UE, Ashby MN, et al. Microbialite genetic diversity and composition relate to environmental variables. FEMS Microbiol Ecol. 2012; 82(3): 724–35. https://doi.org/10.1111/j.1574-6941.2012.01447.x PMID: 22775797
9. Montecchia S, Tosj M, Soria MA, Vogrig JA, Sydorenko O, Correa OS. Pyrosequencing Reveals Changes in Soil Bacterial Communities after Conversion of Yungas Forests to Agriculture. PloS one. 2015;20: 10(3):e0119426. https://doi.org/10.1371/journal.pone.0119426 PMID: 25793893
10. Martínez-Porchas M, Vargas-Albores F. Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Rev Aquacult. 2015. 9(42):56. https://doi.org/10.1111/raq.12102
11. Zeglin LH. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Front Microbiol. 2015; 6 (454),454. https://doi.org/10.3389/fmicb.2015.00454 PMID: 26042102
12. Xie Y, Wang J, Wu Y, Ren C, Song C, Yang J, et al. Using in situ bacterial communities to monitor contaminants in river sediments. Environ Pollut. 2016; 212, 348–357. https://doi.org/10.1016/j.envpol.2016.01.031 PMID: 26866572
13. Lindsay MR, et al. Microbialite response to an anthropogenic salinity gradient in Great Salt Lake, Utah. Geobiology. 2017; 15(1): 131–145. https://doi.org/10.1111/gbi.12201 PMID: 27418462
14. Whittaker RH. Evolution and measurement of species diversity. Taxon. 1972; 21(2/3): 213–251. https://doi.org/10.2307/1218190
15. Legendre P, Legendre L. Numerical Ecology, Third Edition. Elsevier, editor. Amsterdam. 2012. https://doi.org/10.1016/S0304-3800(00)00291-X
16. Budnick WR, Leboucher T, Belliard J, Soininen J, Lavoie I, Pound K, et al. Local and regional drivers of taxonomic homogenization in stream communities along a land use gradient. Global Ecology and Biogeography 2019; 28(11): 1597–1609. https://doi.org/10.1111/geb.12976
17. Magurran AE. Measuring biological diversity. John Wiley and Sons, 2013. https://doi.org/10.2307/4126959
18. Anderson MJ, Ellingsen KE, McArdle BH. Multivariate dispersion as a measure of beta diversity. Ecol Lett. 2006; 9(6): 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x PMID: 16706913
19. Burne RV, Moore LS. Microbialites: organosedimentary deposits of benthic microbial communities. Palaios. 1987; 2: 241–254. https://doi.org/10.2307/3514674
20. Dupraz C, et al. Processes of carbonate precipitation in modern microbial mats. Earth Sci. Rev. 2009; 96(3): 141–162. https://doi.org/10.1016/j.earscirev.2008.10.005
21. Microbialites Riding R., stromatolites, and thrombolites. In: Reitner J., Volker T, editors. Encyclopedia of Geobiology. Springer, Dordrecht, 2011. pp. 635–654. https://doi.org/10.1007/978-1-4020-9212-1_196
22. White RA III, Chan AM, Gavelis GS, Leander BS, Brady AL, Slater GF, et al. Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community. Front Microbiol. 2016; 28(6): 1531. https://doi.org/10.3389/fmicb.2015.01531 PMID: 26903951

23. Valdespino-Castillo PM, Hu P, Merino-Ibarra M, López-Gómez LM, Cerqueda-García D, et al. Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. Front Microbiol. 2018; 9: 510. https://doi.org/10.3389/fmicb.2018.00510 PMID: 29666607

24. Schopf JW, Packer BM. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. Science. 1987; 237(4810): 70–3. https://doi.org/10.1126/science.11539686 PMID: 11539686

25. Schopf JW. Fossil evidence of Archaean life: Philos Trans R Soc Lond B Biol Sci. 2006; 361(1470): 869–85. https://doi.org/10.1098/rstb.2006.1834 PMID: 16754604

26. Nutman AP, Bennett VC, Friend CR, Van Kranendonk MJ, Chivas AR. Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. Nature. 2016; 537(7621): 535–538. https://doi.org/10.1038/nature19355 PMID: 27580034

27. Chagas AA, Webb GE, Burne RV, Southam G. Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy. Earth-Sci. Rev. 2016; 162, 338–363. https://doi.org/10.1016/j.earscirev.2016.09.012

28. Paul VG, Wronkiewicz DJ, Mormile MR, & Foster JS. Mineralogy and microbial diversity of the microbialites in the hypersaline Storr's Lake, The Bahamas. Astrobiology. 2016; 16(4):282–300. https://doi.org/10.1089/ast.2015.1326 PMID: 27082142

29. Falcon L, Escobar-Briones E, Romero D. Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico. Hydrobiologia. 2002. 467(1):71–78. https://doi.org/10.1023/A:1014984629985

30. Beltrán Y, Centeno CM, García-Oliva F, Legendre P,Falcón LI. N2 fixation rates and associated diversity (nifH) of microbialite and mat-forming consortia from different aquatic environments in Mexico. Aquat Microb Ecol. 2012; 67(1): 15–24. https://doi.org/10.3354/ame01572

31. Kawaguchi T, Decho AW. A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO3 polymorphism. J Cryst Growth. 2002; 240(1–2): 230–235. https://doi.org/10.1016/S0022-0248(02)00918-1

32. Dupraz C, Visscher PT. Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol. 2005; 13(9): 429–38. https://doi.org/10.1016/j.tim.2005.07.008 PMID: 16087339

33. Zhu T, Dittrich M. Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. Front Bioeng Biotechnol. 2016; 4: 4. https://doi.org/10.3389/fbioe.2016.00004 PMID: 26835451

34. Visscher PT, Reid RP, Bebout BM, Hoeft SE, Macintyre IG, Thompson JA. Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am. Mineral. 1998; 83(11): 1482–1493. https://doi.org/10.2138/am-1997-11-1236

35. Braissant O, Decho AW, Dupraz C, Glunk C, Przekop KM, Visscher PT. Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology. 2007; 5(2007): 401–411. https://doi.org/10.1111/j.1472-4669.2007.00117.x

36. Saghai A, Zivanovic Y, Zeyen N, Moreira D, Benzerara K, Deschamps P, et al. Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. Front Microbiol. 2015; 6(797): 1–16. https://doi.org/10.3389/fmicb.2015.00797 PMID: 26300865

37. Gischler E, Gibson MA, Oschmann W. Giant Holocene freshwater microbialites, Laguna Bacalar, Quintana Roo, Mexico. Sedimentology. 2008; 55: 1293–1309. https://doi.org/10.1111/J.1365-3091.2007.00946.X

38. Gischler E, Golubic S, Gibson M, Oschamann W, Hudson JH. Microbial mats and microbialites in the freshwater Laguna Bacalar, Yucatan Peninsula, Mexico. In 'Advances in Stromatolite Geobiology'. Reitner J, Sütwe T, Yuen D, editors. ( Springer: Berlin, Germany.). 2011. pp. 187–205. https://doi.org/10.1007/978-3-642-10415-2_13

39. Johnson DB, Beddows PA, Flynn TM, Osburn MR. Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar, Mexico. Geobiology. 2018; 16(3): 319–337. https://doi.org/10.1111/gbi.12283 PMID: 29656514

40. Hernández-Arana HA, Vega-Zepeda A, Ruíz-Zárate MA, Falcón-Alvarez LI, López-Adame H, Herrera-Silveira J, et al. Transverse coastal corridor: from freshwater lakes to coral reefs ecosystems. In Biodiversity and Conservation of the Yucatán Peninsula. Springer, Cham. 2015. pp 355–376. https://doi.org/10.1007/978-3-319-06529-8_14

41. Castro-Contreras SI, Gingras MK, Pecoits E, Abert NR, Petrash D, Castro-Contreras SM, et al. Textural and geochemical features of freshwater. Palaios. 2014; 29(5): 192–209. https://doi.org/10.2110/palo.2013.063

42. Strickland JDH, Parsons TR. A practical handbook of seawater analysis. Fish Res Board Can Bull. 1972.

43. Hansen HP, Koroleff F. Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M, editors. Methods of Seawater Analysis. Wiley-VCH, Weinheim. 1999. pp. 159–228. https://doi.org/10.1002/9783527613984.ch10

44. Lê S, Husson F. FactorMineR: An R Package for Multivariate Analysis. J. Stat. Softw. 2008; 25: 1–18. https://doi.org/10.18637/jss.v025.i01

45. Muller G, Gatsner M. Chemical analysis. Neu Jb Mineral Mh. 1971; 10: 466–469.

46. Olsen SR, Sommers LE. Phosphorus. In Page A.L., et al (ed.) Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI. 1982. pp. 403–430.

47. Walkley A, Black LA. An examination of the Dgtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 1934; 37: 29–38. https://doi.org/10.1097/00010694-193401000-00003

48. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA. 2011; 108: 4516–4522. https://doi.org/10.1073/pnas.1000080107 PMID: 20534432

49. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, et al. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ. 2018; 9: 10. https://doi.org/10.7287/peerj.preprints.27295v1

50. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016; 13(7): 581–3. https://doi.org/10.1038/nmeth.3869 PMID: 27214047

51. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016; 4: 2584. https://doi.org/10.7717/peerj.2584 PMID: 27781170

52. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 20123; 8(4): e61217. https://doi.org/10.1371/journal.pone.0061217 PMID: 23630581

53. Anderson MJ, Willis T. Canonical Analysis of Principal Coordinates: a useful method of constrained ordination for Ecology. Ecology. 2003; 84(2): 511–525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2

54. Zapala M.A. and Schork N.J. Multivariate regression analysis of distance matrices for testing associations between gene expression patterns and related variables. Proc Natl Acad Sci USA. 2006; 103:19430–19435. https://doi.org/10.1073/pnas.0609333103 PMID: 17146048

55. Legendre P, Gauthier O. Statistical methods for temporal and space–time analysis of community composition data. Proc Biol Sci. 2014; 281(1778): 20132728. https://doi.org/10.1098/rspb.2013.2728 PMID: 24430848

56. Legendre P, De Cáceres M. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. Ecol Lett. 2013; 16(8): 951–63. https://doi.org/10.1111/ele.12141 PMID: 23809147

57. Baselga A, Orme CDL. betapart: an R package for the study of beta diversity. Methods Ecol. Evol. 2012; 3(5): 808–812. https://doi.org/10.1111/j.2041-210X.2012.00224.x

58. Dray S, Blanchet G, Borcard D, Guenard G, Jombart T, Larocque G, et al. adespatial: Multivariate multiscale spatial analysis. 2018. R package version 0.1–1. Retrieved from https://CRAN.Rproject.org/package=adespatial.

59. Dray S, Dufour AB. The ade4 package: implementing the duality diagram for ecologists. J. Stat. Softw. 2007; 22(4): 1–20. https://doi.org/10.18637/jss.v022.i04

60. Tobón-Velázquez NI, Vieyra MR, Paytan A, Broach KH, Terrones LMH. Hydrochemistry and carbonate sediment characterization of Bacalar Lagoon, Mexican Caribbean. Mar Freshwater Res. 2018; 70 (3), 382–394. https://doi.org/10.1071/MF18035

61. Lebedeva EV, Mikhalev DV, Nekrasova LA. Evolutionary stages of the karst-anthropogenic system of the Yucatán Peninsula. Geography and Natural Resources. 2017; 38(3): 303–311. https://doi.org/10.1134/S187537281703012X

62. Pérez L, Bugja R, Lorenschat J, Brenner M, Curtis J, Hoelzmann P, et al. Aquatic ecosystems of the Yucatán Peninsula (Mexico), Belize, and Guatemala. Hydrobiologia. 2011; 661(1): 407–433. https://doi.org/10.1007/s10750-010-0552-9

63. Perry E, Velázquez-Oliman G, Marin L. The hydrogeochemistry of the karst aquifer system of the northern Yucatan Peninsula, Mexico. Int Geol Rev. 2002(3):191–221. https://doi.org/10.2747/0020-6814.44.3.191

64. Rasmussen KA, Macintyre IG, Prufert L. Modern stromatolite reefs fringing a brackish coastline, Chetumal Bay, Belize. Geology. 1993; 21(3): 199–202. https://doi.org/10.1130/0091-7613(1993)021<0199:MSRFAB>2.3.CO;2

65. Carrillo L, Palacios-Hernández E, Yescas M, Ramírez-Manguilar AM. Spatial and seasonal patterns of salinity in a large and shallow tropical estuary of the Western Caribbean. Estuar Coast. 2009; 32(5): 906–916. https://doi.org/10.1007/s12237-009-9196-2

66. Sánchez JA, Álvarez T, Pacheco JG, Carrillo L, González RA. Calidad del agua subterránea: acuífero sur de Quintana Roo, México. Tecnol Cienc Agua. 2016; 7(4): 75–96. 7.

