UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS DE LA TIERRA

Comunidades microbianas activas en sedimentos del lago cráter Rincón de Parangueo: hacia la caracterización de nuevos ambientes

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS DE LA TIERRA

PRESENTA

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CDMX, diciembre 2023

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Janet Sánchez Sánchez

Agradecimientos

“Estoy entre aquellos que piensan que la ciencia tiene una gran belleza” **Marie Curie**

Este trabajo no hubiera sido posible sin mi asesor, el Dr. Mariano Cerca, que desde un principio me brindó su apoyo incondicional. Agradezco su entusiasmo, motivación, paciencia e inmenso conocimiento. Su guía me ayudó en todo el tiempo de investigación y redacción de esta disertación. No podría haber imaginado tener un mejor asesor y mentor en este viaje. Además de mi asesor, quisiera extender mi más sincero agradecimiento a mi comité de tesis y jurado para examen de grado: Dra. Rocío Jetzabel Alcántara, Dr. Gilles Levresse, Dr. Alfredo Varela Echavarría, Dra. Bárbara Moguel, Dra. Lilia Montoya Lorenzana y el Dr. Arturo Carlos II Becerra Bracho gracias por su aliento, retroalimentación y orientación durante este proceso.

Mi más sincero agradecimiento al apoyo de Ricardo Carrizosa Elizondo, Vania Ferrer, Dora Carreón Freyre, Jorge Aranda Gómez, Sara Solis Valdez, Carolina Muñoz Torres, Marina Vega González, Alexander Correa, Alexis López Esquivel, Gustavo Olivares y Yosahandy Vázquez del Centro de Geociencias (UNAM), por su apoyo en el trabajo de campo, análisis de microscopía electrónica de barrido, análisis molecular y análisis fisicoquímicos. Luis Aguilar, Alejandro de León y Jair García Sotelo del Laboratorio Nacional de Visualización Científica Avanzada (UNAM), por su asistencia en el análisis bioinformático. Carlos Lozano Flores del Instituto de Neurobiología (UNAM) por su orientación en los experimentos de laboratorio molecular.

Un agradecimiento especial a la Dirección de Medio Ambiente de Valle de Santiago, Marcela Areli Araiza Ortíz y María de Lourdes Rodríguez Campos, y a la Secretaría de Medio Ambiente y Ordenamiento Territorial del Estado de Guanajuato, por su apoyo en las actividades de divulgación científica realizadas en el cráter Rincón de Parangueo.

Expreso mi agradecimiento al Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) por la beca que respaldó mis estudios de posgrado.

Finalmente, agradezco el apoyo incondicional y amor de mi familia: mi esposo, mis padres y mis hermanos. Las palabras no pueden expresar lo agradecida que estoy por su entusiasmo, orgullo, curiosidad y motivación que me apoyaron en el camino. ¡Gracias por apoyar siempre mis sueños!

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ABSTRACT

Geobiology is an emerging discipline that aims to comprehend the interactions between the geosphere and biosphere, as well as how their processes have co-evolved throughout Earth's history. In the fossil record, microbialites, sedimentary structures formed through the interplay of microbial communities and their environment, have dominated 80% of Earth's history. These structures exist at the intersection of chemistry, biology, physics, and geology, providing a splendid opportunity to study the geochemical and biological processes involved in their formation making them an invaluable tool in the field of geobiology. Microbialites reached their peak during the late Mesoproterozoic era (approximately 1.4 billion years ago), but today they are much less abundant. Their presence is now limited to a few environments (*e.g*., marine, hypersaline, and freshwater), often characterized by extreme physicochemical conditions.

This dissertation examines the geobiological processes that enable the formation of microbialites in Rincón de Parangueo, a maar crater created by a phreatomagmatic explosion located in the central region of the Trans-Mexican Volcanic Belt, an extreme environment that has been less studied compared to other modern saline-alkaline environments. Extremophilic microbial communities are present within the crater, giving rise to microbialites along the ancient lake shoreline, microbial mats, and planktonic microorganisms within the hypersaline ponds. Rincón de Parangueo serves as a "natural laboratory" for studying the structure, dynamics, and diversification of extremophilic microbial populations, connections between biotic and abiotic processes, and microorganism-mineral interactions. Through a series of techniques including X-ray diffraction, scanning electron microscopy, high-throughput DNA sequencing methods, bioinformatic analysis, ground-penetrating radar surveys, physicochemical characterization, collection of geological and physicochemical field data, as well as compilation of information from previous studies, we identified the biotic factors (*e.g*., microbial community, microorganism-mineral interaction, metabolic components) and abiotic factors (*e.g*., geology and hydrochemistry) that contribute to the development of microbialites in Rincón de Parangueo.

RESUMEN

La geobiología es una disciplina emergente que busca entender las interacciones entre la geosfera y la biosfera y cómo sus procesos han coevolucionado a lo largo de la historia de la Tierra. En el registro fósil las microbialitas, estructuras sedimentarias formadas mediante la interacción de comunidades microbianas y su ambiente, han dominado el 80% de la historia de la Tierra. Existen en la interfaz de la química, la biología, la física y la geología, proporcionando una magnífica oportunidad para el estudio de los procesos geoquímicos y biológicos implicados en su formación y son una herramienta invaluable en la geobiología. Las microbialitas tuvieron su máximo apogeo a finales del Mesoproterozoico (hace 1,400 millones de años), hoy en día son mucho menos abundantes, su existencia se reduce a pocos ambientes (*e.g.*, marinos, hipersalinos y de agua dulce) que en muchas ocasiones presentan características fisicoquímicas extremas.

En esta disertación se analizan los procesos geobiológicos que permiten la formación de microbialitas en Rincón de Parangueo, un cráter maar formado por una explosion freatomagmática ubicado en la región central de la Faja Volcánica Transmexicana, un ambiente extremo poco estudiado en comparación con otros ambientes salino-alcalinos modernos. Comunidades microbianas extremófilas están presentes dentro del cráter formando microbialitas en la antigua costa del lago, tapetes microbianos y microorganismos planctónicos dentro de las pozas hipersalinas. Rincón de Parangueo es una "laboratorio natural" para el estudio de la estructura, dinámica y diversificación de poblaciones microbianas extremófilas, conexiones entre procesos bióticos y abióticos e interacciones microorganismo-mineral. Mediante una serie de técnicas que incluyen difracción de rayos X, microscopía electrónica de barrido, métodos de secuenciación de ADN de alto rendimiento, análisis bioinformático, sondeo por radar de penetración terrestre, caracterización fisicoquímica, colección de datos geológicos y fisicoquímicos en campo, así como la compilación de información de trabajos previos se lograron identificar los factores bióticos (*e.g.*, comunidad microbiana, interacción microorganismo-mineral, componente metabólico) y abióticos (eg., propiedades fisicoquímicas, componente geológico e hidroquímica) que propician el desarrollo de microbialitas en Rincón de Parangueo.

INTRODUCCIÓN

Rincón de Parangueo es un cráter volcánico de origen freatomagmático ubicado en el centro de México con una edad aproximada de 137,000 años (Aranda-Gómez et al., 2017). Se localiza en el municipio de Valle de Santiago (20°23’N y 101°15’W; 1700 m s.n.m.), en el Estado de Guanajuato. Pertenece al subgrupo de volcanes monogenéticos del campo volcánico Michoacán-Guanajuato, dentro del mayor complejo volcánico en el centro de México, la Faja volcánica Trans-mexicana (Aranda-Gómez et al., 2017). Dentro del cráter existió un lago perenne hasta finales del siglo pasado, pero la extracción excesiva de agua subterránea en la región ha provocado la desecación paulatina del lago y actualmente está a punto de desaparecer (Aranda-Gómez et al., 2017). La disminución del nivel de agua fue acompañada por un aumento en su salinidad y alcalinidad por lo que ahora es considerado un ambiente con condiciones fisicoquímicas extremas. Rincón de Parangueo es un lugar con gran diversidad biológica donde microorganismos extremófilos forman microbialitas, estructuras organo-sedimentarias formadas por la acreción, atrapamiento y precipitación de minerales gracias a la actividad microbiana (Reid et al., 2000; Dupraz, 2004), cuya importancia radica en su analogía con las microbialitas fósiles del Precámbrico que constituyen una de las evidencias más antiguas de vida en la Tierra (Benzerara et al., 2006).

Hoy en día, el desarrollo de microbialitas ocurre solo en pocos lugares del planeta y es aún más escaso su desarrollo dentro de cráteres volcánicos (Tabla suplementaria 1). A pesar de su importancia geobiológica en un contexto nacional e internacional, las microbialitas de Rincón de Parangueo permanecieron prácticamente invisibles para la comunidad científica por muchos años. Nuestro estudio es uno de los primeros en abordar desde un punto de vista multidisciplinario la interacción de factores bióticos y abióticos en el desarrollo de microbialitas en Rincón de Parangueo. Esta disertación compila los resultados de nuestra investigación, el documento incluye artículos científicos y de divulgación publicados en revistas indexadas de circulación nacional e internacional.

En el articulo “ Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico” describimos el entorno geológico donde se han desarrollado las comunidades microbianas extremófilas dentro del cráter, realizamos un mapeo en campo de la morfología de las microbialitas y un reconocimiento de depósitos de carbonatos. Reportamos los resultados obtenidos mediante métodos de secuenciación de alto rendimiento para caracterizar las comunidades microbianas (Bacteria, Arquea y Eucaria) en: 1) microbialitas ubicadas en la falla principal del cráter, 2) tapetes microbianos ubicados en las pozas salino-alcalinas y 3) microorganismos planctónicos en aguas poco profundas de las pozas salino-alcalinas. Discutimos la relación de las comunidades microbianas identificadas con el ambiente geológico donde se desarrollan y describimos las principales características fisicoquímicas de las estructuras organo-sedimentarias.

En el artículo “Mobile mud layer underneath the desiccated maar lake of Rincón de Parangueo and insights into its microbial fingerprints” examinamos las propiedades fisicoquímicas de una capa subsuperficial de lodo móvil saturado a 4 m debajo del fondo desecado del lago en Rincón de Parangueo. Discutimos la movilidad del lodo y la formación de domos, su relación con la subsidencia activa del cráter, la entrada de agua subterránea y gas en los sedimentos. Mediante métodos de secuenciación de ADN de alto rendimiento identificamos la huella microbiana (Bacteria y Arquea) de la comunidad obtenida en el subsuelo y analizamos su conexión con las principales propiedades fisicoquímicas de los sedimentos a profundidad, así como su probable papel en la producción de gas y la sobrepresión del fluido de poro en la capa de lodo.

En el artículo “Geomicrobiology of the crater maar Rincón de Parangueo: exploring the link between an evolving extreme environment and its potential metabolic diversity” abordamos nuevos métodos bioinformáticos para re-evaluar el conjunto de datos obtenidos mediante secuenciación masiva de distintos microambientes dentro del cráter. Identificamos el posible rol de las comunidades microbianas en la generación de las estructuras carbonatadas mediante la predicción del potencial metabólico de la comunidad microbiana (Bacteria y Arquea). Revisamos las características físicas y morfológicas de la secuencia sedimentaria lacustre ubicada en la parte central del cráter. Finalmente, compilamos los resultados de investigaciones recientes sobre las comunidades microbianas, reportamos la evolución de los parámetros fisicoquímicos y la morfología de las estructuras carbonatadas dentro del cráter.

OBJETIVOS

Objetivo general:

Reconocer los componentes clave que permiten la formación de microbialitas en el cráter lago Rincón de Parangueo mediante la identificación de los factores bióticos (*e.g.*, comunidad microbiana, interacción microorganismo-mineral y componente metabólico) y factores abióticos (*e.g.*, propiedades fisicoquímicas, componente geológico). Integrar los resultados con el fin de comprender las características únicas del cráter que lo distinguen como uno de los pocos en el mundo con formación de microbialitas.

Objetivos específicos:

1) Caracterizar la comunidades microbianas desarrolladas en la superficie del cráter Rincón de Parangueo (microbialitas, tapetes microbianos y microorganismos planctónicos dentro de las pozas hipersalinas), mediante métodos de secuenciación de alto rendimiento, utilizando secuencias de amplicones de los genes ADNr 16S y ADNr 18S y métodos bioinformáticos basados en Unidades Taxonómicas Operativas (OTU´s).