67. Sun MY, Dafforn KA, Brown MV, Johnston EL. Bacterial communities are sensitive indicators of contaminant stress. Mar Pollut Bull. 2012; 64(5):1029–38. https://doi.org/10.1016/j.marpolbul.2012.01.035 PMID: 22385752

68. Hanashiro FTT, Mukherjee S, Souffreau C, Engelen J, Brans K, Busschaert P, et al. Freshwater Bacterioplankton Metacommunity Structure Along Urbanization Gradients in Belgium. Front Microbiol. 2019; 10: 743. https://doi.org/10.3389/fmicb.2019.00743 PMID: 31031725

69. Fourqurean JW, Zieman JC, Powell GVN. Phosphorus limitation of primary production in Florida Bay: evidence from C:N:P ratios of the seagrass Thalassia testudinum. Limnol Oceanogr. 1992; 37: 162–171. https://doi.org/10.4319/lo.1992.37.1.0162

70. Archana A, Thibodeau B, Geeraert N, Xu MN, Kao SJ, Baker DM. Nitrogen sources and cycling revealed by dual isotopes of nitrate in a complex urbanized environment. Water Res. 2018; 142: 459–470. https://doi.org/10.1016/j.watres.2018.06.004 PMID: 29913387

71. Elser JJ, Schampel JH, Garcia-Pichel FE, et al. Effects of phosphorus enrichment and grazing snails on modern stromatolitic microbial communities. Freshw Biol. 2005; 50: 1808–1825. https://doi.org/10.1111/j.1365-2427.2005.01451.x

72. Sánchez-Sánchez JA, Álvarez-Legorreta T, Pacheco-Ávila JG, González-Herrera RA, Carrillo-Bribiezca L. Caracterización hidrogeoquímica de las aguas subterráneas del sur del Estado de Quintana Roo, México. Rev Mex Cienc Geol. 2015; 32(1): 62–76.

73. Reid RP, Visscher PT, Decho AW, Stolz JF, Bebout BM, Dupraz C, et al. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites: Nature. 2000; 406(6799): 989–92. https://doi.org/10.1038/35023158 PMID: 10984051

74. Castanier S, Le Métayer-Levrel G, Perthuisot JP. Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view. Sediment Geol. 1999; 126(1–4): 9–23. https://doi.org/10.1016/S0037-0738(99)00028-7

75. Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, et al. Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment. Geol. 2006; 185(3-5): 131–145. https://doi.org/10.1016/j.sedgeo.2005.12.008

76. Oliva-Rivera JJ, Ocaña FA, de Jesús-Navarrete A, de Jesús-Carrillo RM, Vargas-Espósitos AA. Reproducción de pomacea flagellata (mollusca: ampullariidae) en la Laguna de Bacalar, Quintana Roo, México. Rev Biol Trop. 2016; 64(4): 1643–1650. https://doi.org/10.15517/RBT.V64I4.22871 PMID: 29465942

77. Oakley BB, Carbonero F, Van Der Gast CJ, Hawkins RJ, Purdy KJ. Evolutionary divergence and biogeography of sympatric niche-differentiated bacterial populations. ISME J. 2010; 4(4): 488–97. https://doi.org/10.1038/ismej.2009.146 PMID: 20054357

78. Ozturk E, Bal N. Evaluation of ammonia–nitrogen removal efficiency from aqueous solutions by ultrasonic irradiation in short sonication periods. Ultrason Sonochem. 2015; 26: 422–427. https://doi.org/10.1016/j.ultsonch.2015.02.012 PMID: 25753490

79. Aranda-Cirerol N, Comín FA, Herrera-Silveira J. Nitrogen and phosphorus budgets for the Yucatán littoral: an approach for groundwater management. Environ Monit Assess. 2011; 172(1-4): 439–505. https://doi.org/10.1007/s10661-010-1349-z PMID: 20162449

80. Camacho-Cruz KA, Ortiz-Hernández MC, Sánchez A, Carrillo L, Navarrete ADJ. Water quality in the eastern karst region of the Yucatan Peninsula: nutrients and stable nitrogen isotopes in turtle grass, Thalassia testudinum. Environ Sci Pollut Res Int. 2019: 1–17. https://doi.org/10.1007/s11356-018-3003-1

81. Bonilla-Rosso G, Peimbert M, Alcaraz LD, Hernández I, Eguiarte LE, Olmedo-Alvarez G, et al. Comparative metagenomics of two microbial mats at Cuatro Ciénegas Basin II: community structure and composition in oligotrophic environments. Astrobiology. 2012; 12(7): 659–73. https://doi.org/10.1089/ast.2011.0724 PMID: 22920516

82. Vieira RP, Gonzalez AM, Cardoso AM, et al. Relationships between bacterial diversity and environmental variables in a tropical marine environment, Rio de Janeiro. Environ Microbiol. 2008; 10(1): 189–199. https://doi.org/10.1111/j.1462-2920.2007.01443.x PMID: 17892478

83. White RA III, Power IM, Dipple GM, Southam G, Suttle CA. Metagenomic analysis reveals that modern microbialites and polar microbial mats have similar taxonomic and functional potential. Front Microbiol. 2015; 6: 966. https://doi.org/10.3389/fmicb.2015.00966 PMID: 26441900

84. Shiraishi F, Hanzawa Y, Okumura T, Tomioka N, Kodama Y, Suga H, et al. Cyanobacterial exopolymer properties differentiate microbial carbonate fabrics. Sci Rep. 2017; 7(1): 11805. https://doi.org/10.1038/s41598-017-12303-9 PMID: 28924251

85. Spring S, Bunk B, Spröer C, Schumann P, Rohde M, Tindall BJ, et al. Characterization of the first cultured representative of Verrucomicrobia subdivision 5 indicates the proposal of a novel phylum. ISME J. 2016; 10(12): 2801–2816. https://doi.org/10.1038/ismej.2016.84 PMID: 27300277

86. Farías ME, Contreras M, Rasuk MC, Kurth D, Flores MR, Poiré DG, et al. Characterization of bacterial diversity associated with microbial mats, gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile. Extremophiles. 2014; 18(2): 311–29. https://doi.org/10.1007/s00792-013-0617-6 PMID: 24442191

87. Couradeau E, Benzerara K, Moreira D, Gerard E, Kaźmierczak J, Tavera R, et al. Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). PloS one. 2011; 6(12): e28767. https://doi.org/10.1371/journal.pone.0028767 PMID: 22194908

88. Wu H, Li Y, Zhang J, Niu L, Zhang W, Cai W,Zhu X. Sediment bacterial communities in a eutrophic lake influenced by multiple inflow-rivers. Environ Sci Pollut Res Int. 2017; 24(24): 19795–19806. https://doi.org/10.1007/s11356-017-9602-4 PMID: 28685337

89. Roguet A, Eren AM, Newton RJ, McLellan SL. Fecal source identification using random forest. Microbiome. 2018; 6(1): 185. https://doi.org/10.1186/s40168-018-0568-3 PMID: 30336775

90. Simões NR, Nunes AH, Dias JD, Lansac-Tôha FA, Velho LFM, et al. Impact of reservoirs on zooplankton diversity and implications for the conservation of natural aquatic environments. Hydrobiologia. 2015; 758(1): 3–17. https://doi.org/10.1007/s10750-015-2260-y

91. Logue JB, Langenheder S, Andersson AF, Bertilsson S, Drakare S, Lanzén A, et al. Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. ISME J. 2012; 6(6): 1127–36. https://doi.org/10.1038/ismej.2011.184 PMID: 22170419

92. Landeiro VL, Franz B, Heino J, Siqueira T, Bini LM. Species-poor and low-lying sites are more ecologically unique in a hyperdiverse Amazon region: Evidence from multiple taxonomic groups. Divers Distrib. 2018; 24(7): 966–977. https://doi.org/10.1111/ddi.12734

93. Legendre P. Interpreting the replacement and richness difference components of beta diversity. Glob Ecol Biogeogr. 2014; 23(11): 1324–1334. https://doi.org/10.1111/geb.12207

94. Dixon J; Hamilton K, Pagiola Stefano, Segnestam L. Tourism and the Environment in the Caribbean: An Economic Framework. Environment Department working papers; no. 80. Environmental economic series. World Bank, Washington, DC. © World Bank. 2001. Available: https://openknowledge.worldbank.org/handle/10986-/18299.

95. Metcalfe CD, Beddows PA, Bouchot GG, Metcalfe TL, Li H, Van Lavieren H. Contaminants in the coastal karst aquifer system along the Caribbean coast of the Yucatan Peninsula, Mexico. Environ Pollut. 2011; 159(4): 991–7. https://doi.org/10.1016/j.envpol.2010.11.031 PMID: 21232837

96. Lizardi-Jiménez MA, Leal-Bautista RM, Ordaz A, Reyna-Velarde R. Airlift bioreactors for hydrocarbon water pollution remediation in a tourism development pole. Desalin. Water Treat. 2013; 54(1): 44–49. https://doi.org/10.1080/19443994.2013.876670

97. Akçaalan R, Albay M, Koker L, Baudart J, Guillebault D, Fischer S, et al. Seasonal dynamics of freshwater pathogens as measured by microarray at Lake Sapanca, a drinking water source in the northeastern part of Turkey. Environ Monit Assess. 2017; 190(1): 42. https://doi.org/10.1007/s10661-017-6314-7 PMID: 29273852

98. Andersen JH, Carstensen J, Conley DJ, Dromph K, Fleming-Lehtinen V, Gustafsson B, et al. Long-term temporal and spatial trends in eutrophication status of the Baltic Sea. Biol Rev Camb Philos Soc. 2017; 92(1): 135–149. https://doi.org/10.1111/brv.12221 PMID: 26467655

99. Null KA, Knee KL, Crook ED, de Sieyes NR, Rebolledo-Vieyra M, Hernández-Terrones L, et al. Composition and fluxes of submarine groundwater along the Caribbean coast of the Yucatan Peninsula. Cont Shelf Res. 2004; 77: 38–50. https://doi.org/10.1016/j.csr.2014.04.008

100. Álvarez-Góngora C, Herrera-Silveira J. Variations of phytoplankton community structure related to water quality trends in a tropical karstic coastal zone. Mar. Pollut. Bull. 2006; 52(1): 48–68. https://doi.org/10.1016/j.marpolbul.2005.08.006 PMID: 16194550

101. Tapia-González FU, Herrera-Silveira JA, Aguirre-Macedo ML (2008) Water quality variability and eutrophic trends in karstic tropical coastal lagoons of the Yucatán peninsula. Estuar Coast Shelf Sci. 2008; 76(2): 418–430. https://doi.org/10.1016/j.ecss.2007.07.025

**102.** Herrera-Silveira JA, Morales-Ojeda S. Evaluation of the health status of a coastal ecosystem in southeast México: Assessment of water quality, phytoplankton and submerged aquatic vegetation. Mar. Pollut. Bull. 2009; 59(1–3), 72–86. https://doi.org/10.1016/j.marpolbul.2008.11.017 PMID: 19157464

**103.** Hernández-Terrones L, Rebolledo-Vieyra M, Merino-Ibarra M, Soto M, Le-Cossec A, Monroy-Ríos E. Groundwater pollution in a karstic region (NE Yucatan): Baseline nutrient content and flux to coastal ecosystems. Water Air Soil Pollut. 2011; 218: 517–528. https://doi.org/10.1007/s11270-010-0664-x

**104.** De Anda V, Zapata-Peñasco I, Eguiarte LE, Souza V. Toward a Comprehensive Understanding of Environmental Perturbations in Microbial Mats from the Cuatro Cienegas Basin by Network Inference. In Ecosystem Ecology and Geochemistry of Cuatro Cienegas: How to Survive in an Extremely Oligotrophic Site, eds. Elser SV, Garc JF, editors. ía-Oliva ( Berlin: Springer). 2018. pp. 85–97. https://doi.org/10.1007/978-3-319-95855-2_7

**105.** Hunt DE, Ward CS. A network-based approach to disturbance transmission through microbial interactions. Front Microbiol. 2015; 6: 1182. https://doi.org/10.3389/fmicb.2015.01182 PMID: 26579091

## 9.5 Depth related structure and microbial composition of microbialites in a karst sinkhole, Cenote Azul, Mexico.

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# Depth Related Structure and Microbial Composition of Microbialites in a Karst Sinkhole, Cenote Azul, Mexico

Alfredo Yanez-Montalvo[a,b], Bernardo Águila[a], Selene Gómez-Acata[a], Martín Mass-Vargas[c], Nancy Cabanillas-Terán[d], Alejandro Vega-Zepeda[b], Humberto Bahena[b], Héctor Hernández-Arana[b], and Luisa I. Falcón[a]

[a]UNAM, Instituto de Ecología, Parque Científico y Tecnológico de Yucatán, Sierra Papacal, Yucatán, México; [b]El Colegio de la Frontera Sur, Departamento de Sistemática y Ecología Acuática, Chetumal, México; [c]Colegio de Bachilleres, Bacalar, México; [d]CONACYT, El Colegio de la Frontera Sur, Chetumal, México

**ABSTRACT**

Microbialites are sedimentary structures that represent modern models of the oldest life forms, stromatolites (<3.5 Ga), and are relevant for evolutionary and ecological studies. Cenote Azul is a deep (>90 m) karst sinkhole in the Yucatan peninsula characterized by microbialites that develop along its wall and hydrogeochemistry defined by the saturation of carbonate, sulfate and calcium ions. In this study, high throughput sequence analysis of 16S rRNA genes allowed characterization of the prokaryotic communities associated with microbialites in a depth profile. The most represented phyla were Proteobacteria (23.6–30.1%), Planctomycetes (11.6–13.8%), Cyanobacteria (9.7–16.5%), Acidobacteria (6.1–8.3%), Rokubacteria (4.1–7.8%), Chloroflexi (3.3–4.4%), Nitrospirae (3.5–4.6%), Actinobacteria (2.6–5%) Bacteroidetes (1.7–4.1%) and Thaumarchaeota (7.5–11.1%). Phylogenetic distance analyses described two distinct clusters of microbialites: Shallow (5 and 10 m) and Deep (20 and 30 m). The dominant diversity at the phylum level of the prokaryotic community described in this system is similar to that of other microbialites from different environments, but differences are reported at the classification level of order, family and genus. The mineral composition of the Cenote Azul microbialites has calcite as the main constituent mineral (~97%). Finally, this work establishes a baseline on the presence of microbialites and its relation to depth in the sinkholes of the Yucatan peninsula and stimulates the monitoring of these communities as a tool for the conservation of sites with high tourism pressure.