2) Identificar la huella microbiana en muestras del subsuelo en el cráter Rincón de Parangueo (4 metros de profundidad) mediante el uso del gen ADNr 16S, métodos de secuenciación de alto rendimiento y métodos bioinformáticos actualizados.

3) Reevaluar el conjunto de datos generados por secuenciación del gen 16S ADNr a partir de muestras de la superficie y subsuelo del cráter, mediante la utilización de métodos bioinformáticos basados en

Variantes de Secuencia de Amplicones (ASV´s) y bases de datos actualizadas. Interpretar los resultados para obtener información sobre la diversidad y composición de la comunidad microbiana y llevar a cabo una comparación con los resultados de los análisis previos.

4) Identificar el potencial metabólico de la comunidad procariótica en diferentes microambientes dentro del cráter mediante la predicción metabólica indirecta basada en los taxones identificados, para proponer los posibles metabolismos que participan en la precipitación activa de carbonatos en el lago cráter Rincón de Parangueo.

5) Describir el entorno geológico dentro del cráter maar Rincón de Parangueo mediante la compilación de información sobre la disminución del nivel de agua en el lago, subsidencia activa del cráter, entrada de agua subterránea y desgasificación en los sedimentos.

6) Definir las principales características de los microambientes estudiados en la superficie del cráter mediante su descripción morfológica (*e.g.*, macro-, meso- y microestructura), composición mineral y valoración de parámetros fisicoquímicos.

7) Determinar mediante trabajo geológico en campo, extracción de núcleos de sedimentos, caracterización fisicoquímica, cartografía de estructuras de deformación (domos de lodo) e interpretación de perfiles de Radar de Penetración Terrestre, las características del subsuelo a 4 metros de profundidad y las principales causas que provocan su movilidad.

8) Relacionar los datos geológicos, fisicoquímicos y biológicos de cada uno de los microambientes, para definir las características únicas de cada sitio estudiado y la participación de las comunidades microbianas en cada uno de ellos.

MÉTODOS Y TÉCNICAS

1. Métodos moleculares:

a) *Extracción de ácidos ribonucleicos (ADN):*

La extracción de ADN genómico se realizó por triplicado y se manejó de forma independiente usando 30 g de material/biomasa para Microbialita 1, 2, 3; 30 g de material/biomasa para Tapete microbiano 1, 2, 3; 30 g de material/biomasa para sedimentos; 3500 ml para Agua 1 y 500 mL para Agua 2 y 3. La extracción se realizó siguiendo el protocolo de preparación de muestras propuesto por Bey et al. (2010), y el kit de extracción *PowerSoil DNA Isolation (MoBio)* de acuerdo con las instrucciones del fabricante.

b) *Amplificación y secuenciación:*

Mediante el uso de los genes ADNr 16S y ADNr 18S, ampliamente utilizados para la identificación de especies bacterianas y sus relaciones filogenéticas (Valenzuela-González et al., 2015), se llevó a cabo una caracterización de las comunidades microbianas. Los primers utilizados para la amplificación de las muestras fueron los siguientes: TAReuk (Eucaria; Villarino et al., 2018), 28F/519R (Bacteria; Lay et al., 2013) y Arch517F/909R (Arquea; Lay et al., 2013). Las muestras se enviaron para su secuenciación en la plataforma Illumina MiSeq (https://www.illumina.com) al laboratorio de secuenciación genómica RTL (Lubbock TX, USA) (https://rtlgenomics.com/).

c) *Análisis bioinformático:*

Para el primer análisis bioinformático basado en Unidades Taxonómicas Operativas (OTU´s) se utilizó la siguiente metodología: El ensamble de secuencias pareadas (pair-end) se llevó a cabo con el software PEAR Illumina (Zhang et al. 2014). La dereplicación se realizó utilizando el algoritmo USEARCH (Edgar 2010). La selección de unidades taxonómicas operativas (OTU) se realizó con ayuda del algoritmo UPARSE OTU clustering (Edgar 2013). Para la verificación de quimeras se empleó el software de detección de quimeras UCHIME (Edgar et al. 2011). Las secuencias se alinearon utilizando el algoritmo de alineación global USEARCH (Bokulich et al. 2015) y una base de datos de alta calidad propia del laboratorio de secuenciación genómica RTL generada a partir de datos del National Center for Biotechnology Information (NCBI). Para la visualización de los gráficos se utilizó el paquete de software Circos (https://dzone.com/articles/circle-through-your-google).

Para el segundo análisis bioinformático basado en Variantes de Secuencia de Amplicones (ASV´s) se empleó el siguiente procedimiento: se eliminaron los adaptadores y los primers utilizando Trimmomatic v. 0.39 (Bolger et al., 2014). El filtrado de calidad, estimación de errores, proceso de dereplicación, alineamiento de secuencias, eliminación de secuencias quiméricas y selección de ASVs fueron realizados con el software DADA2 v. 1.14.1 (Callahan et al., 2016). La asignación de taxonomía se realizó comparando contra la base de datos SILVA SSU v. 138 (Quast et al., 2012). Las tablas de taxonomía y ASVs, así como los metadatos asociados, se convirtieron en un objeto phyloseq (McMurdie & Holmes, 2013) para un análisis más detallado. Los gráficos de barras y los análisis de diversidad se realizaron con los paquetes phyloseq y ggplot2 v. 3.3.2 (Wickham, 2016) en un entorno R v. 3.6.3 (R Core Team, 2013). El análisis de redes ecológicas (ENA) se construyó con base en el objeto phyloseq y la disimilitud de Bray Curtis. Las gráficas se realizaron con el paquete igraph (Csardi & Nepusz, 2006) y Cytoscape 3.9.1 ambos en el entorno R v. 3.6.3. Las funciones metabólicas inferidas de las comunidades microbianas se predijeron después de obtener los grupos procarióticos (Bacteria y Arquea) generados a partir de los ASVs, utilizando el mapeador local de iVikodak (Nagpal et al., 2019).

2. Métodos físicoquímicos:

a) Microscopía electrónica de barrido:

Se identificaron mediante microscopía electrónica de barrido las comunidades microbianas presentes en muestras de microbialita, sedimentos y tapetes microbianos. Las muestras de tamaño milimétrico fueron montadas en una placa de aluminio cubierta con cinta adhesiva conductora de carbono, fueron observadas en el microscopio electrónico de barrido SEM HITACHI TM1000 a 15 kV con un detector de retrodispersión en el Laboratorio de Geoquímica de Fluidos Corticales en el Centro de Geociencias, UNAM.

b) Difracción de Rayos X:

Muestras seleccionadas de microbialitas y sedimentos fueron analizadas en el Laboratorio de Geoquímica de Fluidos Corticales en el Centro de Geociencias, UNAM. La preparación de la muestra consistió en su trituración en un mortero de ágata para obtener polvos secos de grano fino (<0,002 mm). Las muestras se analizaron usando el difractómetro Miniflex Rigaku con radiación de cobre Kα 1.5406 Å. Los parámetros especificados fueron: voltaje 30 KV, corriente 15 Ma, ángulo de comienzo 20° y ángulo de finalización 80°, en pasos de 0.02

grados y 0.06 segundos para el punto medida. Las fases cristalinas se identificaron usando la base de datos PDF-2 (International Centre for Diffraction Data, https://www.icdd.com).

c) *Radar de penetración terrestre (RPT):*

Se reprocesaron y reinterpretaron dos perfiles de RPT de aproximadamente 1,000 m de largo, que fueron registrados y reportados previamente en la parte central del antiguo lago (Cerca et al., 2014). Para generar los perfiles se utilizaron pulsos electromagnéticos de alta frecuencia que fueron transmitidos al subsuelo con el equipo SIR-20 (Geophysical Survey Systems, Inc) el cual posee una antena receptora de 200 MHz, utilizando un tacómetro calibrado para recoger 40 trazas por metro (Rocha-Treviño, 2015).

d) *Geoquímica (aniones y cationes):*

Muestras seleccionadas de sedimentos del antiguo lago fueron filtradas al vacío para obtener salmuera (agua de poro), la cual fue analizada en el Laboratorio de Geoquímica Ambiental en el Centro Geociencias UNAM. El análisis de cationes se llevó a cabo según el método EPA 200.7 (EPA, 2001) con un espectrofotómetro de emisión óptica (Thermo iCAP 6500 Duo Plasma) de acoplamiento inductivo, usando un estándar interno de Itrio (1 ppm) para evaluar la señal del instrumento (rango de error de ± 10 %). Los aniones fueron determinados de acuerdo con el método EPA 300.1 (Hautman and Munch, 1997), con el equipo Thermo-Dionex1100 brand HPLC-IC con supresor ASRS-Ultra 300 de 4 mm, columna IonPac AS14A y detector electroquímico ED50.

e) *Contenido de humedad del suelo por gravimetría*:

El método gravimétrico es un método directo de medición de la humedad del suelo, mediante la diferencia de peso entre la muestra húmeda y seca (peso constante). Este parámetro se determinó empleando el método AS-05 NOM-021-RECNAT-2000 en el Laboratorio de Mecánica de Geosistemas (LAMG), UNAM. Las muestras y su contenedor fueron pesados individualmente y se introdujeron al horno de precisión por 24 horas a 100-105 °C. Las muestras se consideraron secas cuando su peso permaneció constante a una temperatura de 100-105 °C, transcurrido el tiempo se pesaron nuevamente. La diferencia entre el peso húmedo y el peso seco de la muestra fue el valor de su contenido de agua.

f) Densidad aparente:

La densidad aparente es la masa de suelo seco existente en una determinada unidad de volumen y su valor se relaciona con la proporción de poros existente en dicho volumen (Agostini et al., 2014). La técnica está basada en la medición de dos parámetros: el volumen de los sólidos en la muestra y el volumen ocupado por el espacio poroso. La determinación de la densidad aparente se realizó mediante el método más comúnmente conocido como "método del cilindro". El procedimiento consistió en introducir un cilindro metálico en el sedimento y determinar la masa de suelo seco que quedó en su interior después de su secado en horno de precisión a una temperatura de 100-105 °C por 48 horas.

g) *pH:*

La medición del pH está basado en el potencial eléctrico producido por los iones H^+ en una solución, los iones son detectados por un electrodo de hidrógeno y son comparados con el potencial constante que produce un electrodo patrón (Fassbender & Bornemisza, 1987). Los valores de pH se determinaron mediante el método AS-02 NOM-021-RECNAT-2000 con el potenciómetro Thermo Scientific STAR A211, en el Laboratorio de Edafología del Centro de Geociencias, UNAM. Diez gramos de muestra fueron tamizados en tamices de acero inoxidable (Advantech) con un tamaño de malla #10, posteriormente se adicionaron 25 ml de agua destilada y se agitaron por 1 hora a 200 rpm, transcurrido el tiempo se midió el pH.

h) *Conductividad eléctrica:*

Mide la capacidad que tiene un material para conducir la electricidad, depende de la concentración total de iones presentes en la solución, la movilidad y valencia de los iones, así como la temperatura a la cual se realiza la medición (Solís-Castro et al., 2018). El método utilizado para medir la conductividad fue el propuesto por la NOM-021-RECNAT-2000. Se tamizaron 10 gramos de muestra en tamices de acero inoxidable (Advantech) con un tamaño de malla #10, se adicionaron 50 ml de agua destilada, se agitaron por 1 hora a 200 rpm y se dejaron reposar por 24 horas. Transcurrido el tiempo se obtuvo la medición con el potenciómetro Thermo Scientific STAR A211, en el Laboratorio de Edafología del Centro de Geociencias, UNAM.

i) *Densidad real:*

Los valores de densidad real se determinaron mediante el método AS-04 NOM-021-RECNAT-2000 en el Laboratorio de Mecánica de Geosistemas (LAMG), UNAM. Su valor es determinado por el cociente del peso de la muestra entre el volumen de la muestra, a su vez el volumen de la muestra es determinado indirectamente mediante la medición de la masa y la densidad del agua desplazada por la muestra. La metodología fue la siguiente: se agregaron 5 gramos de muestra a los picnómetros y se pesaron; se agregó agua destilada y fueron colocados al vacío durante 15 min para eliminar el aire; se dejaron reposar en el desecador por 30 min; posteriormente, fueron aforados con agua destilada y pesados nuevamente. Por último, los picnómetros fueron saturados con agua destilada y pesados.

j) *Determinación de la textura del suelo mediante el procedimiento de Bouyoucos:*

El tamaño de partícula en muestras de sedimentos fue determinada mediante el método AS-09 NOM-021-RECNAT-2000. La preparación de la muestra consistió en la eliminación de la materia orgánica. Para ello, se pesaron 75 g de muestra, se agregaron 100 ml de agua destilada, 20 ml de H_2O_2 30% w/w y se dejó reposar en baño María a temperatura ambiente hasta que terminó la efervescencia. Se colocaron 50 gramos de muestra libre de materia orgánica en una mezcladora con 20 ml de hexametafosfato de sodio (calgon) y 100 ml de agua destilada. Se mezcló por 15 minutos y se transfirió a una probeta de 1000 ml. La probeta se aforó con agua destilada y se agitó por un minuto. Las lecturas fueron tomadas con el hidrómetro de bouyoucos a diferentes tiempos. Para el caso de las partículas mayores a 0.002 mm (limo y arena), el tiempo de lectura fue de 2 horas, para las partículas mayores a 0.05 mm el tiempo de lectura fue de 40 segundos.

RESULTADOS

Los resultados obtenidos en esta tesis permitieron la publicación de tres artículos científicos y un artículo de divulgación en revistas indexadas de circulación nacional e internacional:

Artículos científicos en revistas indexadas:

1) **Sánchez-Sánchez**, J., Cerca, M., Alcántara-Hernández, R. J., Lozano-Flores, C., Carreón-Freyre, D., Levresse, G., Vega, Marina., Varela-Echavarría, Alfredo., Aranda-Gómez, J. J. (2019). Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico. *FEMS microbiology ecology*, 95(5), fiz051.

2) **Sánchez-Sánchez**, J., Cerca, M., Alcántara-Hernández, R.J, Aranda-Gómez, J.J., Carreón-Freyre, D., Lozano-Flores, C., Muñoz-Velasco, I., Levresse, G., Varela- Echavarría, A. (2021). Mobile mud layer underneath the desiccated maar lake of Rincón de Parangueo and insights into its microbial fingerprints. *Revista Mexicana de Ciencias Geológicas*, 38(3), 178-192

3) **Sánchez-Sánchez**, J., Cerca, M., Moguel, B. B., Muñoz-Velasco, I., Alcántara-Hernández, R. J., Carreón-Freyre, D., & Levresse, G. (2023). Geomicrobiology of the Rincón de Parangueo maar crater: Exploring the link between an evolving extreme environment and its potential metabolic diversity. *Freshwater Biology*, 00, 1–21.

Artículo de divulgación científica:

1) **Sánchez-Sánchez,** J., & Cerca, M. Explorando la vida en un ambiente extremo: extremófilos en el cráter volcánico Rincón de parangueo, Guanajuato. (2023). *Enseñanza y Comunicación de las Geociencias*, v. 2, núm. 1, p. 5-9

FEMS Microbiology Ecology, 95, 2019, fiz051

doi: 10.1093/femsec/fiz051
Advance Access Publication Date: 10 April 2019
Research Article

RESEARCH ARTICLE

Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico

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One sentence summary: Extant microbial communities of Rincon de Parangueo maar crater, Mexico

Editor: Tillman Lueders.

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ABSTRACT

Rincon de Parangueo is a maar where a perennial lake was present until the 1980s. A conspicuous feature of the lake's sediments is the presence of bioherms and organo-sedimentary deposits produced by microbial communities. The gradual lake desiccation during the last 40 years has produced dramatic environmental changes inside the maar basin, which resulted in the formation of a highly saline-alkaline system with extant microorganisms. In this paper we succinctly describe the geologic setting where the microbial communities have developed inside of the maar crater and the results obtained from high-throughput sequencing methods to characterize the microbial component (Bacteria, Eukarya and Archaea) in endolithic mats of calcareous sediments, and microbial mats and free-living microorganisms in the soda ponds. The studied sites displayed different microbial communities with a diverse number of phylotypes belonging to Bacteria and Eukarya, contrasting with a much less diverse component in Archaea. The sequences here detected were related to environmental sequences from sites with extreme life conditions such as high alkalinity (alkaliphiles), high salinity (halophiles) and high temperature (thermophiles). Moreover, our results indicate an important unexplored endemic microbial biodiversity in the vestiges of the former lake that need to be studied.

Keywords: maar lake; alkaline sediments; soda ponds; microbial community; microbialites; phylotype

Received: 11 January 2019; **Accepted:** 9 April 2019

INTRODUCTION

Growth of modern and extant microbial stromatolites in volcanic crater lakes is sustained only in exceptional cases documented around the world. Examples of these microbial environments include the quasi-marine alkaline lake inside the craters of Niuafo'ou in Tonga (Kazmierczak and Kempe 2006) and Satonda in Indonesia (Kempe *et al.* 1996), the soda Lake Van in Turkey (López-García *et al.* 2005), in the thermal springs of Yellowstone (Lau *et al.* 2005; Berelson *et al.* 2011) and the alkaline lake in the maar crater of Alchichica in eastern Mexico (Tavera and Komárek 1996; Couradeau *et al.* 2011; Kazmierczak *et al.* 2011). In addition to the well-documented case of Alchichica, the occurrence of microbialites in Mexico has been reported in several maar or tectonic lakes in the Trans Mexican Volcanic Belt, such as Rincon de Parangueo, La Alberca, Quechulac, Alberca Los Espinos, Patzcuaro, Aljojuca, La Preciosa, Joya de Yuriria, Tecuitlapa and Atexcac (Aranda-Gomez *et al.* 2017; Zeyen *et al.* 2017; Chacón *et al.* 2018). All these lakes contain or contained until recently, salty and highly alkaline lakes inside small endorheic basins.

The Rincon de Parangueo (RP) maar, in the Valle de Santiago Volcanic field (Fig. 1), had a perennial lake until the 1980s, when overexploitation of the feeding aquifer provoked a dramatic decrease of the water level and land subsidence (Aranda-Gómez *et al.* 2013; Rocha-Treviño 2015). Currently, RP behaves as a playa lake that increases its size during the rainy season. Later, during the dry season, the water level is greatly reduced by evaporation and a small amount of infiltration. The gradual water level reduction in the lake has been accompanied by marked changes in salinity and pH of the water (Table 1). Partial desiccation of the lake has completely exposed organo-sedimentary structures, which grew near the former coast and were formed by characteristic microbial communities. These microbialites include biohermal thrombolites, stromatolites and oncolites formed by active trapping, binding and induced carbonate precipitation (mainly aragonite and hydromagnesite). The rapid lake desiccation from the early 1980s led some authors to consider RP microbialites as fossils (Zeyen *et al.* 2017). However, the recent report of living hygroscopic cyanobacteria in the non-lithified crustal layers of individual thrombolites (Chacón *et al.* 2018) suggests the presence of active microorganisms in the subaerial sediments. In any case, RP is an example of a fast and drastic transforming environment where macro- and micro-geobiological interactions directly influence biological succession (Chacón *et al.* 2018); it represents a natural laboratory for the study of extant and recently modified microbial communities. The study of the microbial component of present-day communities and their relationships with the recent sedimentary record is relevant because of the unique characteristics of the environment that prevailed during the lake period. Nowadays, most of the lacustrine area has been transformed in a subaerial alkaline soil surface. This recent dramatic environmental evolution and the co-existence of microbial mats in sediments and alkaline ponds turn RP into an amazing natural laboratory to analyze the microbial genetic component living in such different niches inside it.

MATERIALS AND METHODS

Stratigraphy

Two field campaigns were carried out in 2016 and 2017. The geological map presented by Aranda-Gómez *et al.* (2017) served as a base map for field work, which consisted of mapping major organo-sedimentary structures and larger bioherms. Field data were integrated with the description of one stratigraphic profile logged across the crater. Stratigraphy of the calcareous sequence and organo-sedimentary structures was described at outcrops and shallow trenches (<2 m) excavated along the profile. Sediment samples were collected at the trenches for each representative layer to measure selected physical properties such as fine grain size distribution (ASTM D422-63 2007), bulk density (ASTM D7263-09 2018), gravimetric water content, pH and electric conductivity (EC) in a saturated paste of soils prepared using distilled water (NOM-021-SEMARNAT-2000).

X-ray diffraction and scanning electron microscope observations

For scanning electron microscope (SEM) observations, millimeter-sized carbonate fragments were mounted on an aluminum plate covered with carbon conductive adhesive tape. Samples were observed in a HITACHI TM1000 SEM at 15 kV with a backscattered detector. For X-ray diffraction (XRD), the fine grain (<0.002 mm) fractions of samples were crushed in an agate mortar and the dry powders were loaded on aluminum plates; the samples were analyzed using a Miniflex Rigaku diffractometer using copper radiation Kα 1.5406 Å, between a 2θ angle of 5 to 80 degrees in steps of 0.02 degrees and 0.06 s for the measured spot. Crystalline phases were identified using the PDF-2 database (International Centre for Diffraction Data, 2005).

Sampling strategy for microbial analysis

Microbial communities were characterized within the crater in endolithic microbial mats of sediments, microbial mats and free-living microorganisms in the soda ponds. Collection of the samples for DNA extraction was done in November 2016. The goal was to characterize the microbial communities in relation to their geological environment, thus we selected three different environments for DNA sampling. (i) Lithifying microbial mats (Microbialite samples) living at the surface and in the massive carbonates (endolithic) and located at the walls of the annular fracture (Fig. b and c). (ii) Microbial mats (Mat samples) collected along the shore of each the three hypersaline alkaline water ponds (pH 10, EC $\sim$30 mS cm^{-1}). (iii) Aquatic microbes (Water samples) from each of the three small lacustrine remnants. Mat 1 and Water 1 were collected from the largest pond, while Mat 3 and Water 3 from the smallest (see Fig. b and c). Each sample was taken with sterile gloves and tweezers to minimize the risk of contamination, using 50 mL sterile conical tubes (Corning Company, USA), which were kept on ice during transport and stored at –20°C until processing.

Nucleic acid extraction and analysis

Metagenomic DNA extraction was done in triplicate and handled as independent (using 30 g of material/biomass for Microbialite 1, 2, 3 and Mat 1, 2, 3; 500 mL for Water 2, 3; 3500 ml for Water 1) following the sample preparation protocol reported by Bey *et al.* (2010), and the PowerSoil extraction kit DNA Isolation (MoBio) also following the manufacturer's instructions. Samples were sent for sequencing to the RTL genomics company at Lubbock (TX, USA) (http://rtlgenomics.com). The microbial community was analyzed using the 16S ribosomal RNA