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## Introduction

Stromatolites constitute the oldest fossil record of life (~3500 MYA) dating back to the Archean eon (Grotzinger and Knoll 1999; Schopf 2006). Microbialites are rock-like structures, formed by microbial communities present in mineral-saturated water bodies. These communities have the physical capacity and metabolic ability to trap, bind and promote the precipitation of inorganic minerals (Burne and Moore 1987; Dupraz et al. 2011). Microbialites were abundant all over the planet during the Precambrian, participating in the oxidation of the atmosphere and the development of the biogeochemical cycles that allowed the evolution of eukaryotic life (Dupraz et al. 2009; Falkowski et al. 2008). Currently, the most extensive studies of marine microbialites are from Shark Bay in western Australia (Babilonia et al. 2018; Logan 1961) and Highborne Cay in Bahamas (Khodadad and Foster 2012; Reid et al. 1999), while lacustrine environments that harbor microbialites include Lake Van in Turkey (Kempe et al. 1991), Bacalar lagoon, Alchichica crater-lake and Cuatro Ciénegas in Mexico (Alcántara-Hernández et al. 2017; Beltrán et al. 2012; Centeno et al. 2012; Couradeau et al. 2011; Gischler et al. 2008; Valdespino-Castillo et al. 2018; Winsborough and Golubic 2007; Yanez-Montalvo et al. 2020), Pavilion Lake in Canada (Laval et al. 2000), among others (Chagas et al. 2016). The increase in studies of modern microbialites has allowed the exploration of the role of microbes in the formation and maintenance of these structures and to better understand the ancient record of life (Omelon et al. 2013).

Microbialite formation depends on the balance between physical, chemical and biological reactions (Dupraz et al. 2009), and the interaction of a wide variety of microbial metabolisms(Archaea/Bacteria) including phototrophs, methanogens, sulfur oxidizers, denitrifiers, and those involved in carbonate precipitation. In microbialites, microbes remain in a layer of exopolymeric substances (EPS) that provides adhesion, protection and adsorbed cations ($Ca^{2+}$, $Mg^{2+}$) (Braissant et al. 2007; Dupraz et al. 2004).

CONTACT Luisa I. Falcón falcon@ecologia.unam.mx UNAM, Instituto de Ecología, Parque Científico y Tecnológico de Yucatán, Sierra Papacal, Yucatán 97302, Mexico

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The organic mineralization occurs within the EPS, oxygenic photosynthesis contributes to create an alkaline environment that promotes the formation of carbonate nucleation sites (Dupraz et al. 2009; Obst et al. 2009; Paulo and Dittrich 2013). In the same EPS matrix, processes such as anoxygenic photosynthesis and sulfate reduction are involved in the precipitation of minerals (Braissant et al. 2007). In contrast, processes such as sulfide oxidation or fermentation, promote the dissolution of carbonates (Dupraz and Visscher 2005). Microbialite accretion is related to the biological and metabolic balance of microbial metabolisms.

Characterization of biological attributes and diversity allows a better understanding of microbialite formation and their responses to the environment, which is useful when considering these communities as bioindicators of environmental disturbance and health (Lindsay et al. 2017; McDevitt-Irwin et al. 2017; Meziti et al. 2016; Yanez-Montalvo et al. 2020). The presence of microbialites in freshwater bodies represents an important opportunity for ecological studies. These are long-lived sessile microbial assemblages, and therefore, are exposed to changes caused by intense anthropogenic activities (the increase of greenhouse gases and temperature, habitat modification and incorporation of nutrients, among others) (Dupraz et al. 2011; Foster et al. 2019; Lindsay et al. 2017).

Lacustrine and marine microbialites around the world are mainly constituted by Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes and Planctomycetes (Breitbart et al. 2009; Centeno et al. 2012; Chagas et al. 2016; Suosaari et al. 2016; Warden et al. 2016; White et al. 2015, 2016; Yanez-Montalvo et al. 2020). In general, microbialites that develop in different environments have a similar composition at the phylum level, but a unique composition at the genus and species levels, in relation to biogeographic processes, environmental conditions and habitat modification (Centeno et al. 2012; Valdespino-Castillo et al. 2018; Yanez-Montalvo et al. 2020). Additionally, stromatolites that develop in depth gradients, such as Alchichica (Mexico) and Pavilion (Canada) lakes, the microbial composition includes an the significant abundance of Acidobacteria, Nitrospirae and Archaea, has been documented (Russell et al. 2014; Valdespino-Castillo et al. 2014; White et al. 2016).

The Yucatan peninsula (YP) in Mexico, is a vast limestone platform with karstic environments, formed during the Miocene-Pleistocene, composed of limestone, dolomite, and anhydrite (Perry et al. 2009; Bauer-Gottwein et al. 2011). Perry et al. (2002) divided the YP into six hydrogeochemical/physiographic regions based on its geological characteristics. The karstic nature of each region is characterized by exceptional permeability due to the dissolution of the rock and connection of groundwater that flows through systems, where caves, canals and sinkholes intervene (Bauer-Gottwein et al. 2011). Sinkholes, also called cenotes (in Mayan, ts'onot), are formed by processes involving the collapse of subterranean caverns, with an opening in the surface, coupled with the dissolution of the limestone platform by carbonic acid (Cervantes-Martínez et al. 2009; Gabriel et al. 2009; Schmitter-Soto et al. 2002). Sinkhole research in the YP has focused on the hydrochemistry of waters, connectivity and zooplankton composition (Schmitter-Soto et al. 2002; Pérez-Ceballos et al. 2012; Montes-Ortiz and Elías-Gutiérrez 2018). However, no investigation about the microbial community composition of microbialites.

The eastern evaporative region of the YP is very peculiar and presents connectivity processes, in terms of hydrology, biogeochemistry and ecology (Perry et al. 2002). The Coastal Transversal Corridor is a hydrodynamic model for southern Quintana Roo (QR) proposed by Hernández-Arana et al. (2015) as a strategy for research and conservation of interconnected ecosystems including coral reefs, coastal lagoons, mangrove forests, floodplains, cenotes and karst lakes. In addition, this region has different aquatic environments that harbor microbialites, including Chetumal Bay, Muyil lagoon, Chichancanab lagoon and Bacalar lagoon (Beltrán et al. 2012; Centeno et al. 2012; Gischler et al. 2008; Johnson et al. 2018; Rasmussen et al. 1993; Valdespino-Castillo et al. 2018; Yanez-Montalvo et al. 2020). Bacalar lagoon is one of the freshwater sites with the highest presence of microbialites in the world (Gischler et al. 2008). In Bacalar lagoon, microbialites are located in reef patches throughout the 40 km of the lagoon and show spatial differentiation along a north-south gradient (Yanez-Montalvo et al. 2020). The Bacalar lagoon system has four sinkholes: Brujas, Esmeralda, Cocalitos and Xulha, within the main water body (Gischler et al. 2008, 2011) and the Cenote Azul (CA), located, on the land side, at a distance of approximately 200 m. This research focuses on the CA sinkhole, which has particular hydrogeochemical and biological characteristics (Perry et al. 2002) and where, Hernández-Arana et al. (2015) reported microbialites at a depth of 18 m. In this study, we aimed to explore the prokaryotic composition of the microbialites from the CA sinkhole, following a depth gradient. We hypothesize that changes in the structure and microbial composition of microbialites will be mainly related to depth as seen in other environments (Águila 2018; Russell et al. 2014). To this aim, we followed the next strategy: (a) describe the hydrogeochemical parameters of the water column, (b) analyze the mineral composition of the microbialites, and (c) describe the composition and structure of the microbialites following a high-throughput sequencing approach of the V4 hypervariable region of the 16S rRNA gene.

## Materials and methods

### *Study site*

The CA is an open karst sinkhole, located in Quintana Roo in southeastern YP, Mexico (18°38′48″N, 88°24′42″W). It is close (<200 m) to Bacalar lagoon (Figure 1), has a circular shape, with a depth >90 m, and a diameter of ~100 m, making it one of the largest sinkholes in the region (Cervantes-Martínez et al. 2009; Perry et al. 2002). Cenote Azul is considered an oligotrophic, well-mixed water system, with no halocline, and an average annual temperature of 29.2 ± 0.9 °C (Cervantes-Martínez et al. 2002; Schmitter-Soto et al. 2002).

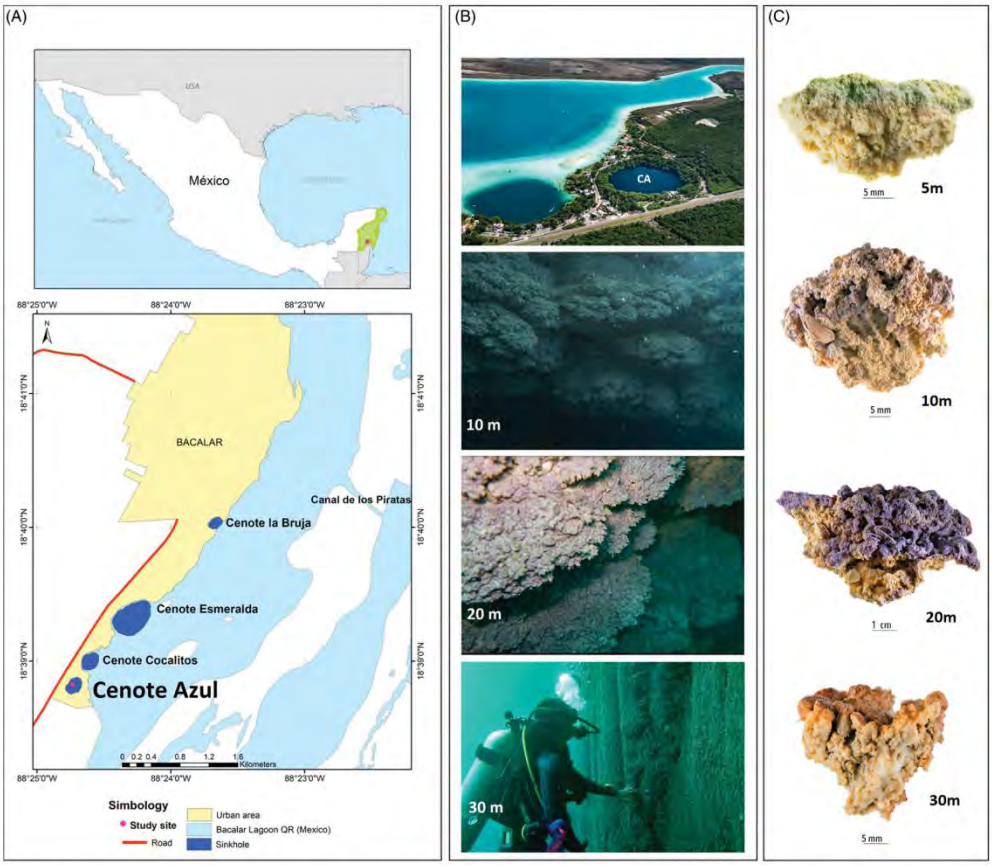


Figure 1. Geographical location of the sampling site. (a) Location of the study site and its separation in reference to Bacalar lagoon. (b) Panoramic view of the CA and photograph of the microbialites inside the sinkhole. (c) Photograph of microbialite fragments in the depth profile of the study.

### Sample collection

Microbialite samples were obtained in a bathymetric profile, at 30, 20, 10 and 5 m using autonomous diving equipment. At each depth, the microbialites had similar physical characteristics including color and texture. Five microbialite fragments ($10 \times 5 \times 2$ cm) were collected in each depth, with separation of approximately one meter per sample, using a sterile chisel and a sledgehammer (Supplementary Figure 1). The fragments were placed individually in nets previously labeled for each depth. Samples were transferred to sterile containers and stored in refrigeration until arrival in the laboratory. Water samples (2.5 L) were collected at each depth with a Niskin bottle and used for genetic (1 L) and physicochemical characterization (1.5 L). All samples were placed in refrigeration at 4 °C for transport to the laboratory and then stored in a freezer at −80 °C (microbialites). The transfer time of the samples from the workstation to storage (−80 °C) was less than two hours. All microbialites were sampled under collector permit PPF/DGOPA-113/14. Field studies did not involve endangered or protected species.

### Water chemistry analysis

Major cations and anions were analyzed in the Institute of Geology (UNAM, LANGEM-PT-LCL) by ion chromatography with conductometric detection on Waters 1525 binary HPLC pumps, autosampler (model No. 717) and conductivity detector (model No. 432). An IC-Pak Anion HR (Waters) stationary column was used with 5 µm amino packing. Anionic analysis used a mobile phase consisting of a mixture of acetonyl, butanol and a solution of gluconate/sodium borate in water with one mL/min flux rate. Major cations were run with a mobile phase consisting of dipicolinic acid and nitric acid (1.7 mM each) in water, with a flux

rate of 0.9 mL/min. Volume of sample inserted to the chromatograph was 10 µL. Temperature, pH, and conductivity were measured *in situ* with a multiparametric probe.