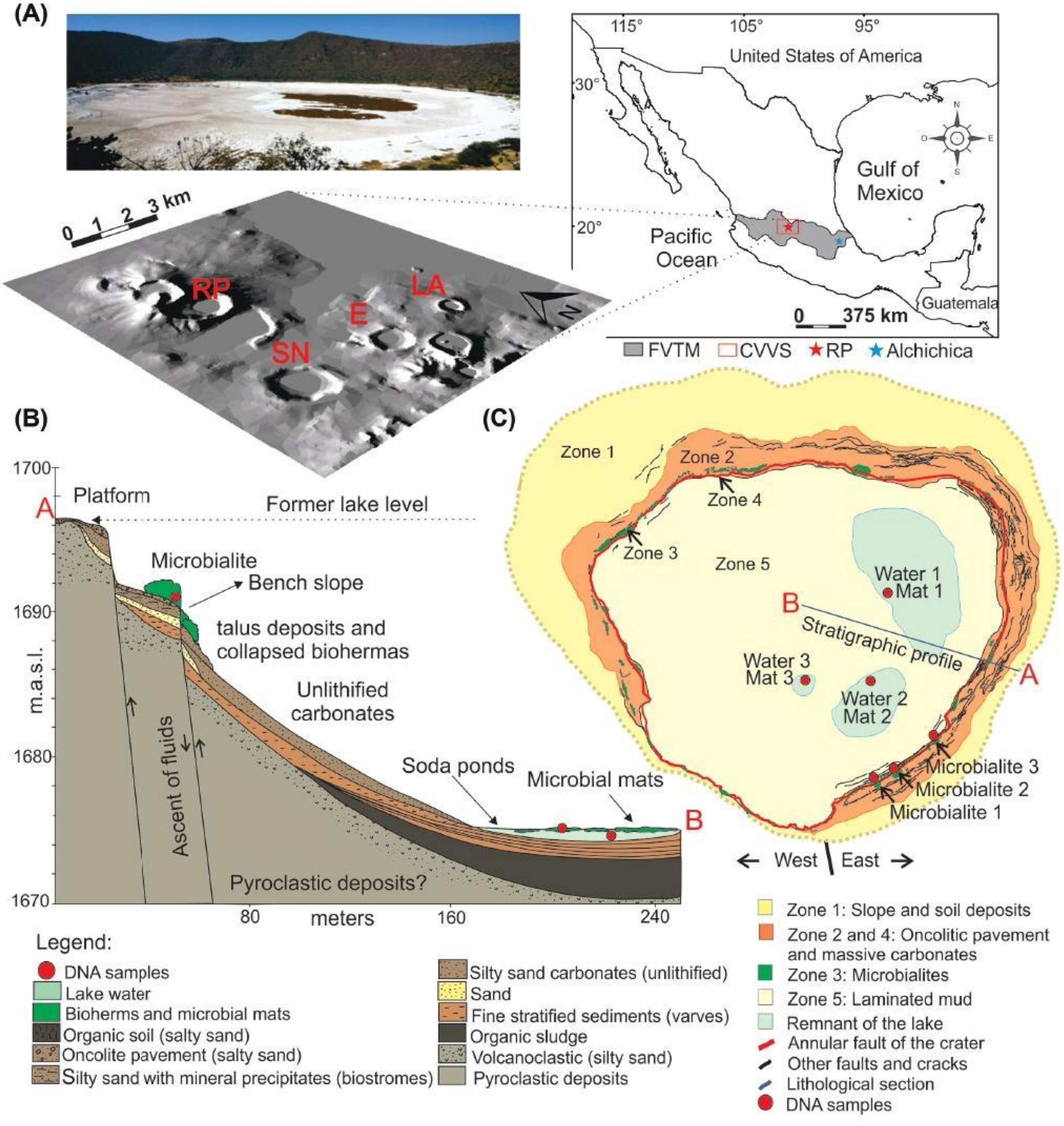

Figure 1 (A) Geographic location of crater maar Rincon de Parangueo (RP) in the Valle de Santiago Volcanic Field , within the Trans Mexican Volcanic Belt (TMVB). Panoramic view shows the remnant lakes in the maar crater. The digital elevation map shows the topographic elevations and the craters in the Valle de Santiago area, the craters maar of Rincon de Parangueo (RP), Hoya La Alberca (LA), Hoya Estrada (HE) and Hoya San Nicolas (SN). **(B)** Stratigraphic sequences observed along the AB profile showing the carbonated sequence, grain size distribution and the DNA site samples. **(C)** Mapping of organo-sedimentary structures shows five zones with concentric distribution, the remnant alkaline ponds in the central part of the crater, the annular fault, and other faults and fractures. Collection sites of nine samples: Microbialite 1, 2, 3; Water 1, 2, 3; Mat 1, 2, 3.

(16S rRNA) for Bacteria and Archaea, and the 18S ribosomal RNA (18S rRNA) for Eukarya (Table 2). DNA sequencing was performed on an Illumina MiSeq platform. The forward and reverse reads were merged together using the PEAR Illumina paired-end read merger (Zhang *et al.* 2014). Prefix dereplication was performed using the USEARCH algorithm (Edgar 2010). Operational taxonomic unit (OTU) selection was performed using the UPARSE OTU selection algorithm (Edgar 2013). Chimera checking was performed on the selected OTUs using the UCHIME chimera detection software executed in *de novo* mode (Edgar *et al.* 2011). The sequences were aligned using the USEARCH global alignment algorithm (Bokulich *et al.* 2015) using a high-quality database from the NCBI database. Visualization of the data was carried out through a series of graphs using the Circos software package, using the website https://dzone.com/articles/circle-through-your-google. The sequences here generated are available in the NCBI database under the BioProject PRJNA510628.

Table 1. Compilation of data obtained from the water of Rincon de Parangueo through time.

Date	T (°C)	pH	EC (μS cm^{-1})	References
1982	30	–	25,000	Green 1986
1995	–	–	70 000–80 000	Escolero-Fuentes and Alcocer-Duran 2004
1999	18.1	10.2	165 000	Aranda-Gómez *et al.* 2013
2002	–	–	187 500	Escolero-Fuentes and Alcocer-Duran 2004

RESULTS

Geologic setting and description of the microbialites

RP is a Quaternary maar (<0.137 Ma: Aranda-Gómez *et al.* 2013) located at the central part of the Trans Mexican Volcanic Belt (Fig. 1a). The geology of the maar and its surroundings has been widely described by Aranda-Gómez *et al.* (2013, 2014, 2017). The presence of a perennial lake was reported inside the crater in 1900 (Ordoñez 1900). Overexploitation of the regional aquifer in the early 1980s caused the gradual shrinking of the lake and by 2000 the desiccation process was 'completed' according to Escolero-Fuentes and Alcocer-Duran (2004). However, Aranda-Gómez *et al.* (2013) pointed out that since 2009 there is always at least a remnant pond inside the crater, which is probably fed by a perched aquifer inside the maar. The morphology of the lake has been affected by rapid (~1 m year^{-1}) active subsidence and a large set of semicircular faults and fractures with throws mainly toward the center of the crater, forming a 15 m high topographic scarp (Rocha-Treviño 2015; Aranda-Gomez *et al.* 2017). These young structures mean that nowadays both the bottom of the lake and the organo-sedimentary structures located near the former shore are well exposed. Comprehensive reviews of the geology and of the modern microbialites found in RP crater have been presented previously by Aranda-Gómez *et al.* (2017) and Chacón *et al.* (2018), respectively. Here, we summarize the information reported by these authors along with some observations that will be discussed.

The lacustrine carbonate sequence reported at Rincon de Parangueo crater has a simple concentric distribution of facies (Zones 1–5 in Fig. 1c). Starting from an area close to the inner crater wall, Zone 1 was described by Aranda-Gomez *et al.* (2017) as a talus deposits, composed of volcanic rock blocks and, in places, a silty-sand organic soil. Microbialites are present in Zone 1 as thin carbonate crusts attached to basaltic blocks, tree roots and other organic detritus derived from the vegetation. Zone 2 is a nearly flat or gently inclined 'oncolite pavement' that formed a platform bench (e.g. Platt and Wright 1991), which occurred in the sublittoral zone of the lake. The oncolites of Zone 2 vary in size from 2 to 8 cm in diameter, and are generally rounded or elliptical. Oncolites are embedded in a matrix of carbonaceous silty-sand sediment. Oncolites are concentric laminar structures that grow around nuclei (Wade and Garcia-Pichel 2003). Thus, microbialites in this zone nucleated around rock fragments, intraclasts derived from lake sediments, or wood fragments derived from roots or tree branches. These structures can contain voids due to the decomposition of the wood on which they were formed, preserving only the external structure. Zone 3 is an annular alignment of domical biohermal thrombolites, reaching up to 1 m in height (Fig. 2 A and B) and, in places, tens of meters in length. This zone occurs at the limit between the sublittoral and talus zones of the former lake. It also coincides with a present-day main annular fault scarp. Zone 4 is a narrow and highly deformed steep zone beyond Zone 3, indicated by an arrow in the map, made of tilted blocks bounded by tensional joints and high angle normal and reverse faults. The blocks are made of thinly laminated calcareous mud. Finally, Zone 5 is the innermost one corresponding to the pelagic central basin, which was filled by thinly laminated sediments (organic and detritic varves) deposited nearly horizontally. Layered sediments or 'varves' are composed by micro-layers of tan-colored carbonaceous micritic mud and silty-clayey sediments with a larger percentage of organic matter. Some silt and fine sand layers are also interbedded in the sequence (Kienel *et al.* 2009). Currently the finely laminated sediments can be found up to a depth of 120 cm, and below this sequence there is a massive, organic-rich, mud layer with a high water content (>200%).

Modern bioherms form a discontinuous rim (Zone 3: Fig. 1c) constituted by clusters of porous thrombolites, which are aligned roughly parallel to the marginal facies of the paleo-lake (Chacón *et al.* 2018). Their mesostructures are characterized by a thrombolitic nuclei surrounded by stromatolitic crusts (Fig. 2E) that according to Chacón *et al.* represent the last accretion stages formed at intertidal conditions. The stromatolitic crust is composed by fine laminations made of aragonite with minor hydromagnesite, where Chacón *et al.* (2018) observed a greenish layer of living hygroscopic cyanobacteria in all samples. Fig. H shows the characteristic pattern of aragonite and hydromagnesite (Hy) peaks found in XRD analyses of the microbialite. Microstructures associated with lithified coccoid cyanobacteria

Table 2. Primers used for analyzing microbial genetic abundance from different samples inside Rincon de Parangueo maar.

Life domain	Primer	Sequences	Reference
Eukarya	TAReuk	Forward CCAGCASCYGCGGTAATTCC Reverse ACTTTCGTTCTTGATYRA	Villarino *et al.* 2018
Bacteria	28F/519R	Forward GAGTTTGATCNTGGCTCAG Reverse GTNTTACNGCGGCKGCTG	Fan, McElroy and Thomas 2012; Lay CY *et al.* 2013
Archaea	Arch517F/909R	Forward GCYTAAAGSRNCCGTAGC Reverse TTTCAGYCTTGCGRCCGTAC	Lay CY *et al.* 2013

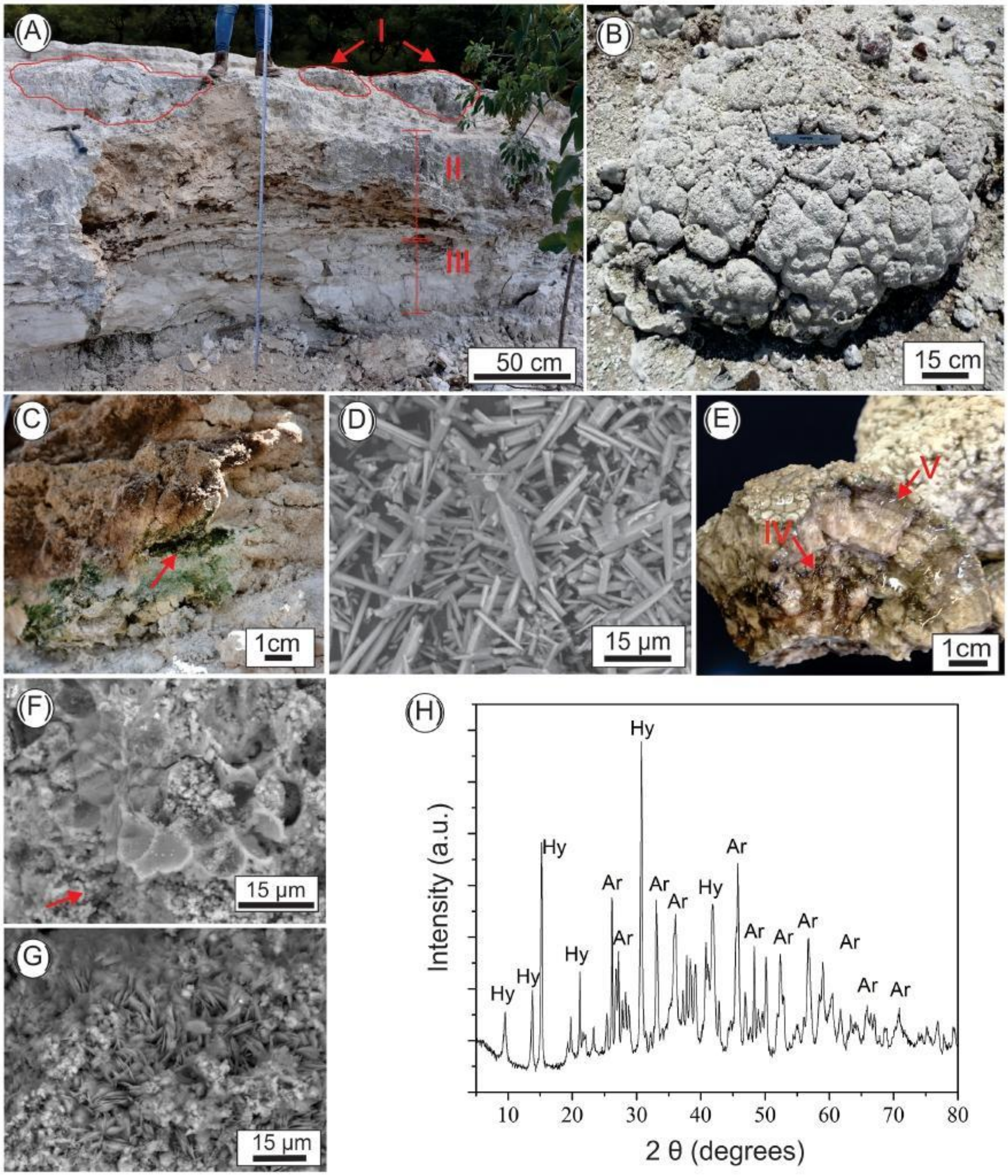

Figure 2 Microbial mats in sediments and bioherms. **(A)** Sequence of carbonates exposed at the wall of the annular fault of the crater, in the upper part are the bioherms (I), in the middle part the presence of biostromes (II) with a high content of aragonite in which the presence of microbial communities was observed. Finally, in the lower part a zone of massive carbonates (III). **(B)** Well-defined domical bioherm. **(C)** Occurrence of endolithic living microbial mats observed as dark brown and greenish mats (red arrow) distributed on the surface of the fault, mainly on the surface of the massive non-lithified layer. **(E)** Aspect of the thrombolite (IV) and laminar mesostructures (V); note the presence of a greenish layer of cyanobacteria in the laminar crust. Scanning electron microscopy images: **(D)** crystal structures of aragonite formed in the microbial mats located in the middle part of the carbonate sequence; **(F** and **G)** structures associated to lithified probable coccoid Cyanobacteria (red arrow). (H) X-ray diffraction pattern of microbialite showing peaks characteristic of aragonite (Ar) and hydromagnesite (Hy).

were observed within a matrix of globular aragonite at the stromatolitic crusts (Fig. F and G). In addition to the thrombolite, stromatolite and oncolite morphologies previously reported by Chacón *et al.* (2018), here we describe chimney-like structures that occur along the annular fault (Fig. B). The microstructure observed in samples from the chimneys (Fig. E) include needle-like aragonite crystal associated with gypsum.

The topographic scarp formed by the downthrow displacement along the ring-fault system (Fig. 3A) has ruptured and down-faulted part of the biohermal thrombolites, exposing their internal structures and showing a complex network of precipitation/dissolution pores within large biohermal crusts (Fig. 3C and D). The recently formed, subsidence-related, fault planes commonly contain large areas covered by a crust made of globular aragonite, which is interpreted as a 'dripstone', a type of speleothem formed by aqueous solutions that flow along the fault planes and tensile fractures (Fig. 3A). Globular aragonite nanoprecipitates probably associated to lithified coccoid

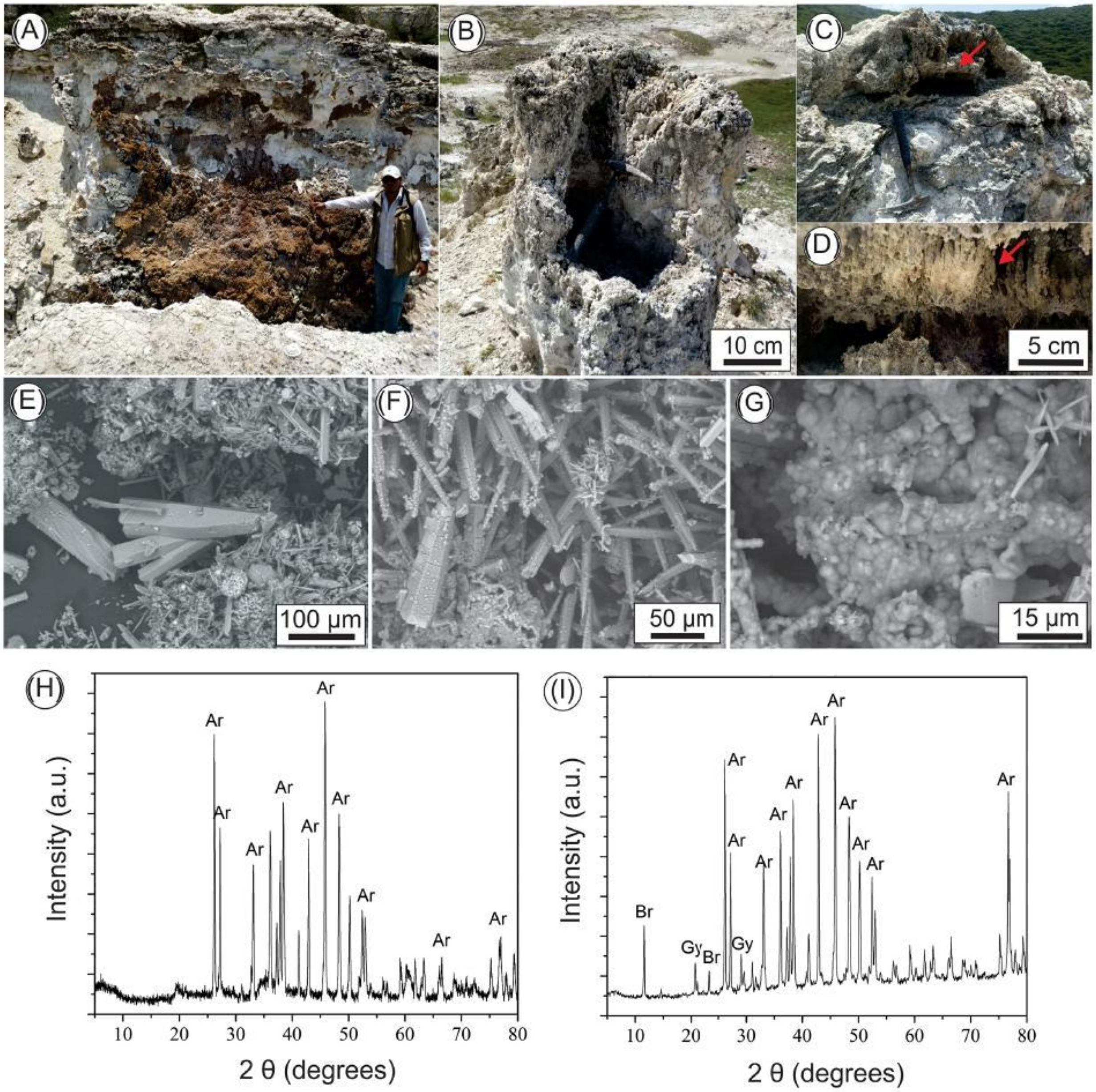

Figure 3 Mineral precipitation and chimney-like structures associated with the annular fault. (A) Microbialite cut by the annular fault of the crater, contains areas with brown mineral precipitation. (B) Chimney structure located in the annular fault zone with mineralized zones. (C) Bioherm cut by the annular fault of the crater, domic structure with cavities. (D) Zoom of the previous figure showing the aspect of the macropore and speleothems formed by dissolution. Scanning electron microscopy images: (E) Crystalline structures of aragonite (Ar) formed in the chimney; (F) crystalline structures of aragonite found in the internal part of the domic structure (C); (G) carbonate nanoprecipitates associated to lithified probable coccoid cyanobacteria. X-ray diffraction pattern: (H) the brown area in the image (A) showing peaks characteristic only of aragonite (Ar); (I) the chimney showing peaks characteristic of aragonite (Ar), gypsum (Gy) and brushite (Br).

cyanobacteria were found on the fault planes (Fig. 3F and G). XRD patterns obtained in samples of the brownish crusts precipitated as thin coatings on the walls of dissolution pores within the bioherms show only characteristic peaks of aragonite (Fig. H), whereas the diffraction pattern of the precipitates at the chimney flanks indicates the presence of aragonite and secondary peaks of gypsum (Fig. 3I).

Endolithic microbial mats in the calcareous sediments

The occurrence of living endolithic microbial mats was observed as thin layers of dark brown and greenish areas irregularly distributed on the surface of faults and open fractures, mainly on the surface of the massive, non-lithified mud carbonate layers (Fig. C). SEM images obtained of samples from these mats show acicular aragonite crystals related to the microbial activity (Fig. D).

The sedimentary sequence hosting the microbial mats was described from trenches and natural outcrops located on profile A–B in the eastern part of the lake basin (Fig. 1b and Table 3). Near the former coast of the lake, the sedimentary succession is composed of four layers. (i) The base is a pyroclastic deposit that was accumulated during the formation of the maar or shortly after. This, in turn, is covered by three successions made of calcareous sediments. (ii) Resting directly atop the pyroclastic deposits there is an irregular layer up to 2 m thick, made of massive, soft and friable, calcareous silty-sand, with

Table 3. Selected physical properties measured in the geologic record of the carbonate sediments and pyroclastic deposits at the margins of the Rincon de Parangueo maar.

Unit	Bulk density (g cm^{-3})	Gravimetric water (%)	EC (μS cm^{-1})	pH
Oncolite pavement	0.75	50.58	653	9.6
Unlithified carbonates	0.84	75.19	983	9.7
Pyroclastic deposits	1.01	63.42	1248	9.4

few oncolites embedded. This unit, described as a calcareous tufa by Chacón *et al.* (2018), is alkaline (pH ~9.8), with a low bulk density of ~0.9 g cm^{-3} (Table 3) and with high porosity (>50%). This layer is also partially saturated, with a mean gravimetric water content of 65% and with a mean EC value of 615 μs cm^{-1} (ranging from 460 to 740 μs cm^{-1}). The high porosity of the samples was corroborated by the SEM studies. (iii) The second calcareous unit is a set of several layers of mud with abundant oncolites and it forms the 'pavement' that covers the calcareous platform near the former lake coast (Fig. A). These sedimentary layers have a pH around 9.4, bulk density of 0.7 g cm $^{-3}$, gravimetric water content of 48% and EC of 687 μs cm^{-1}. The calcareous sediments unit is relevant since samples of lithifying living mats of endolithic microbial communities were collected on the walls of the tensional fractures. (iv) The biohermal thrombolites cover the third calcareous unit.

Microbial mats in the hypersaline ponds

Currently, the most obvious living microbial mats in the RP crater are those of the small soda ponds at the bottom of the crater. Water depth of the ponds is <0.5 m and the bottom is a soft, saturated mud. The size of the ponds and the color of the brine vary during the year and at any given time there are differences between the ponds. Fig. 4A shows the water color of the smaller pond at the time of sampling for this research. Microbial mats form in the water and accrete to the shore where they intermingle with the evaporites, forming a thin (~1 cm) crust around the shrinking shore. In the lower left corner of Fig. 4A, a thin cumulate of microbial mat and salt crystals forms a 'raft' that floats on the water. The mesoscopic relationships among microbes and evaporites are shown in Fig. 4B. A clean mixture of trona and halite generally tend to be colorless or white crystalline aggregate. Thus, the pink and green colors in the sample are attributed to the presence of different microbial communities living in the raft. Well developed, euhedral crystals of halite and trona crystals form the substrate where the bacterial mat was growing. It is unknown whether the microbial communities play a roll in evaporite precipitation or if they are opportunistic colonies that settled and grew atop the recently precipitated evaporites. Fig. 4C shows an SEM image of the crystalline structure of halite and trona obtained from a portion of the samples shown in Fig. 4B. An XRD (Fig. 4D) pattern of the same sample shows characteristic peaks of halite, trona and thermonatrite.