### X-Ray diffraction (XRD)

All determinations were done in the X-Ray Diffraction Laboratory, UNAM. An EMPYREAN Diffractometer equipped with an Fe filter, a cobalt thin tube focus and PIXcel3D detector was used to analyze the mineral composition of the microbialites. XRD analysis conditions were as follows: samples were homogenized with an agate mortar, sieved through a mesh <75 µm, and measured using an aluminum sample-holder (non-oriented fractions). All measures were carried in the angular range of $2\theta$ from 5° to 80° in step scanner mode with a "step scan" of 0.003° with a scan speed of 40 sec per step. The diffraction patterns were analyzed with the HighScore software (version 4.5) with reference patterns from the ICDDPDF-2 and ICSD databases.

### Total DNA extraction and 16S rRNA amplification

Microbialite samples were extracted using the DNeasy PowerSoil® Kit (Qiagen) following the manufacturer's protocols. Duplicate samples for each depth were used for the extraction ($n = 10$ extraction/depth). Total DNA was eluted in a final volume of 30 µl molecular grade water and stored at −20 °C prior to PCR amplification. DNA was verified by gel electrophoresis and amplified (prokaryotic hypervariable region V4, 16S rRNA) using primers 515 F/806R, according to established protocols (Caporaso et al. 2011). PCR amplification conditions were performed in three independent reactions applied to each sample. All PCRs were run with the following program: 98 °C for 30 s followed by 35 cycles of 95 °C for 30 s, 52 °C for 40 s, and 72 °C for 90 s, with a final elongation step of 12 min at 72 °C, and kept at 4 °C. PCR products were pooled and purified with Ampliclean magnetic beads (NimaGen, NDL). The quantification of the purified amplicon library was performed with a QUBIT® fluorometer (Promega, USA). The final concentration of libraries per sample was 20 ng/µl and were sequenced on a 2 × 300 Illumina MiSeq platform (Yale Center for Genome Analysis, CT, USA).

Water from each sampling depth (1 Lt) was filtered into 0.22 µM DURAPORE (Millipore) membranes and total DNA was extracted following the protocol of the DNeasy PowerWater® Kit (Qiagen). All used membranes per depth were pooled together to obtain a representative composition. DNA was extracted, the 16S rRNA region V4 and amplified and sequenced as mentioned above.

### Bioinformatic processing of illumina 16S rRNA sequences and biostatistical analysis

The sequences generated in this study (V4 region, 16S rRNA) belonging to 40 microbialite samples (10 samples/depth) and four water column samples have been submitted to the Sequence Read Archive (SRA, Leinonen et al. 2011) and deposited in GenBank under BioProject PRJNA630412. Raw sequences were imported and processed in QIIME2 (QIIME 2, v.2018.6) (Bolyen et al. 2019), sequences were clustered into ASVs and were assigned taxonomy using the SILVA database (release 132–99% OTUs, 515–806 region) (Quast et al. 2013). Based on quality plots, forward and reverse reads were truncated at their 3′ end at the 200 sequencing positions, respectively. Chimeric sequences were removed using the dada2 pipeline (Callahan et al. 2016). ASV's (amplicon sequence variants) were grouped to 100% sequence similarity. Sequences were aligned using MAFFT (Katoh and Standley 2013), and rooted trees were constructed using FastTree for analysis of phylogenetic diversity (Price et al. 2009).

Subsequently, all sequence data were analyzed in the R statistical environment (version 3.6.3), with Phyloseq R (McMurdie and Holmes 2013), ggplot2 (v 2.1.0) (Ginestet 2011), and vegan (v2.3-3) (Oksanen et al. 2016), ampvis2 (Andersen et al. 2018) and BiodiversityR (Kindt and Kindt 2019) packages were used for data visualization and statistical testing. ASVs representing less than 1000 sequences across the dataset, all singletons plus chloroplast and mitochondrial sequence reads were eliminated using customized R scripts.

The prokaryotic (Bacteria + Archaea) genetic composition of microbialites was described along the depth profile, using sequences representing >2% of relative abundance at the phylum level. The biological diversity used to determine the similarity in the community structure of microbialites was explored using the alpha diversity metrics (Shannon and Simpson, observed ASV's and Chao1). Beta diversity was analyzed using a matrix of community composition (ASV's matrix) to obtain the community turnover along the depth profile. The dissimilarity in community structure between samples (Beta diversity) was evaluated with a Principal Coordinates Analysis (PCoA) with weighted UniFrac distance metrics (Lozupone et al. 2011) and with the Local Contribution to Beta Diversity (LCBD) metrics proposed by Legendre and De Cáceres (2013). Venn diagrams were constructed using the software Venny 2.1 (Oliveros 2007–2015). To visually inspect the structure of the prokaryotic community in the depth profile, a heatmap was generated by pheatmap (R package 1.0.7) (Kolde and Kolde 2015). For heatmap analysis, normalized counts were log transformed with the DEseq2 package (Love et al. 2014). Additionally, the composition of prokaryotes in the water column was analyzed (Supplementary Figure 2) and the sequences were deposited in BioProject PRJNA631060.

### Statistical analysis

Discrimination between prokaryotic community composition along the depth gradient were resolved with a Canonical Analysis of Principal Coordinates (Anderson and Robinson 2003; Anderson and Willis 2003) and their *a priori* classification according to the depth profile, based on Bray–Curtis dissimilarities and 999 permutations. All the indices for alpha diversity were compared using tests of

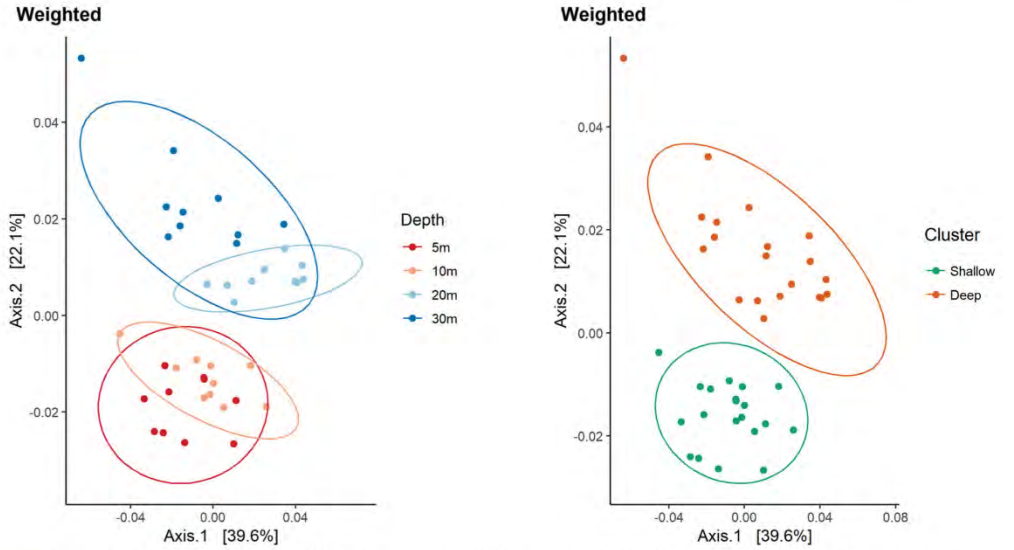


Figure 2. The analysis of the main coordinates (PCoA) based on the weighted UniFrac distances. (a) Microbialites in the depth profile. (b) Separation into two clusters of microbialites according to their phylogenetic assemblage.

Mann–Whitney–Wilcoxon. For beta-diversity, PERMANOVA analyses were performed using the Adonis function of the vegan package, based on the weighted UniFrac matrix. All correlations and tests with p-value $< 0.05$ were considered significant after 999 permutations.

## Results

### *Hydrogeochemical characteristics of the Cenote Azul sinkhole*

The physico-chemical parameters of the water column along the sampled depth profile are summarized in Supplementary Table 1. CA exhibited a homogeneous composition in all parameters with a range of values for pH (7.9–8.5), conductivity (2.31–2.29 µS/cm) and total dissolved solids (1.12–1.3 ppt) typical of the YP groundwater (Perry et al. 2002, 2012; Sánchez-Sánchez et al. 2015). The ions in the water column also had homogeneous values $SO_4^{2-}$ >$HCO_3^-$ >$Cl^-$ >$NO_3^-$ and $Ca^{2+}$ >$Mg^{2+}$ >$Na^+$ >$K^+$ (Supplementary Tables 2 and 3). Nitrates were only detected at 5 m ($NO_3^-$ = 28.1 mg/L) along with the highest value for chlorine (95.2 mg/L). Some ions and compounds were not detected. For these, the following detection limit ranges were used: ammonium (LD = 0.2 mg/L), nitrites (LD = 2 mg/L), phosphates (LD = 0.2 mg/L), bromine (LD = 0.8 mg/L), fluorine (LD = 5 mg/L). No significant gradient in hydrogeochemical parameters was recorded.

### *Mineralogy of microbialites of Cenote Azul*

The mineral composition of the microbialites in the depth profile are shown in Supplementary Table 4. Microbialites from 5 m were not as hard as the rest (10–30 m). Calcite made up 90–97% of the mineral composition of all microbialites. Traces of other minerals were found including quartz and hematite, with a smaller percentage of magnesite, while rhodochrosite was detected only at 10 m.

### *Diversity and structure of the prokaryotic community of the microbialites of Cenote Azul*

An initial dataset of 7,080,965 16S rRNA sequences was recovered, where a total of 5,032,627 high-quality reads were obtained from the 40 samples after quality filtering, ranging from 15,458 to 420,654 with a mean of 127,002 reads per sample. A total of 19,306 ASVs were observed for all samples. Water column samples had an initial dataset of 223,336 16S rRNA sequences; a total of 91,288 high-quality reads were obtained from eight samples after quality filtering, ranging from 6,134 to 17,143 with a mean of 11,829 reads per sample. A total of 1,687 ASVs were registered for water column samples (Supplementary Figure 2), which are different in composition to microbialites.

Principal coordinate analysis (PCoAs) using weighted UniFrac distances were performed to investigate the community structure of the microbialites (Figure 2). Based on the PCoAs, the weighted UniFrac matrix explained up to 61.7% of the variation in genetic composition related to depth (Figure 2(a)) and forming two clusters: Shallow (5 and 10 m) and Deep (20 and 30 m) (Figure 2(b)). To statistically support the observed clustering of the prokaryotic diversity in the above PCoAs, the communities of the two profiles were examined with a PERMANOVA test using the weighted UniFrac matrix (Supplementary Table 5). The

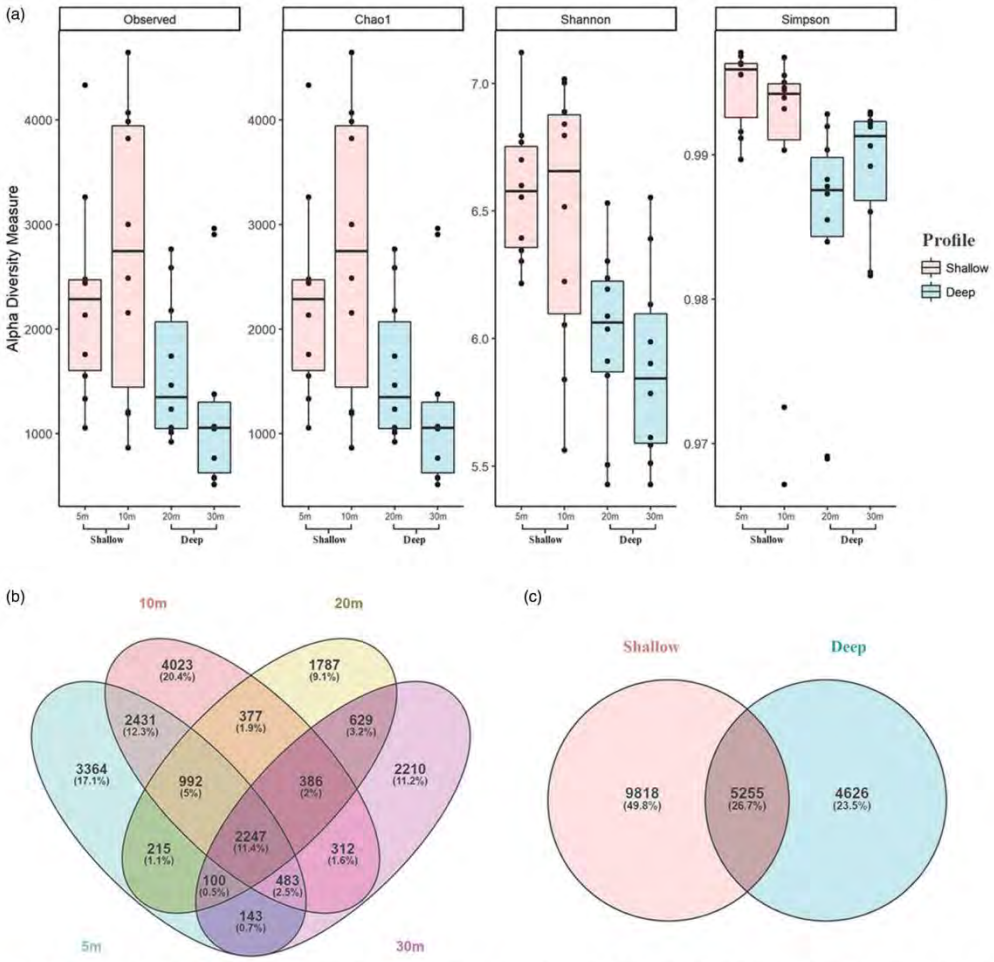


**Figure 3.** Diversity indices and the ASVs shared between the clusters. (a) Boxplot representation of the $\alpha$-diversity and richness metrics for communities at depth profile and cluster. (b) Venn diagrams of unique and shared ASVs in the depth profile and (c) in depth cluster.

microbialites from the four sampled depths were distributed in high percentages for the first two components and gave significantly different results ($p < 0.05$), suggesting depth as an explanatory factor in the structure of the microbialite genetic diversity (Figure 2(a)). Notably, the cluster distribution between Shallow and Deep was also significant ($p < 0.05$) supporting these depth clusters, which also have different phylogenetic assemblages (Figure 2(b)).