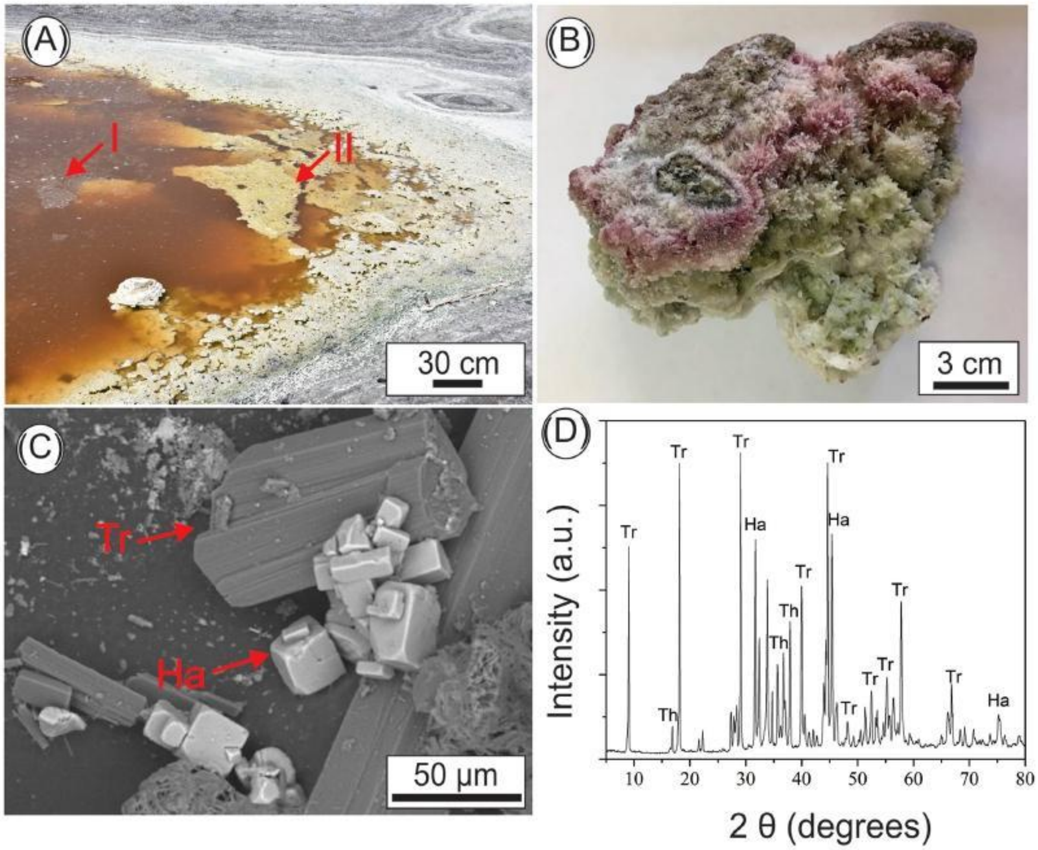

Figure 4. Microbial mats and communities in the hypersaline ponds. (A) Small pond located within the remnant area of the lake. Salt precipitation in the middle of the pond (I) and microbial mats developing along with salt precipitates (II). (B) Hand sample of the microbial mat formed in the pond. Note the difference in the color between the hand sample and the water. (C) Scanning electron microscopy image: the crystalline structure of salts precipitated in the remnant of the lake was identified. (D) X-ray diffraction pattern showing peaks characteristic of halite (Ha), trona (Tr) and thermonatrite (Th).

Microbial analysis results

The number of filtered DNA sequences obtained for each of the samples is shown in Table 4. The minimum number of sequences obtained for a sample was about 6000, but most samples had at least 10 000 sequences. The relative abundance of Bacteria, Eukarya and Archaea at the phylum level is shown in Fig. 5, and the relationships at order level are shown for Bacteria (Fig. 6), Eukarya (Fig. 7) and Archaea (Fig. 8). Table 5 summarizes the meaning of the abbreviations used in the Figs 6–8.

Bacterial 16S rRNA

The 16S rRNA phylogenetic analyzes revealed a diverse bacterial component within the analyzed samples. The bar charts in Fig. 5A show the relative abundances of the bacterial community at the phylum level. A remarkable aspect is the high abundance of Cyanobacteria and Proteobacteria observed in all samples. Other phyla identified in lesser abundance were Actinobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Chloroflexi, Spirochaetes, Verrucomicrobia and Deinococcus-Thermus. In particular, Deinococcus-Thermus (one of the most extremophilic phylum of bacteria) was found in minor quantities in eight out of nine samples. The comparison of results at the order level indicates different relative abundances for each sample type, i.e. Microbialite, Mat and Water. In the ribbon graph of Fig. 6 the order Oscillatoriales was predominant in Microbialite samples (4.2–28%) and Mat samples (27–51%). Chroococcales show higher percentages in Microbialite (4–18%). Nostocales presents high percentages in Water (~32%) and Microbialite (1–13%) samples. In Mat, high percentages of unclassified Cyanobacteria were also found (15–29%). Rhodobacterales was unclassified in all samples, i.e. Microbialite (7–33%), Mat (11–22%) and Water (1–23%). Rhizobiales was found in Microbialite (1–4%) and Mat (0.8–0.9%). The Burkholderiales order was mainly identified in Microbialite (0.9–6%), in a similar way to Xanthomonadales (0.9–20%). Another important observation is the high percentage of unclassified bacterial sequences. For graphic simplicity the different types of unrecognized sequences (no hit, unknown and unclassified) were grouped together as unclassified (Fig. 5A), the graphics showing the complete distribution are presented in the supplementary material available online.

Eukarya 18S rRNA

Phylogenetic analyzes for the 18S rRNA genes showed different phyla in the samples (Fig. 5B). For the Microbialite samples, the most abundant phyla were Chlorophyta (green algae) and Nematoda. In Mat samples, Bacillariophyta (diatomea) represents the highest percentage followed by Chlorophyta. In Water samples, the phylum Chlorophyta was the most abundant, followed by Rotifera. Some other phyla were recognized in lesser proportion such as Arthropoda, Chordata, Ascomycota, Basidiomycota, Chytridiomycota and Streptophyta.

The relative abundances at order level are presented in Fig. 7. Unclassified order of Bacillariophyceae (diatomea) was found in Water (7–47%) and Mat samples (8–15%), whereas unclassified orders of the Bacillariophyta phylum were found in high percentages in Mat samples (43–54%). High abundances of Chlamydomonates were found in Water samples (4–99%). Unclassified orders (0.6–53%) of the class Trebouxiophyceae were obtained in Microbialites. The Ulvophyceae class showed high percentages of the Ulvales order in the Mats (26–34%), and high abundances of the orders Floscularia cea (15–40%) and Ploima (0.2–22%) were found in the Water samples. As in the case of Bacteria, a high percentage of the Eukarya sequences could not be classified (Fig. 5B).

Table 4. Number of total sequences obtained from different samples inside Rincon de Parangueo.

Sample	Filtered sequences Bacteria	Eukarya	Archaea
Microbialite 1	10230	13 399	16 211
Microbialite 2	23 588	11 105	27 958
Microbialite 3	29 682	11 000	18 367
Mat 1	7275	8963	16 874
Mat 2	12 615	23 939	21 561
Mat 3	9601	14 821	16 702
Water 1	8616	18 254	13 595
Water 2	6090	10 430	16 409
Water 3	21 192	27 555	27 950
TOTAL	128 889	139 466	175 627

Archaea 16S rRNA

Lower numbers of Archaeal phyla were recorded in comparison with Bacteria and Eukarya. Euryarchaeota was found as the predominant phylum in all samples (Fig. 5C). The percentage of this phylum in the case of Microbialites was 0.2–7%, in Mat 15–20% and Water 30–73%. A high percentage of unclassified Archaea sequences were obtained in all samples: Microbialite 33–39%, Mat 1.8–86% and Water 3.8–18%. Five families of the classes Halobacteria and Methanomicrobia were found: Halobacteriaceae, Haloferacaceae, Natrialbaceae, Methanocalculaceae and Methanosarcinaceae. At the order level (Fig. 8), Halobacteriales sequences were obtained in high percentages in Water (52–89%) and Microbialite (34–38%) samples. Natrialbales was identified in the sample Water 1 (10.9%). Additionally, an unidentified genus was reported for Microbialite samples (18–23%). For Mat samples, unclassified domain sequences were found in high percentages (13–90%).

DISCUSSION

Geological observations

RP has the best developed ring of modern domic thrombolites among the 17 maar craters in the Valle de Santiago volcanic field, where only the La Alberca, Cíntora and Joya de Yuriria crater lakes have poorly developed microbialites encrusted in the basaltic rocks (Aranda-Gómez *et al.* 2013, 2017; Zeyen *et al.* 2017; Chacón *et al.* 2018). At RP the thickness of the sequence of oncolite-bearing and massive calcareous sediments reach >3 m near the paleolake margin. The factors that favored calcareous sedimentation in the recent past may include (i) the young eruptive age of the maar and associated degassing, both trough sediments and water, (ii) the structural characteristics (fracturing) of the crater, (iii) the water depth during the paleolake period and (iv) the groundwater inflow from the regional aquifer. The RP crater represents the youngest eruption reported so far in the region with a maximum eruption age of 137 ± 89 kyears, inferred from cross-cutting stratigraphic relations with

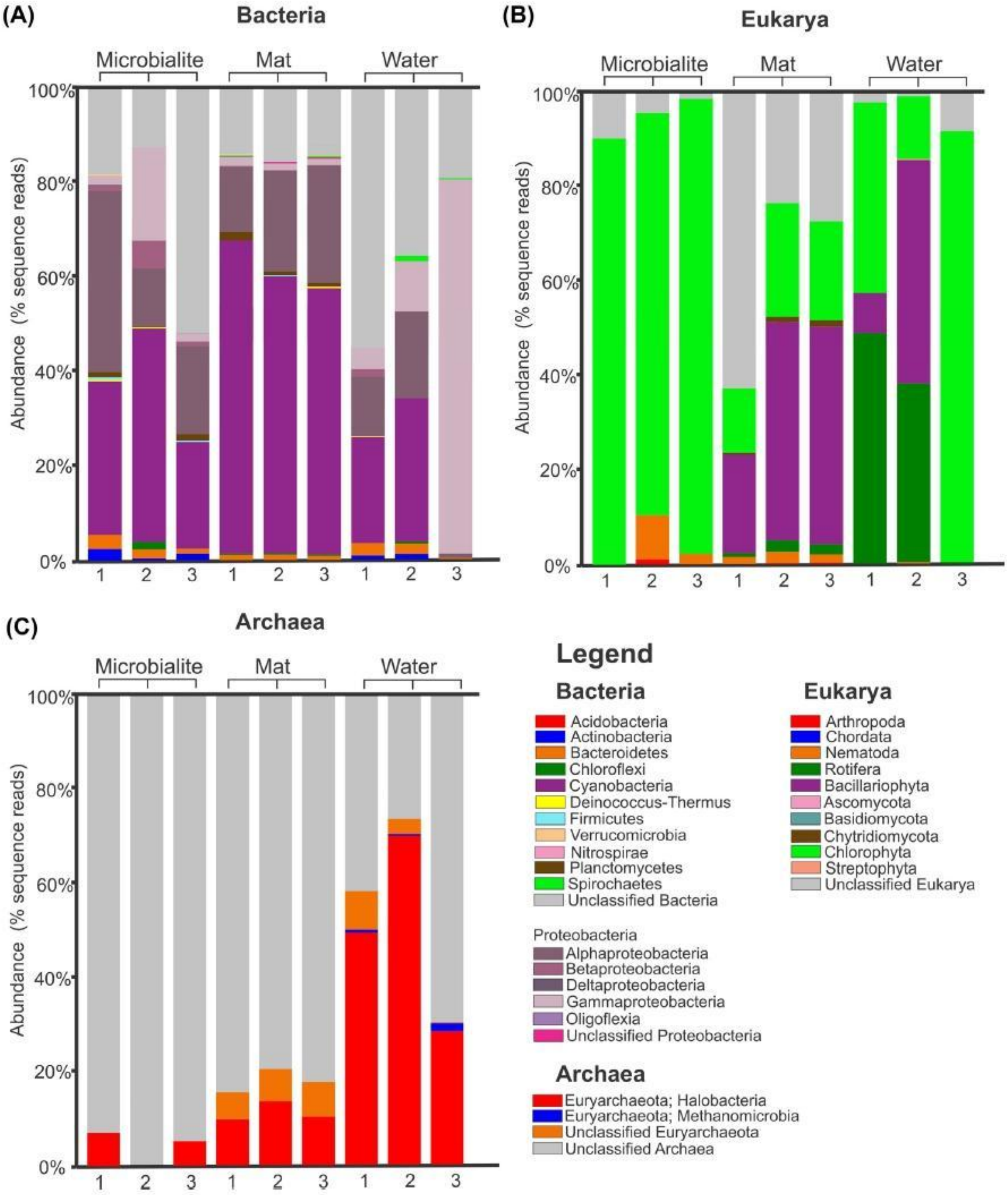

Figure 5. Relative abundances of the obtained phylotypes of Bacteria, Eukarya and Archaea. (A) Bar chart shows a high abundance of Cyanobacteria and Proteobacteria (this last presented at class level) for all samples, and sequences belonging to unclassified Bacteria (no hit, unknown and unclassified were grouped together). In Water samples the unclassified percentage is higher. (B) Bar chart of Eukarya shows the highest abundance of Chlorophyta in Microbialite, Bacillariophyta in Mat samples and Rotifera in two samples of Water. Mat samples show the highest number of unidentified sequences. (C) Relative abundances of Archaea at class level. Only Euryarchaeota was found as the dominant phylum. Water samples showed the highest percentage of unclassified sequences for Halobacteria and Methanomicrobia classes. Mat samples showed the highest percentage of unclassified sequences of the Euryarchaeota phylum. The Microbialite samples presented high percentages of unclassified Archaea sequences.

dated pyroclastic deposits of the Santa Rosa tuff-ring (Aranda-Gómez *et al.* 2017). Repeated volcanic eruptions in the same area may have intensely fractured some of the pre-RP rocks, and a hydraulic connection between the crater lake and the regional aquifer has been proposed by the correlation of groundwater drawdown and subsidence in the crater (Cerca *et al.* 2015). The close spatial association of the large domical biohermal thrombolites and the annular fault suggest that this structure might have controlled groundwater flow into the paleolake. The presence of chimney-like structures and the thrombolitic texture of most microbialites also suggests an upward groundwater flow during the lake period that might have helped to maintain a stable depth water column. Other authors reported the presence of springs associated to groundwater inflow observed

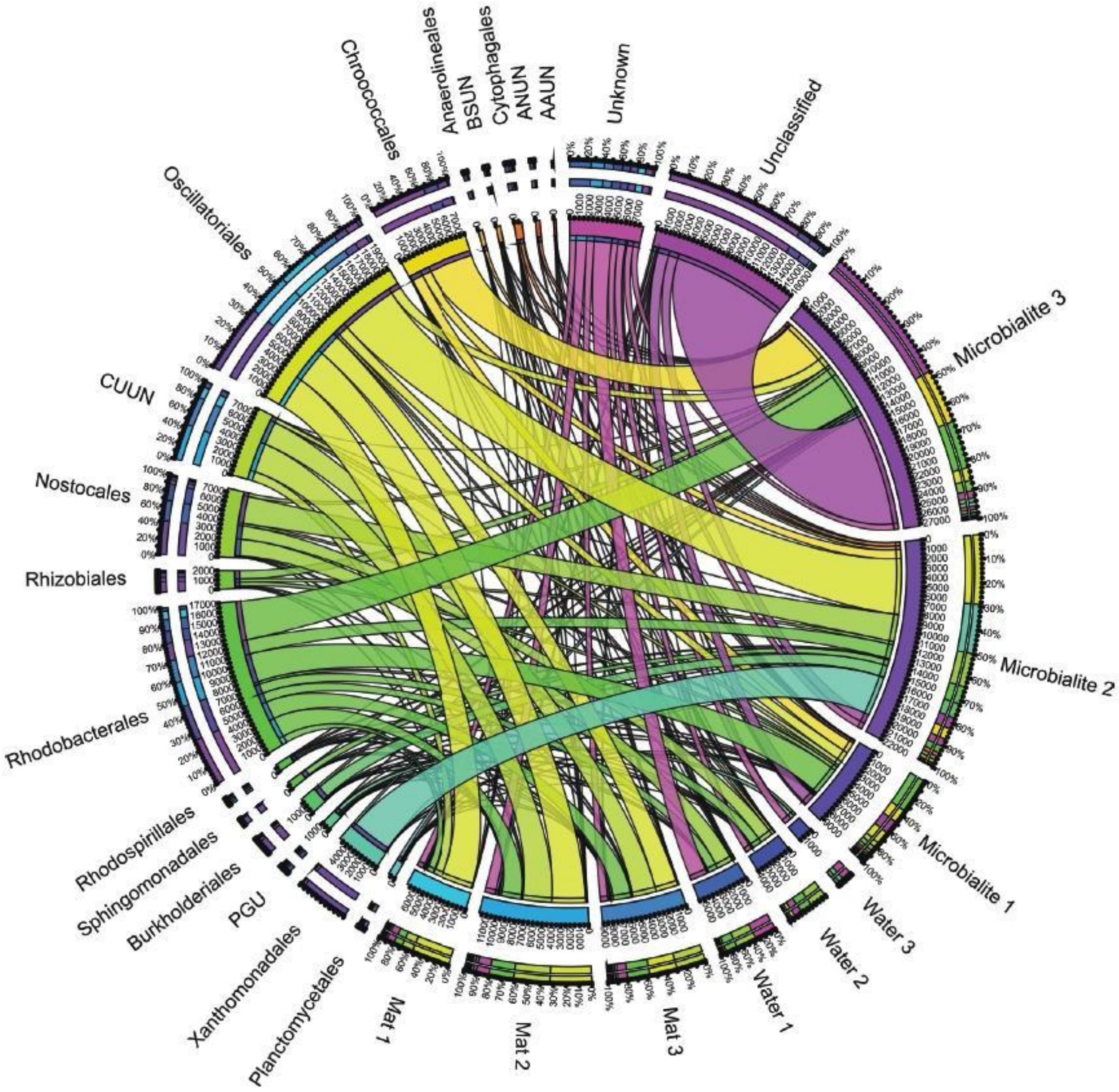

Figure 6. Bacteria distribution in the samples at the order level. In microbialites, the orders Oscillatoriales, Chrococcales, Nostocales, Rhodobacterales, Rhizobiales, Burkholderiales and Xanthomonadales showed high percentages. In Mats, the orders Oscillatoriales, Rhodobacterales and Rhizobiales, and unidentified cyanobacteria, presented high percentages. In Water samples, only Nostocales and Rhodobacterales presented high percentages. Water 3 showed a high percentage of Chromatiales. The meanings of the abbreviations used in the figure are presented in Table 5.

during the lake period in the past century (Escolero-Fuentes and Alcocer-Duran 2004). In Alchichica, a similar groundwater inflow has been inferred from aragonite precipitation (Kazmierczak *et al.* 2011), which is a common mineral phase in RP. This fluid might have increased the CO_2 concentrations, and greatly favored cyanobacterial growth and the consequential deposition of carbonated organo-sedimentary structures. Although there is no evidence of recent volcanic activity at the RP maar, a preliminary compositional analysis made on the degassing soils of the maar sediments is dominated by CO_2 and traces of H_2S and CH_4 (Levresse pers. comm.).

On the other hand, this study characterizes for the first time the microbial component of living communities key to understand the consequences of the profound habitat perturbation or loss of aquatic habitat caused by groundwater overdraft. Shrinkage of the lake in the inside RP maar has lead microbial communities to develop in a new subaerial environment during the last 40 years (Aranda-Gómez *et al.* 2013, 2017). We found that desiccation is the main environmental pressure imposed to the originally subaquatic communities, resulting in the compartmentalization of the environment in (i) subaerial sediments (resembling alkaline desert soils) and (ii) progressively more alkaline ponds.

Phyla distribution in RP

RP represents a unique habitat for extant microbial communities under extreme conditions of salinity, alkalinity and thermal stress, and this evolving habitat is reflected by some components of the microbial communities found inside the crater. Very few studies using molecular methods to describe the three life domains have been carried out in crater lakes (e.g. Couradeau *et al.* 2011). In the case of RP, several phylotypes of Bacteria and Eukarya were identified, contrasting with a considerably smaller

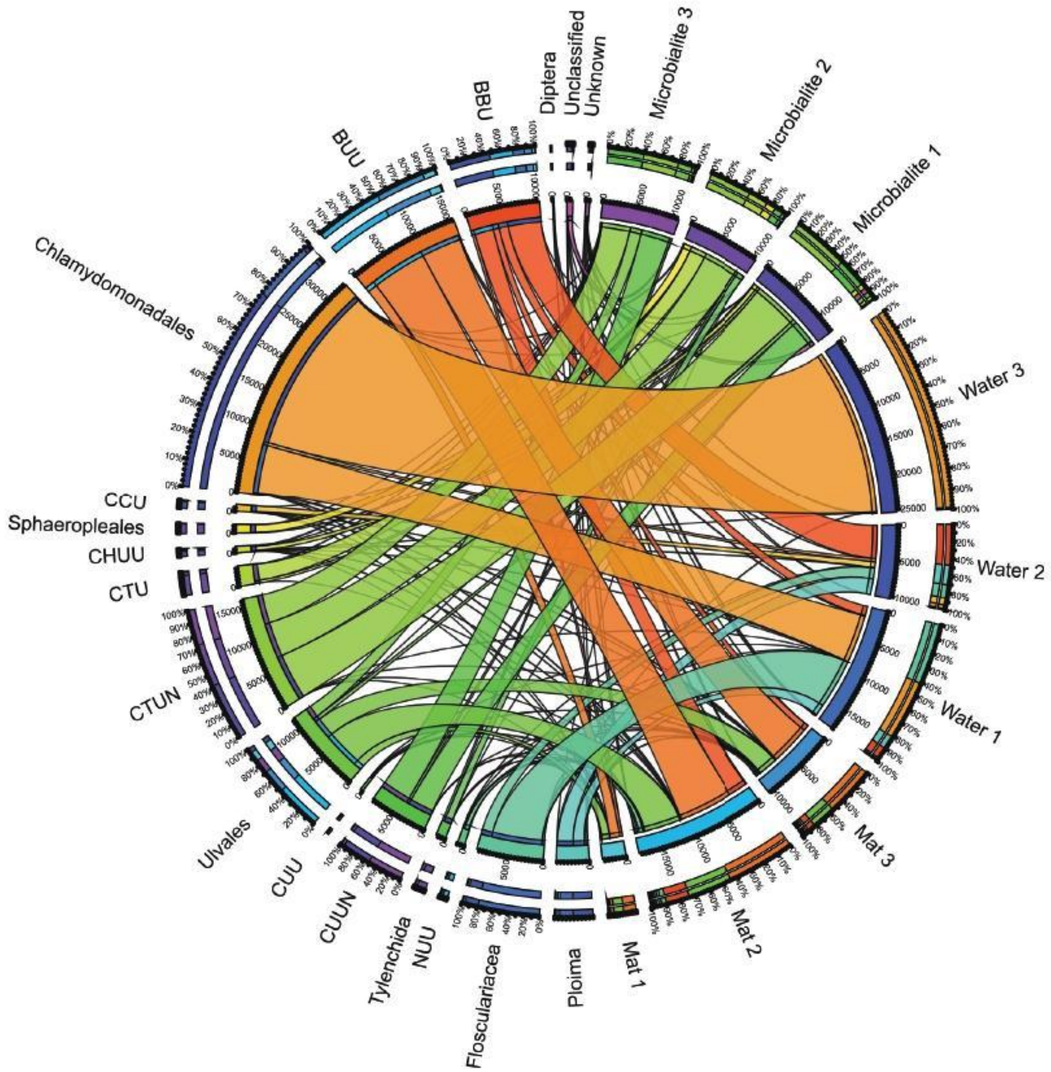

Figure 7. Eukarya distribution in the samples at the order level. In Microbialite samples the class Trebouxiophyceae with unclassified orders were found in high abundances. In Mat samples the Ulvales order, the class Bacillariophyceae with order unclassified and the Bacillariophyta phylum with unclassified order were found in high abundances. In Water samples the orders Floscularíacea, Ploima, Chlamydomonates and Bacillariophyceae with an unclassified order showed high abundances. The meanings of the abbreviations used in the figure are presented in Table 5.