A classification analysis (CAPdiscrim) based on the composition in the different sampling points (5,102,030 m) showed an *a priori* classification success of the samples, in significant proportions of 93% (Supplementary Table 5). The exploration of community diversity and distribution is presented in Figure 3. Shannon, Simpson, Observed and Chao1 indexes were calculated to estimate and compare richness and diversity in all microbialites (Figure 3(a)), which were significantly different ($p < 0.05$) between the Shallow and Deep clusters.

Beta diversity analysis showed a change in the community structure between Shallow and Deep clusters, first visualized in the PCoA based on the UniFrac matrix, and later corroborated through the LCBD index ($p < 0.05$). Additionally, the LCBD index showed that the most significant changes occur in the prokaryotic assemblages at depths of 5 and 30 m, $p < 0.05$. The microbialites sampled at 10 m showed the highest values of diversity and richness. Venn diagram allowed us to represent the core of ASVs, i.e., those ASVs that are shared in all microbialites, was represented by

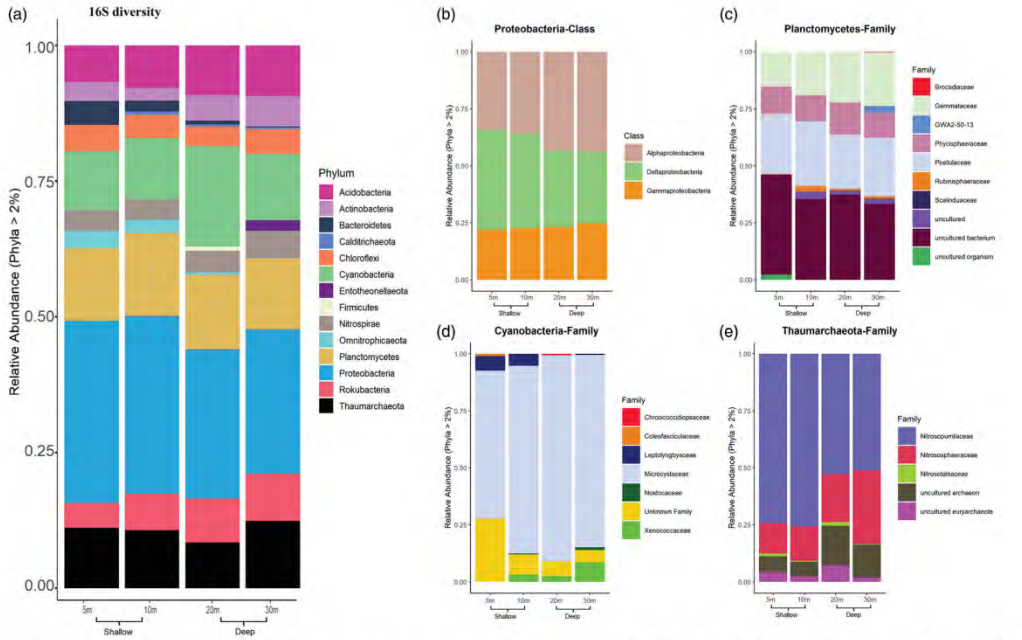


**Figure 4.** Distribution and relative abundance of the dominant prokaryotic communities from microbialites. Classified at the (a) phylum level; (b) Proteobacteria (class level). (c) Planctomycetes (family level). (d) Cyanobacteria (family level), (e) Thaumarchaeota (family level). All recovered sequences grouped in ASVs with a frequency higher than 2%.

11.4% (Figure 3(b)), where microbialites at 10 m have the highest percentage of shared ASVs (20.4%), while the lowest percentage is at 20 m (9.1%). Further, the ASVs representing the Shallow profile (49.8%) was higher than the deep profile and the core between clusters was 26.7% (Figure 3(c)).

A total of 54 different prokaryotic phyla constitute the microbiome associated with microbialites in CA. The core composition was represented (>2% abundance) by Proteobacteria (23.6–30.1%), Planctomycetes (11.6–13.8%), Cyanobacteria (9.7–16.5%), Acidobacteria (6.1–8.3%) Rokubacteria (4.1–7.8%), Chloroflexi (3.3–4.4%), Nitrospirae (3.5–4.6%), Actinobacteria and (2.6–5%) Bacteroidetes (1.7–4.1%) with Thaumarchaeota (7.5–11.1%) as the dominant archaea, all of them representing a total of 85.7% of the prokaryotic community (Figure 4). The core of the prokaryotic community composition is homogeneously distributed, where only some phyla show greater abundance, for example, Proteobacteria, Bacteroidetes and Omnitriophicaeota in the Shallow profile microbialites, and for the Deep microbialites including Cyanobacteria, Acidobacteria, Rokubacteria and Actinobacteria. The other phyla such as Thaumarchaeota, Planctomycetes and Chloroflexi are contributing similarly to both depth clusters.

Within Proteobacteria, Alpha (39.3%), Delta (37.6%) and Gamma (23.2%) were the dominant classes (Figure 4(b)). Planctomycetes groups a vast diversity, including previously uncultured organisms that constitute close to half the diversity within this phylum. The deepest microbialites (30 m) exhibit the presence of Brocadiaceae, which could suggest the relevance of anammox in the system (Figure 4(c)). Cyanobacteria showed dominance of Microcystaceae (approx 70%), followed by a previously undescribed family, which shall merit further investigation, and members of Xenococcaceae which increased with depth (Figure 4(d)). Another relevant component at the phylum level is the Thaumarchaeota (Archaea domain), in which Nitrosopumilaceae and Nitrososphaeraceae, represented up to 80% of the abundance (Figure 4(e)).

### *Prokaryotic community similarities*

Changes in the structure of the prokaryotic community were represented in a hierarchical heat map (Figure 5(a)) that shows the differential abundances between the clusters (Shallow and Deep). A hierarchical dendrogram groups the samples in the depth profile and shows the grouping of the proposed clusters (Shallow and Deep). A mean sea-level line, based on the Caribbean sea-level curve proposed by Toscano and Macintyre (2003) was included to relate microbialites found in the depth profile to possible timelines of formation for these structures (Figure 5). The geomorphology of the microbialite wall in the CA sinkhole (Figure 5(b)) is represented based on the divers observations.

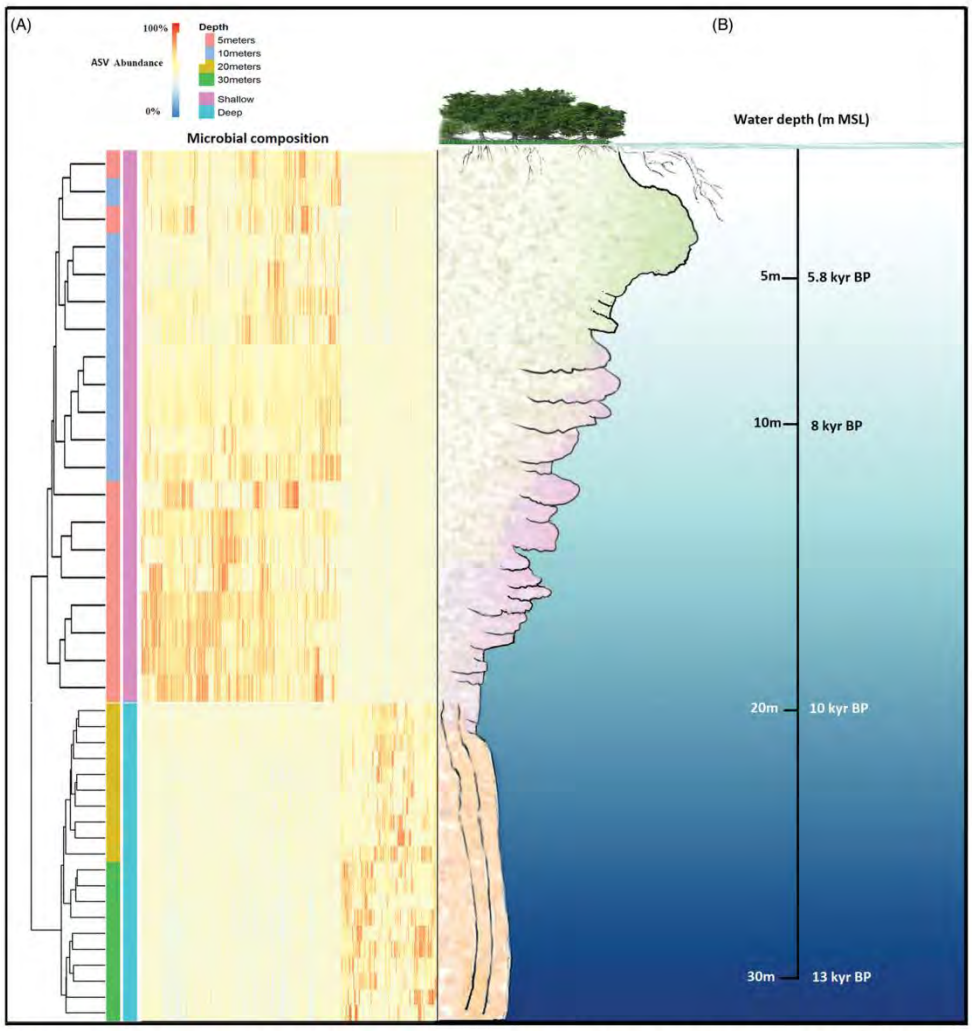


**Figure 5.** Structure of the prokaryotic community and the geomorphic representation of the wall of microbialites in the Cenote Azul sinkhole. (a) Heat map generated with DESeq2 that represents the community in the Cenote Azul sinkhole. The hierarchical dendrogram shows the differentiation of the community in the four sampling sites (5 m, 10 m, 20 m and 30 m) and the two depth profiles (Shallow and Deep). (b) Geomorphic representation of the microbialite wall and the distribution of the prokaryotic community. The line representing mean sea level was generated based on the local curve of Caribbean sea-level from Toscano and Macintyre (2003).

## Discussion

This is the first report on the microbialites that develop in the Cenote Azul (CA) sinkhole providing a baseline on microbialite research in the region that opens the opportunity to monitor an extreme karst system, located in an area of accelerated tourism growth. This study corroborated the presence of a wall of microbialites in the CA (Hernández-Arana et al. 2015), a karstic sinkhole, which extends >90 m depth and found that the prokaryotic communities of the microbialites are phylogenetically associated in two clusters designated as Shallow (5 and 10 m) and Deep (20 and 30 m). While these microbialites depicted a depth related gradient, as noted in the hypothesis, additionally we suggest that differences in microbial assemblages in CA may be associated with local impacts of natural and anthropogenic surface processes, as well as, with the geomorphology, landscape evolution and the hydrological history of sea level in the southeast of the YP.

CA is a unique system for the study of microbialites along a depth gradient, with dissolved oxygen and

deuterium concentrations near the meteoric water line, creating slow evaporation processes (Perry et al. 2002, 2009). The hydrogeochemical variables indicate disconnection with the local coastal aquifer suggesting this sinkhole is recharged from a remote area as far as Esmeralda and Chichancanab lagoons (Perry et al. 2002). Saturation of sulfate ions, bicarbonates, calcium, and the unusual presence of chlorine, coincide with those reported for the water systems in the area, including Bacalar Lagoon (Castro-Contreras et al. 2014; Perry et al. 2012).

Calcite ($CaCO_3$) constitutes 90–97% of the mineral composition of microbialites, corroborating previous studies, where microbialites, in the southern region of QR, are reported to be composed mostly by calcium carbonate (Valdespino-Castillo et al. 2018; Yanez-Montalvo et al. 2020). In the YP, it is common for water bodies such as cenotes (sinkholes), lagoons and the aquifer to be saturated with bicarbonate ions as a consequence of the processes of dissolution of the limestone (Perry et al. 2002). Valdespino-Castillo et al. (2018) suggest through SEM observations coupled to SR-FTIR imaging that calcite-rich microbialites in Bacalar lagoon are associated with organic structures formed of lipid and protein amides that deposit in the organomineral fabrics. The mineral composition of the CA sinkhole analyzed does not differ from the microbialites in Bacalar lagoon, which have the highest content of $C_{org}$ reported for these structures in the YP. Further research is required to better understand the dynamics of carbonate precipitation and formation in depth microbialites. Calcite, the main mineral of CA microbialites, occurs through the precipitation of calcium carbonate, which requires supersaturation of the carbonate ion in the system, availability of $Ca^{2+}$ ion and the appearance of nucleation centers in the EPS, produced mainly by Cyanobacteria (Benzerara et al. 2014; Dupraz et al. 2009; Dupraz and Visscher 2005; Obst et al. 2009). Other bacterial phyla such as Chloroflexi, Alpha-, Delta-proteobacteria and Planctomycetes present in CA microbialites, have been described in microbialites and are postulated to promote the precipitation of carbonate minerals through photosynthesis (oxygenic and anoxygenic) and sulfate reduction (Bundeleva et al. 2012; Gallagher et al. 2012; Visscher et al. 2000).

The main phyla reported as constituents of CA microbialites (Proteobacteria, Planctomycetes, Cyanobacteria, Thaumarchaeota, Rokubacteria, Chloroflexi, Nitrospirae, Actinobacteria and Bacteroidetes) (Figure 4) are similar to those reported around the world for both marine: Highborne Cay, Bahamas (Baumgartner et al. 2009), Shark Bay, Australia (Suosaari et al. 2016) and freshwater microbialites: Cuatro Ciénegas, Mexico (Souza et al. 2012), Salar de Atacama, Chile (Farías et al. 2014), as well as those reported in depth such as Lake Van, Turkey (López-García et al. 2005), Pavilion lake, Canada (Russell et al. 2014), Lake Alchichica, Mexico (Centeno et al. 2012; Couradeau et al. 2011), including the microbialite reef that develops in Bacalar lagoon (Centeno et al. 2012; Johnson et al. 2018; Valdespino-Castillo et al. 2018; Yanez-Montalvo et al. 2020). Although the spatial arrangement based on phylogenetic distance (weighted UniFrac) indicates that there are two large clusters (Shallow and Deep), when we analyze their composition at higher taxonomic levels, these differences are not appreciable, thus suggesting that their composition is similar at the phylum level, but the greatest differences are found at the genus and species level (Figure 4), this idea has been discussed previously (Centeno et al. 2012; De Anda et al. 2018; Lindsay et al. 2017; Yanez-Montalvo et al. 2020). In this study, the CA does not present an environmental gradient along the depth profile related to hydrogeochemistry, but previous research has suggested that light availability and temperature decline rapidly after 10 m depth in YP open sinkhole systems (Cervantes-Martínez et al. 2002; Schmitter-Soto et al. 2002).

In microbialites, oxygenated photosynthesis by cyanobacteria is a common metabolic process for carbonate precipitation in freshwater and marine environments (Chagas et al. 2016). Cyanobacteria, both filamentous and unicellular, have been found to be more abundant in thrombolytic-type microbialites (Chagas et al. 2016). Thrombolites are the main types of microbialites reported for Bacalar lagoon (Beltrán et al. 2012; Centeno et al. 2012; Johnson et al. 2018; Valdespino-Castillo et al. 2018; Yanez-Montalvo et al. 2020) and this study. CA microbialites have a strong component of unicellular colonial Microscyteceae (Figure 4(d)). Two sequences of this family, (uncultured) are among the 10 most abundant genus-level taxa (Supplementary Figure 3). These sequences may belong to an uncultured *Cyanothece* sp. or *Aphanothece* sp., which were observed abundantly by optical microscopy as endoliths in all microbialite samples. Microscyteceae are reported to be efficient calcifiers (Bundeleva et al. 2014). Other families such as Chroococcidiospidaceae are tolerant to high amounts of UV radiation and extreme temperatures (Das and Singh 2017; Lacap-Bugler et al. 2017) and were found in the Deep microbialite cluster. Coleofasciculaceae, a family within Oscillatoriales, was found only in Shallow samples, along with a high percentage of unclassified sequences that could be affiliated to filamentous Synechococcales which were confirmed with microscopy. Leptolyngbyaceae was more abundant in the Shallow CA-microbialites, while Pleurocapsales were more abundant in the Deep microbialites. Pleurocapsales have been reported to be important for carbonate mineral precipitation and microbialite formation in some oligotrophic lake systems (Gérard et al. 2013; Power et al. 2011). *Scytonema* sp., was also common in Shallow samples. This distribution pattern of species of the family Leptolyngbyaceae in Shallow microbialites and the change toward species of the family Xenococcaceae in the Deep profiles, is a phenomenon reported in microbialites from Lake Alchichica (Águila 2018; Saghaï et al. 2015) and Lake Pavilion (Russell et al. 2014).

Cyanobacteria and Proteobacteria are the dominant phyla in microbialites (Baumgartner et al. 2009; Foster et al. 2009; Khodadad and Foster 2012), and together comprised approximately 50% of the prokaryotic diversity in CA microbialites, regardless of depth. Delta- (41–43%) and Alpha-proteobacteria (43%) are the most abundant classes,

in Shallow and Deep profiles, respectively, while Gammaproteobacteria (22–24%) are distributed homogeneously, these groups were also dominant in Pavilion Lake microbialites, a similar karst system (Russell et al. 2014). Certainly, Cyanobacteria and Proteobacteria are the major known diazotrophs in these communities, where the potential for $N_2$-fixation becomes relevant to sustain these complex microbial assemblages (Alcántara-Hernández et al. 2017; Beltrán et al. 2012). Bacalar lagoon microbialites are known to fix $N_2$ during the day, suggesting the relevance of cyanobacterial heterocyst-forming filamentous colonies (Beltrán et al. 2012). CA microbialites harbor Nostocales corresponding to *Fischerella* sp., but probably many other $N_2$-fixers are part of these assemblages.

Alpha-proteobacteria are a group of bacteria with a high metabolic diversity (Havemann and Foster 2008; Ley et al. 2006). Alphaproteobacteria with anoxygenic and heterotrophic phototrophic metabolisms are among the most abundant in microbialites (Gérard et al. 2013). For CA, Rhizobiales, non-sulfur purple photoheterotrophic bacteria, were very abundant (Supplementary Figure 4). This group has been reported to play an important role in Highborne Cay and Shark Bay microbialites (Foster and Green 2011). Alpha-proteobacteria have also been studied for the P-assimilation potential in microbialites, in conjunction with Acidobacteria (Valdespino-Castillo et al. 2014), another common constituent of CA microbialites. Another relevant anoxygenic phototroph, Chloroflexi (Shih et al. 2017) was a constant component of microbialites along all sampled depths in CA microbialites.

Further, Delta-proteobacteria, host the group of sulfate-reducing bacteria (SRB), which reduce sulfate to sulfur, oxidize organic carbon to bicarbonate, and contribute to form an alkaline environment within microbialite fabrics, which in turn is conducive to carbonate mineral precipitation (Baumgartner et al. 2006; Couradeau et al. 2011). Deltaproteobacteria were present in all CA microbiates. Sulfur-cycling Deltaproteobacteria are also typically associated with microbialites and their surrounding sediments (Warden et al. 2016; White et al. 2016). Sulfate reducing bacteria are observed in close proximity to cyanobacteria in oxic portions of hypersaline mats in Guerrero Negro Lagoon, Baja California, Mexico (Baumgartner et al. 2006; Canfield and Des Marais 1991), and in the Bahamas (Reid et al. 2000) suggesting tight biogeochemical cycling between these two groups of organisms (Visscher et al. 2000; Visscher and Stolz 2005). The most abundant Deltaproteobacteria in CA microbialites included several NB1-j, Myxococcales, Desulfarculales and Syntrophobacterales (Supplementary Figure 4). Most sulfate reducers are found in the Deltaproteobacteria class and additionally, some Firmicutes, Nitrospirae, and Archaea are known as sulfate-reducers (Loy et al. 2002; Muyzer and Stams 2008).

In the CA microbialites, distribution of the abundance of the Gammaproteobacteria is independent of the depth profile (Figure 4(b)). Bacteroidetes and sulfate-reducing (Deltaproteobacteria) are reported in microbialites as well as Alpha-proteobacteria and Planctomycetes (Centeno et al. 2012; Couradeau et al. 2011). Betaproteobacteria, Delta- and Gamma-proteobacteria, Actinobacteria, Acidobacteria, Nitrospirae, and Flavobacteria are observed in Pavilion Lake microbialites (Russell et al. 2014). Cuatro Ciénegas microbialites also harbor populations of Delta- and Gamma-proteobacteria and Nitrospirae (Breitbart et al. 2009; Nitti et al. 2012), another group with possible relevance to N-cycling. Nitrospirae, a phylum involved in the nitrification process, was a constant component of CA microbialites. So far, we do not know if the *Nitrospira*, found in great abundance in the microbialites of CA (Figure 4), has the potential to do complete nitrification (Comammox), which will be clarified when a metagenomic approach is followed in this system (Ehrich et al. 1995; Koch et al. 2019). *Nitrospira* were also found along the depth gradient, constituting approx. 25% of accounted diversity. Planctomycetes were represented by approx. 50% of previously undescribed groups. We found an abundance of families that have been described as having interesting metabolisms and in-depth sample studies, such as Gemmataceae, a family of aerobic chemo-organotrophs (Kulichevskaya et al. 2020), Pirellulaceae, which are ammonia oxidizers and Phycisphaeraceae are the main components (Kellogg 2019; Mohamed et al. 2010). Interestingly, Brocadiaceae, reported as a family with known anammox bacteria (Pereira et al. 2017), were detected in the microbialites from 30 m.

Thaumarchaeota, the most abundant archaea known on Earth, includes all recognized archaeal ammonia oxidizers (Kimble et al. 2018). This archaeal phylum is distributed in all the depths of the CA, and its main families (Nitrosopumilaceae, Nitrososphaeraceae), have been recorded as nitrifying groups (Wong et al. 2015). This phylum has been reported in oligotrophic aquatic environments such as caves and also in microbialites in Lake Salda (Turkey) and thrombolites in Highborne Cay, Bahamas (Balci et al. 2020; Mobberley et al. 2015). Interestingly Rokubacteria was found in all samples, increasing their abundances toward 30 m. Rokubacteria is a recently described group, with a potential role in methane oxidation, reported to be found in soils, the rhizosphere, volcanic muds, oil wells, and aquifers (Chernov et al. 2019; Kroeger et al. 2018). They have small cells with large genomes with a high percentage of GC and with general metabolic strategies (mixotrophic) in oligotrophic environments; they also have a high genomic heterogeneity between individuals (Becraft et al. 2017).

CA is a deep sinkhole (>90 m), and we do not know how much deeper microbialites develop along its wall. Similar systems, such as Pavilion lake (Canada), Alchichica crater lake (Mexico) and Lake Van (Turkey) have already reported the presence of microbialites in depths up to 45 m (Águila 2018; López-García et al. 2005; Russell et al. 2014). Interestingly, CA microbialites were different in composition and abundance with the presence of groups such as Acidobacteria, Nitrospirae, Rokubacteria and Thaumarchaeota; the genetic diversity was higher for CA microbialites ($H' = 5.9–6.5$), compared to those of Bacalar lagoon ($H' = 3.3–5.7$), a system separated to CA by 200 m

(Yanez-Montalvo et al. 2020). The particularity of CA in comparison to Bacalar lagoon was manifested in a comparative study of zooplankton, where it showed the presence of species exclusive to CA, and concluded that these systems had low connectivity, despite their proximity (Montes-Ortiz and Elías-Gutiérrez 2018).

The phylogenetic separation of the prokaryotic communities along the depth gradient found in CA microbialites might be related to the sea-level variation that YP has experienced (Figure 5). The current structure of YP was reached in the Pleistocene era (López-Ramos 1975; Torrescano-Valle and Folan 2015); during the last glacial maximum (LGM), and different eustatic changes have been experienced at spatial and temporal scales, where the sea-level was lower than present (about 130 m) (Back and Hanshaw 1970; Ward and Halley 1985). Several studies have described the possible evolution of sea-level in the Caribbean during the Holocene, their measurements were based on approximately 737 points covering a period of 12 ky BP, taken from samples of mangrove peat, microbial mats, beach rock and corals (Cuevas et al. 2008; Gabriel et al. 2009; Khan et al. 2017). The Holocene records of sea-level have been highly variable, depending on the coastal environment features and its relation to sinkholes. Numerous works have described coastal environmental changes that influence sea-level in sinkholes in northern and southern QR including the Rio Hondo region and Chetumal Bay, near the Belizean border (Torrescano and Islebe 2006; Gabriel et al. 2009; Aragón-Moreno et al. 2018). The works carried out for the QR area agree with those indicated in the calibrated sea-level curve of Toscano and Macintyre (2003). Based on sea level changes and environmental coastal interactions with sinkholes which indicates that sea-level 10 ky BP was around −25 to −30 m, and may indicate that the microbialites that develop >30 m in the CA are older (Deep profile) than those in the Shallow cluster. The communities at 30 m have had to adapt to the changing ecological conditions, bathymetry and light intensity and hydrogeochemical changes throughout the Holocene period, but also the accretion rate of these communities have balanced with the sea-level changes as we can tell due to the continuous microbialite structure along the depth gradient.

In this study of microbialites occurring in the CA, most of the variables measured in the water column were homogeneous. Only in the most surficial sample (5 m), where the microbialites were more friable and green color (Figure 1), the environmental conditions varied by the presence of the nitrate and an increase in chlorine ions. The presence of these ions is in concentrations comparable to Bacalar lagoon, and their values may be associated with natural runoff and anthropogenic activities in the region. The depth of 10 m has more solid microbialites and lavender color, it is the maximum depth where the Secchi disk is observed. Previous research has suggested that the availability of light and temperature decreases rapidly after 10 m depth in YP open-sink systems (Schmitter-Soto et al. 2002; Cervantes-Martínez et al. 2002). The available light and other environmental conditions can make the 10 m niche conducive to a more transient microbial assembly, since at this depth the highest number of ASVs were detected.

As a whole, this study proposes that the Shallow profile (5 and 10 m) is a dynamic ecosystem of environmental variables, the microbial assemblies at this depth gradient present greater biological interactions and environmental forces. Interestingly, the Deep profile (20 and 30 m), is a habitat with a stable dynamism, the mean alpha diversity, richness and particulate ASVs were lower compared to the Shallow profile (Figure 3). We consider that a prokaryotic community acclimated to the natural and historical conditions of the site is maintained in this range, constituting an extreme niche due to its oligotrophic conditions, hydrogeochemistry and presenting practically no recent anthropogenic alterations. It is recognized that microbial assemblages from extreme environments contain community members that may represent autochthonous or indigenous species, adapted to the set of physical, chemical and biological parameters of the site (Panikov 2010).

Microbialites in the CA sinkhole support a diversity of prokaryotic associated with the biogeochemical cycles of N, C, S and P. We consider that the good health status (based on diversity and species composition) of the microbialites in the CA responds to the current water quality. The model of microbialites as bioindicators of microbial communities becomes relevant in systems such as CA, which are in regions with intense human activities including tourism, lack of wastewater treatment and agriculture based on an intense use of synthetic fertilizer compounds. Generating the basic knowledge on the prokaryotic composition associated with CA microbialites (in southern QR) provides a guideline for future work focused on their metabolic potential and how these communities respond to changing temporalities, anthropogenic regional pressures and global climate change, which will allow the development of effective strategies for their management and sustainability.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## References


Águila B. 2018. Caracterización de cianobacterias en microbialitas del lago cráter Alchichica en un gradiente de profundidad. Dissertation, National Autonomous University of Mexico (UNAM), México.

Alcántara-Hernández RJ, Valdespino-Castillo PM, Centeno CM, Alcocer J, Merino-Ibarra M, Falcón LI. 2017. Genetic diversity associated with N-cycle pathways in microbialites from Lake Alchichica Mexico. Aquat Microb Ecol 78(2):121–133.

Andersen SK, Kirkegaard RH, Karst SM, Albertsen M. 2018. ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. bioRxiv. 10–11.

Anderson MJ, Robinson J. 2003. Generalized discriminant analysis based on distances. Aust NZ J Stat 45(3):301–318.

Anderson MJ, Willis TJ. 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84(2):511–525.

Aragón-Moreno AA, Islebe GA, Torrescano-Valle N, Arellano-Verdejo J. 2018. Middle and late Holocene mangrove dynamics of the Yucatan Peninsula, Mexico. J S Am Earth Sci 85:307–311.

Babilonia J, Conesa A, Casaburi G, Pereira C, Louyakis AS, Reid RP, Foster JS. 2018. Comparative metagenomics provides insight into the ecosystem functioning of the Shark Bay Stromatolites, Western Australia. Front Microbiol 9:1359.

Back W, Hanshaw B. 1970. Comparison of chemical hydrogeology of the carbonate peninsulas of Florida and Yucatan. J Hydrol 10(4): 330–368.

Balci N, Gunes Y, Kaiser J, On SA, Eris K, Garczynski B, Horgan BH. 2020. Biotic and abiotic imprints on Mg–rich stromatolites: lessons from Lake Salda SW Turkey. Geomicrobiol J 37:1–25.

Bauer-Gottwein P, Gondwe BR, Charvet G, Marín LE, Rebolledo–Vieyra M, Merediz–Alonso G. 2011. The Yucatán Peninsula karst aquifer Mexico. Hydrogeol J 19(3):507–524.

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop KM, Visscher PT. 2006. Sulfate reducing bacteria in microbial mats: changing paradigms new discoveries. Sediment Geol 185(3–4):131–145.

Baumgartner LK, Spear JR, Buckley DH, Pace NR, Reid RP, Dupraz C, Visscher PT. 2009. Microbial diversity in modern marine stromatolites, Highborne Cay, Bahamas. Environ Microbiol 11(10): 2710–2719.

Becraft ED, Woyke T, Jarett J, Ivanova N, Godoy-Vitorino F, Poulton N, Brown JM, Brown J, Lau MCY, Onstott T, et al. 2017. Rokubacteria: genomic giants among the uncultured bacterial phyla. Front Microbiol 8:2264.

Beltrán Y, Centeno CM, García–Oliva F, Legendre P, Falcón LI. 2012. N2 fixation rates and associated diversity (nifH) of microbialite and mat–forming consortia from different aquatic environments in Mexico. Aquat Microb Ecol 67(1):15–24.

Benzerara K, Skouri-Panet F, Li J, Férard C, Gugger M, Laurent T, Couradeau E, Ragon M, Cosmidis J, Menguy N, et al. 2014. Intracellular Ca-carbonate biomineralization is widespread in cyanobacteria. Proc Natl Acad Sci USA 111(30):10933–10938.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al–Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8):852–885.

Braissant O, Decho AW, Dupraz C, Glunk C, Przekop KM, Visscher PT. 2007. Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology 5(4):401–411.

Breitbart M, Hoare A, Nitti A, Siefert J, Haynes M, Dinsdale E, Edwards R, Souza V, Rohwer F, Hollander D. 2009. Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico. Environ Microbiol 11(1):16–34.

Bundeleva IA, Shirokova LS, Bénézeth P, Pokrovsky OS, Kompantseva EI, Balor S. 2012. Calcium carbonate precipitation by anoxygenic phototrophic bacteria. Chem Geol 291:116–131.

Bundeleva IA, Shirokova LS, Pokrovsky OS, Bénézeth P, Ménez B, Gérard E, Balor S. 2014. Experimental modeling of calcium carbonate precipitation by cyanobacterium *Gloeocapsa* sp. Chem Geol 374–375:44–60.

Burne RV, Moore LS. 1987. Microbialites: organosedimentary deposits of benthic microbial communities. Palaios 2(3):241–254.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583.

Canfield DE, Des Marais DJ. 1991. Aerobic sulfate reduction in microbial mats. Science 251(5000):1471–1473.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108(Supplement_1):4516–4522.

Castro-Contreras SI, Gingras MK, Pecoits E, Aubet NR, Petrash D, Castro-Contreras SM, Dick G, Planavsky N, Konhauser KO. 2014. Textural and geochemical features of freshwater. Palaios 29(5): 192–209.

Centeno CM, Legendre P, Beltrán Y, Alcántara-Hernández RJ, Lidström UE, Ashby MN, Falcón LI. 2012. Microbialite genetic diversity and composition relate to environmental variables. FEMS Microbiol Ecol 82(3):724–735.

Cervantes-Martínez A, Elías-Gutiéerrez M, Suárez-Morales E. 2002. Limnological and morphometrical data of eight karstic system-scenotes' of the Yucatan Peninsula Mexico during the dry season (February–May 2001). Hydrobiologia 482(1/3):167–177.

Cervantes-Martínez A, Mezeta-Barrera M, Gutiérrez-Aguirre MA. 2009. Limnología básica del lago cárstico turístico Cenote Azul en Quintana Roo México. Hidrobiológica 19(2):177–180.

Chagas AA, Webb GE, Burne RV, Southam G. 2016. Modern lacustrine microbialites: towards a synthesis of aqueous and carbonate geochemistry and mineralogy. Earth Sci Rev 162:338–363.

Chernov TI, Zhelezova AD, Tkhakakhova AK, Bgazhba NA, Zverev AO. 2019. Microbiomes of virgin soils of Southern Vietnam tropical forests. Microbiology 88(4):489–498.

Couradeau E, Benzerara K, Moreira D, Gérard E, Kaźmierczak J, Tavera R, López-García P. 2011. Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline Lake Alchichica (Mexico). PLoS One 6(12):e28767.

Cuevas D, Sherman C, Ramirez W, Hubbard DK. 2008. Environmental factors controlling community structure, morphology and linear extension of Mid-Holocene reef corals from Canada Honda, Southwestern Dominican Republic. Proc 11th Int Coral Reef Symp 1:21–25.

Das SK, Singh D. 2017. Chroococcidiopsis a Cryptoendolithic Cyanobacterium from Larsemann Hills East Antarctica. Nelumbo 59(1):105–109.

De Anda V, Zapata-Peñasco I, Souza V. 2018. Towards a comprehensive understanding of environmental perturbations in microbial mats from the Cuatro Ciénegas Basin by network inference. In: Elser, SV, García-Oliva, JF, editors. Ecosystem Ecology and Geochemistry of Cuatro Ciénegas: How to Survive in an Extremely Oligotrophic Site. Berlin: Springer, p85–97.

Dupraz C, Reid RP, Braissant O, Decho AW, Norman RS, Visscher PT. 2009. Processes of carbonate precipitation in modern microbial mats. Earth Sci Rev 96(3):141–162.

Dupraz C, Reid RP, Visscher PT. 2011. Modern microbialites. In: Reitner, J, Thiel, V, editors. Encyclopedia of Geobiology. Berlin: Springer, p617–634.

Dupraz C, Visscher PT. 2005. Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol 13(9):429–438.

Dupraz C, Visscher PT, Baumgartner LK, Reid RP. 2004. Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island Bahamas). Sedimentology 51(4):745–765.

Ehrich S, Behrens D, Lebedeva E, Ludwig W, Bock E. 1995. A new obligately chemolithoautotrophic, nitrite-oxidizing bacterium,

*Nitrospira moscoviensis* sp. nov. and its phylogenetic relationship . Arch Microbiol 164(1):16–23.
Falkowski PG, Fenchel T, Delong EF. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science 320(5879):1034–1039.
Farías ME, Contreras M, Rasuk MC, Kurth D, Flores MR, Poiré DG, Novoa F, Visscher PT. 2014. Characterization of bacterial diversity associated with microbial mats, gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile. Extremophiles 18(2):311–329.
Foster JS, Green SJ. 2011. Microbial diversity in modern stromatolites. In: Tewari, V, Seckbach, J, editors. Stromatolites: Interaction of Microbes with Sediments. Dordrecht: Springer, p383–405.
Foster JS, Green SJ, Ahrendt SR, Golubic S, Reid RP, Hetherington KL, Bebout L. 2009. Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. ISME J 3(5):573–587.
Foster JS, Reid RP, Visscher PT, Dupraz C. 2019. Editorial: characterizing modern microbialites and the geobiological processes underlying their formation. Front Microbiol 10:2299.
Gabriel JJ, Reinhardt EG, Peros MC, Davidson DE, van Hengstum PJ, Beddows PA. 2009. Palaeoenvironmental evolution of Cenote Aktun Ha (Carwash) on the Yucatan Peninsula, Mexico and its response to Holocene sea-level rise. J Paleolimnol 42(2):199–213.
Gallagher KL, Kading TJ, Braissant O, Dupraz C, Visscher PT. 2012. Inside the alkalinity engine: the role of electron donors in the organomineralization potential of sulfate-reducing bacteria. Geobiology 10(6):518–530.
Gérard E, Ménez B, Couradeau E, Moreira D, Benzerara K, Tavera R, López-García P. 2013. Specific carbonate-microbe interactions in the modern microbialites of Lake Alchichica (Mexico). ISME J 7(10): 1997–2009.
Ginestet C. 2011. ggplot2: elegant graphics for data analysis. J R Stat Soc Ser 174(1):245–246.
Gischler E, Gibson MA, Oschmann W. 2008. Giant Holocene freshwater microbialites Laguna Bacalar Quintana Roo Mexico. Sedimentology 55(5):1293–1309.
Gischler E, Golubic S, Gibson M, Oschamann W, Hudson JH. 2011. Microbial mats and microbialites in the freshwater Laguna Bacalar Yucatan Peninsula Mexico. In: Reitner, J, Sütwe, T, Yuen, D, editors. Advances in Stromatolite Geobiology. Berlin, Germany: Springer, p187–205.
Grotzinger JP, Knoll AH. 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Ann Rev Earth Planet Sci 27(1):313–358.
Havemann SA, Foster JS. 2008. Comparative characterization of the microbial diversities of an artificial microbialite model and a natural stromatolite. Appl Environ Microbiol 74(23):7410–7421.
Hernández-Arana HA, Vega-Zepeda A, Ruíz-Zárate MA, Falcón-Alvarez LI, López-Adame H, Herrera-Silveira J, Kaster J. 2015. Transverse coastal corridor: from freshwater lakes to coral reefs ecosystems. In: Islebe, GA, Calmé, S, León-Cortés, JL; Schmook, B, editors. Biodiversity and Conservation of the Yucatán Peninsula. Cham: Springer, p355–376.
Johnson DB, Beddows PA, Flynn TM, Osburn MR. 2018. Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar Mexico. Geobiology 16(3):319–337.
Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol E 30(4):772–780.
Kellogg CA. 2019. Microbiomes of stony and soft deep-sea corals share rare core bacteria. Microbiome 7(1):90.
Kempe S, Kazmierczak J, Landmann G, Konuk T, Reimer A, Lipp A. 1991. Largest known microbialites discovered in Lake Van Turkey. Nature 349(6310):605–608.
Khan NS, Ashe E, Horton BP, Dutton A, Kopp RE, Brocard G, Engelhart SE, Hill DF, Peltier WR, Vane CH, et al. 2017. Drivers of Holocene sea-level change in the Caribbean. Quat Sci Rev 155: 13–36.
Khodadad CL, Foster JS. 2012. Metagenomic and metabolic profiling of nonlithifying and lithifying stromatolitic mats of Highborne Cay, The Bahamas. PLoS One 7(5):e38229.
Kimble JC, Winter AS, Spilde MN, Sinsabaugh RL, Northup DE. 2018. A potential central role of Thaumarchaeota in N-Cycling in a semi-arid environment Fort Stanton Cave Snowy River passage New Mexico USA. FEMS Microbiol Ecol 94(11):fiy173.
Kindt R, Kindt MR. 2019. Package 'BiodiversityR'. Package for community ecology and suitability analysis, 2-11. Accessed March 25, 2020. https://CRAN.R-project.org/package=BiodiversityR.
Koch H, van Kessel MA, Lücker S. 2019. Complete nitrification: insights into the ecophysiology of comammox *Nitrospira*. Appl Microbiol Biotechnol 103(1):177–189.
Kolde R, Kolde MR. 2015. Package 'pheatmap'. R Package. 1(7). Accessed March 26, 2020. https://cran.r-project.org/package=pheatmap.
Kroeger ME, Delmont TO, Eren AM, Meyer KM, Guo J, Khan K, Rodrigues JLM, Bohannan BJM, Tringe SG, Borges CD, et al. 2018. New biological insights into how deforestation in Amazonia affects soil microbial communities using metagenomics and metagenome-assembled genomes. Front Microbiol 9:1635.
Kulichevskaya IS, Naumoff DG, Miroshnikov KK, Ivanova AA, Philippov DA, Hakobyan A, Rijpstra WIC, Damsté JSS, Liesack W, Dedysh SN. 2020. *Limnoglobus roseus* gen. nov., sp. nov., a novel freshwater planctomycete with a giant genome from the family Gemmataceae. Int J Syst Evol Microbiol 70(2):1240–1249.
Lacap-Bugler DC, Lee KK, Archer S, Gillman LN, Lau MCY, Leuzinger S, Lee CK, Maki T, McKay CP, Perrott JK, et al. 2017. Global diversity of desert hypolithic cyanobacteria. Front Microbiol 8:867.
Laval B, Cady SL, Pollack JC, McKay CP, Bird JS, Grotzinger JP, Ford DC, Bohm HR. 2000. Modern freshwater microbialite analogues for ancient dendritic reef structures. Nature 407(6804):626–629.
Legendre P, De Cáceres M. 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. Ecol Lett 16(8):951–963.
Leinonen R, Sugawara H, Shumway M. 2011. The sequence read archive. Nuc Acids Res 39(Database issue):D19–D21.
Ley RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin M, Pace NR. 2006. Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72(5):3685–3695.
Lindsay MR, Anderson C, Fox N, Scofield G, Allen J, Anderson E, Bueter L, Poudel S, Sutherland K, Munson-McGee JH, et al. 2017. Microbialite response to an anthropogenic salinity gradient in Great Salt Lake, Utah. Geobiology 15(1):131–145.
Logan BW. 1961. Cryptozoon and associate stromatolites from the recent Shark Bay Western Australia. J Geol 69(5):517–533.
López-García P, Kazmierczak J, Benzerara K, Kempe S, Guyot F, Moreira D. 2005. Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey. Extremophiles 9(4):263–274.
López-Ramos E. 1975. Geological summary of the Yucatan Peninsula. In: Nairn, AEM, editor. The Gulf of Mexico and the Caribbean. Boston, MA: Springer, p257–282.
Love M, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. Genome Biol 15(12):550–550.
Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer K-H, Wagner M. 2002. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microbiol 68(10): 5064–5081.
Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J 5(2):169–172.
McDevitt-Irwin JM, Baum JK, Garren M, Thurber VLR. 2017. Responses of coral-associated bacterial communities to local and global stressors. Front Mar Sci 4:262.

McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8(4):e61217.
Meziti A, Tsementzi DA, Kormas K, Karayanni H, Konstantinidis KT. 2016. Anthropogenic effects on bacterial diversity and function along a river-to-estuary gradient in Northwest Greece revealed by metagenomics. Environ Microbiol 18(12):4640–4652.
Mobberley JM, Khodadad CLM, Visscher PT, Reid RP, Hagan P, Foster JS. 2015. Inner workings of thrombolites: spatial gradients of metabolic activity as revealed by metatranscriptome profiling. Sci Rep 5:12601.
Mohamed NM, Saito K, Tal Y, Hill RT. 2010. Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. ISME J 4(1):38–48.
Montes-Ortiz L, Elías-Gutiérrez M. 2018. Faunistic survey of the zooplankton community in an oligotrophic sinkhole Cenote Azul (Quintana Roo Mexico) using different sampling methods and documented with DNA barcodes. J Limnol 77(3):428–440.
Muyzer G, Stams AJ. 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6(6):441–454.
Nitti A, Daniels CA, Siefert J, Souza V, Hollander D, Breitbart M. 2012. Spatially resolved genomic, stable isotopic, and lipid analyses of a modern freshwater microbialite from Cuatro Ciénegas, Mexico. Astrobiology 12(7):685–698.
Obst M, Wehrli B, Dittrich M. 2009. $CaCO_3$ nucleation by cyanobacteria: laboratory evidence for a passive, surface-induced mechanism. Geobiology 7(3):324–347.
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2016. Vegan: community ecology package. R package version 2.3-3. Accessed January 25, 2020. http://vegan.r-forge.r-project.org.
Oliveros JC. 2007–2015. Venny. An interactive tool for comparing lists with Venn's diagrams. Accessed May 15, 2020. http://bioinfogp.cnb.csic.es/tools/venny/index.html.
Omelon CR, Brady AL, Slater GF, Laval B, Lim DS, Southam G. 2013. Microstructure variability in freshwater microbialites Pavilion Lake Canada. Palaeogeogr Palaeoclimatol Palaeoecol 392:62–70.
Panikov NS. 2010. Microbial Ecology. In: Wang, L, Ivanov, V, Tay, JH, editors. Environmental Biotechnology. Handbook of Environmental Engineering, vol 10. Totowa, NJ: Humana Press, p121–191.
Paulo C, Dittrich M. 2013. 2D Raman spectroscopy study of dolomite and cyanobacterial extracellular polymeric substances from Khor Al-Adaid sabkha (Qatar). J Raman Spectrosc 44(11):1563–1569.
Pereira AD, Cabezas A, Etchebehere C, Chernicharo CADL, Calábria de Araújo J. 2017. Microbial communities in Anammox reactors: a review. Environ Technol Rev 6(1):74–93.
Pérez-Ceballos R, Pacheco-Ávila J, Euán-Ávila J, Hernández-Arana H. 2012. Regionalization based on water chemistry and physicochemical traits in the "Ring of Cenotes", Yucatan, Mexico. J Caves K 74(01): 90–3102.
Perry E, Oliman GV, Wagner N. 2012. Preliminary investigation of groundwater and surface water geochemistry in Campeche and southern Quintana Roo. In: Spring, UO, editor, Water Resources in Mexico. Berlin Heidelberg: Springer. p87–97.
Perry EC, Paytan A, Pedersen B, Velazquez-Oliman G. 2009. Groundwater geochemistry of the Yucatan Peninsula Mexico: constraints on stratigraphy and hydrogeology. J Hydrol 367(1–2):27–40.
Perry EC, Velazquez-Oliman G, Marin L. 2002. The hydrogeochemistry of the karst aquifer system of the northern Yucatan Peninsula Mexico. Int Geol Rev 44(3):191–221.
Power IM, Wilson SA, Dipple GM, Southam G. 2011. Modern carbonate microbialites from an asbestos open pit pond, Yukon, Canada. Geobiology 9(2):180–195.
Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol 26(7):1641–1650.
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(Database issue):D590–D596.
Rasmussen KA, Macintyre IG, Prufert L. 1993. Modern stromatolite reefs fringing a brackish coastline Chetumal Bay Belize. Geol 21(3): 199–202.
Reid RP, Macintyre IG, Steneck RS. 1999. A microbialite/algal ridge fringing reef complex, Highborne Cay, Bahamas. Atoll Res Bul 465: 1–18.
Reid RP, Visscher PT, Decho AW, Stolz JF, Bebout BM, Dupraz C, Macintyre IG, Paerl HW, Pinckney JL, Prufert-Bebout L, et al. 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406(6799):989–992.
Russell JA, Brady AL, Cardman Z, Slater GF, Lim DS, Biddle JF. 2014. Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada. Geobiology 12(3): 250–264.
Saghaï A, Zivanovic Y, Zeyen N, Moreira D, Benzerara K, Deschamps P, Bertolino P, Ragon M, Tavera R, López-Archilla AI, et al. 2015. Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. Front Microbiol 6:797.
Sánchez-Sánchez JA, Álvarez-Legorreta T, Pacheco-Ávila JG, González-Herrera RA, Carrillo-Bribiezca L. 2015. Caracterización hidrogeoquímica de las aguas subterráneas del sur del Estado de Quintana Roo, México. Rev Mex Cienc Geol 32(1):62–76.
Schmitter-Soto JJ, Comín FA, Escobar-Briones E, Herrera-Silveira J, Alcocer J, Suárez-Morales E, Elías-Gutiérrez M, Díaz-Arce V, Marín LE, Steinich B. 2002. Hydrogeochemical and biological characteristics of cenotes in the Yucatan Peninsula (SE Mexico). Hydrobiologia 467(1/3):215–228.
Schopf JW. 2006. Fossil evidence of Archaean life. Philos Trans R Soc B 361(1470):869–885.
Shih PM, Ward LM, Fischer WW. 2017. Evolution of the 3-hydroxypropionate bicycle and recent transfer of anoxygenic photosynthesis into the Chloroflexi. Proc Natl Acad Sci USA 114(40):10749–10754.
Souza V, Siefert JL, Escalante AE, Elser JJ, Eguiarte LE. 2012. The Cuatro Ciénegas basin in Coahuila, Mexico: an astrobiological Precambrian Park. Astrobiology 12(7):641–647.
Suosaari EP, Reid RP, Playford PE, Foster JS, Stolz JF, Casaburi G, Hagan PD, Chirayath V, Macintyre IG, Planavsky NJ, et al. 2016. New multi-scale perspectives on the stromatolites of Shark Bay Western Australia. Sci Rep 6(1):20557–20513.
Torrescano-Valle N, Folan WJ. 2015. Physical settings environmental history with an outlook on global change. In: Islebe, GA, Calmé, S, León-Cortés, JL; Schmook, B, editors. Biodiversity and Conservation of the Yucatan Peninsula. Cham: Springer, p9–37.
Torrescano N, Islebe GA. 2006. Tropical forest and mangrove history from southeastern Mexico: a 5000 yr pollen record and implications for sea level rise. Veget Hist Archaeobot 15(3):191–195.
Toscano MA, Macintyre IG. 2003. Corrected western Atlantic sea-level curve for the last 11,000 years based on calibrated 14C dates from Acropora palmata framework and intertidal mangrove peat. Coral Reefs 22(3):257–270.
Valdespino-Castillo PM, Alcántara-Hernández RJ, Alcocer J, Merino-Ibarra M, Macek M, Falcón LI. 2014. Alkaline phosphatases in microbialites and bacterioplankton from Alchichica soda lake, Mexico. FEMS Microbiol Ecol 90(2):504–519.
Valdespino-Castillo PM, Hu P, Merino-Ibarra M, López-Gómez LM, Cerqueda-García D, González-De Zayas R, Pi-Puig T, Lestayo JA, Holman H-Y, Falcón LI. 2018. Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. Front Microbiol 9:510.
Visscher PT, Reid RP, Bebout BM. 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology 28(10): 919–922.
Visscher PT, Stolz JF. 2005. Microbial mats as bioreactors: populations processes and products. In: Noffke, N, editor. Geobiology: Objectives Concepts Perspectives. Amsterdam: Elsevier, p87–100.
Ward WC, Halley RB. 1985. Dolomitization in a mixing zone of near-seawater composition, late Pleistocene, northeastern Yucatan Peninsula. J Sediment Res 55(3):407–420.

Warden JG, Casaburi G, Omelon CR, Bennett PC, Breecker DO, Foster JS. 2016. Characterization of microbial mat microbiomes in the modern thrombolite ecosystem of Lake Clifton, Western Australia Using Shotgun Metagenomics. Front Microbiol 7: 1064.

White RA, Chan AM, Gavelis GS, Leander BS, Brady AL, Slater GF, Suttle CA. 2016. Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community. Front Microbiol 28(6):1531.

White RA, Power IM, Dipple GM, Southam G, Suttle CA. 2015. Metagenomic analysis reveals that modern microbialites and polar microbial mats have similar taxonomic and functional potential. Front Microbiol 6:966.

Winsborough BM, Golubic S. 2007. The role of diatoms in stromatolite growth - two examples from modern fresh-water settings. J Phycol 23(2):195–201.

Wong HL, Smith DL, Visscher PT, Burns BP. 2015. Niche differentiation of bacterial communities at a millimeter scale in Shark Bay microbial mats. Sci Rep 5(1):15607.

Yanez-Montalvo A, Gómez-Acata S, Águila B, Hernández-Arana H, Falcón LI. 2020. The microbiome of modern microbialites in Bacalar Lagoon, Mexico. PLoS One 15(3):e0230071.