number of Archaea. An important percentage of the sequenced DNA was unclassified (i.e. we grouped in the analyses the results of no hit, unknown or unclassified), revealing an important unexplored microbial component that needs to be examined in future studies. Overall, the phylotypes identified in RP are related to extreme life conditions and the environmental DNA sequences isolated are represented by alkaliphile, halophile and thermophile microbes (Table 6). The most abundant and characteristic Bacteria of known soda lakes, such as Cyanobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes, Deinococcus-Thermus and Verrucomicrobia (e.g. Grant and Sorokin 2011), were also found in RP. In particular, Bacteria is associated with high salinity and alkalinity of sediment and water, whilst Archaea was associated with salinity. At the order level, the Eukarya component was different in aquatic/benthic environments (Mat and Water) from in those in subaerial conditions (Microbialite). In general, the RP bacterial community was qualitatively similar to the one reported in Alchichica crater lake, where Proteobacteria was identified as the most abundant phylum, with high percentages of Cyanobacteria, Bacteroidetes, Acidobacteria, Chloroflexi and Firmicutes (Centeno et al. 2012). In contrast, the bacterial community of Lake Van crater lake was mainly composed by Firmicutes and Proteobacteria, followed by Cyanobacteria and Actinobacteria (López-García et al. 2005). We have documented a clear distribution of phyla depending on the studied niches in RP. Finally, it is important to remark that the resolution achieved with the genomic technique allows us to make suitable inferences at the phylotype level and the discussion on the relationship between assembly and specific species functions has yet to be investigated in subsequent studies.

Endolithic microbial communities

The high alkalinity caused by the presence of carbonates, bicarbonates and hydroxides, water availability, as well as the high concentration of salts are the main parameters controlling the microbial component in the subaerial environment. The massive soft and friable, silty-sand, non-lithified calcareous sediments represent a habitable substrate for holding microbial communities composed mainly of Cyanobacteria and Proteobacteria. We have described the presence of extant microbial

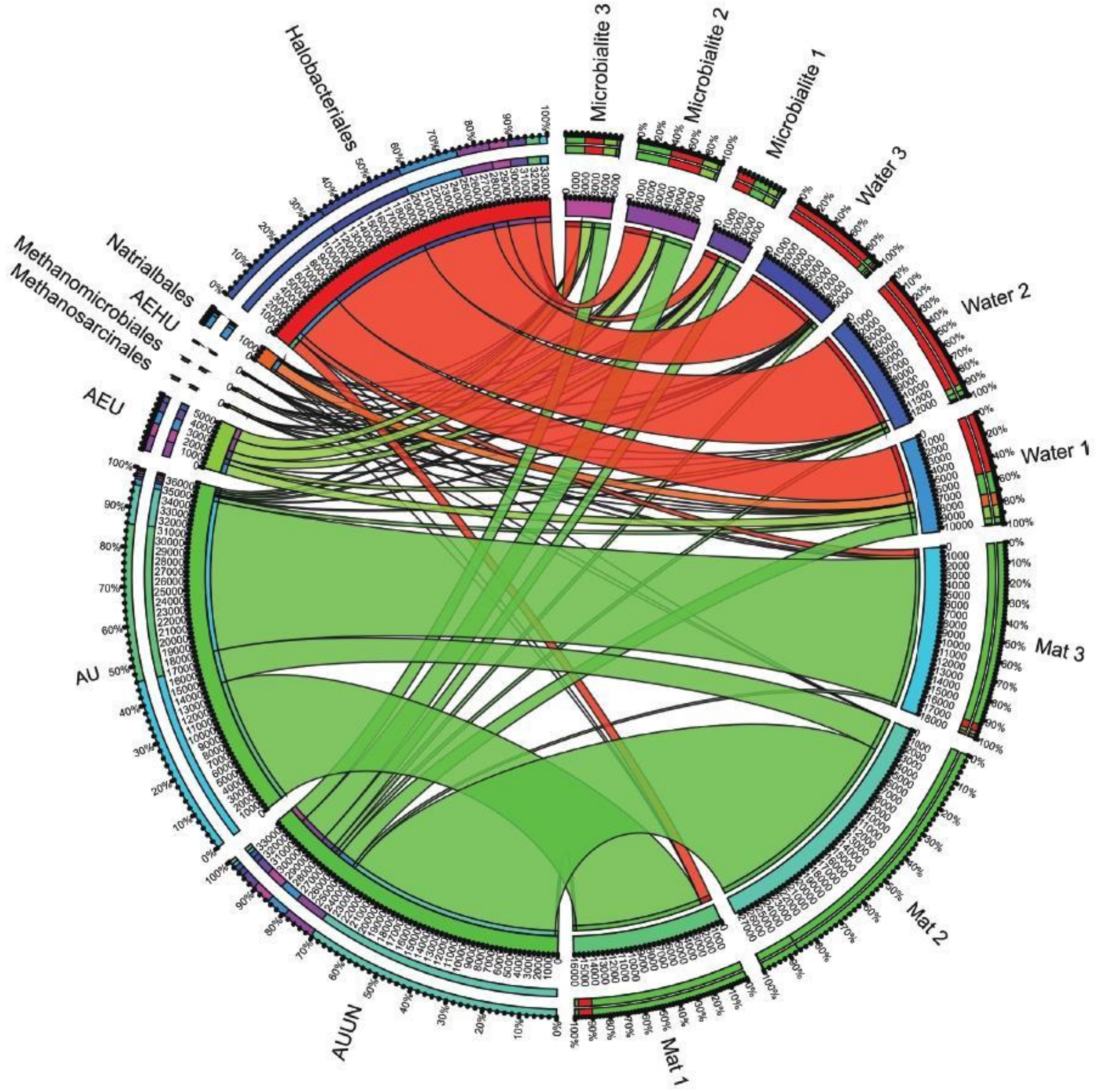

Figure 8. Archaea distribution in the samples at the order level. Halobacteriales presented high percentages in Water and Microbialite samples. High percentages of unclassified sequences of Archaea were observed in all samples. The meanings of the abbreviations used in the figure are presented in Table 5.

mats at the surface, pores and fractures of the non-lithified calcareous sediments. A similar process was observed recently by Chacón *et al.* (2018) for extant microbial communities occupying the pore space near the outer layer of the stromatolites. Calcareous sediments provide an appropriate habitat for endolithic microbial communities since they have a very low density, as they are highly porous and alkaline. Macropores formed by dissolution were also described in this work. The sediment is unsaturated but with high values of gravimetric water content that remain stable through the year. Weathering can provide the needed solutes and CO_2 can be available from gases seeping through the sediments (Fig. 9A and B).

The abundance of phylotypes found within the Microbialite samples is comparable to that obtained for the aquatic samples. In the case of Bacteria, the similarity in proportions of Cyanobacteria and Proteobacteria is notable. For Archaea, the Microbialite samples showed the presence of Halobacteriales and minor quantities of Methanomicrobia. In the case of Eukarya, the Microbialite samples show a major percentage of green algae (Chlorophyta). Algae and bacteria might strategically coexist in mutualism to survive high salinity, lower available free-water, extremely high and low temperature, low light and inorganic carbon conditions, and even high ultraviolet radiation (Ramanan ar *et al.* 2016). Fungi were also observed (Basidiomycota). An organized community structure of cyanobacteria (Chroococcales and Synechococcales), algae and other heterotrophic bacteria such as Actinobacteria and Proteobacteria has been reported in similar extreme environments, such as the Atacama Desert, where microbes are packed into the porous gypsum soil to sustain in such extreme habitat (Wierzchos *et al.* 2015). Our observations suggest that adaptation of the microbial community took advantage of the high porosity and water retention of the soft and friable carbonates to avoid extreme evapotranspiration rates on the former lake bottom. Here we suggest that the bioreceptive characteristics (porosity, available water content, alkalinity) of the calcareous sediments favored short-term adaption strategies to the environmental changes.

Table 5. Summary of the abbreviations for the group of unclassified sequences used in Figures 6–8.

Figure 6—Bacteria	
AAUN	Unclassified Actinobacteria Actinobacteria
ANUN	Unclassified Nitriliruptoria Actinobacteria
BSUN	Unclassified Sphingobacteriia, Bacteroidetes
CUUN	Unclassified Cyanobacteria
PGU	Unclassified Gammaproteobacteria
Figure 7—Eukarya	
NUU	Unclassified Nematoda
BBU	Unclassified Bacillariophyceae Bacillariophyta
BUU	Unclassified Bacillariophyta
CUU	Unclassified Chytridiomycota
CTU, CTUN	Unclassified Trebouxiophyceae Chlorophyta
CUUN	Unclassified Ulvophyceae Chlorophyta
CHUU	Unclassified Chlorophyta
Figure 8—Archaea	
AEHU	Unclassified Halobacteria
AEU	Unclassified Euryarchaeota
AU, AUUN	Unclassified Archaea

The complete figures using all the sequences obtained can be consulted in the supplementary material available online.

Hypersaline ponds

Despite their small size and increasing salinity, the remnant soda ponds are a refuge for bacterial communities with a comparable abundance of microbes to those in the sediments. The microbial association of cyanobacteria, diatoms and algae, as well as other bacteria such as Proteobacteria, Chloroflexi, Actinobacteria, Firmicutes and Bacteroidetes, is characteristic of these alkaline ponds. Cyanobacteria were mainly found in the Mat samples, which can be related to their importance in the formation of carbonates and microbialites, not only because they fix CO_2 and N_2, but also because they produce O_2. Heterotrophs such as Bacteroidetes, Planctomycetes and Actinobacteria were found in the samples and can degrade extracellular polymeric substances, releasing the sequestered cations and promoting the formation of carbonates (Couradeau et al. 2011). Cyanobacteria predominates in the mats of the three ponds, whereas in the Water samples Proteobacteria is dominant. For Eukarya the difference is the predominance of diatom and the presence of Rotifera in water. For Archaea the proportion of Halobacteriales increases in the Water samples. Halobacteriales constituted the largest fraction in the sequences, which coincides with reports of archaeal diversity in similar soda lakes located at Lonar Lake Buldhana, India (Antony, Murrell and Shouche 2012) and the Kenyan Rift valley (Mwirichia et al. 2010). Haloarchaea is a group of aerobic or facultatively anaerobic microorganisms that require high salt concentrations for growth. It has been reported that lakes with a high content of halite (NaCl) contain high amounts of this type of microorganism. Haloarchaea promotes the crystallization of halite in environments with high pH, and it is believed that this their cells can serve as sites for the nucleation of halite crystals and promote their growth (Grant and Sorokin 2011).

This group of microorganisms (i.e. Cyanobacteria, Proteobacteria, Haloarchaea and all those described above) confirms not only the highly alkaline and highly saline environment of the RP lake remnants, but also some of the geological observations such as the production of halite and trona crystals observed at the base of the Mats in the remnant ponds and the production of carbonates in the Microbialite samples. Although the environmental conditions are currently different for the crater maar Alchichica in eastern Mexico, Centeno et al. (2012) reported a similar phyla composition of the microbial community. Our results provide evidence for the primary importance of preserving the ponds as a genomic reservoir of the microbial diversity in this extreme environment.

Some insights at lower taxonomic levels

Microbial communities in the different niches studied are similar at the phylum level; however, at the order level we found striking differences (Table 7 and Fig. 9C–E). For this discussion we note that all the orders yielded different percentages of unknown and unclassified hits. The samples yielded a larger number of Bacteria and Eukarya orders, including microalgae and primary producers. For instance, the order Pleurocapsales (only found in Microbialite) is a calcifying microbe forming stromatolites in alkaline environments such as Lake Van (Lopez-Garcia et al. 2005) and Alchichica (Couradeau et al. 2013), where it has been suggested to be directly involved in the precipitation of aragonite, contributing to microbialite formation. The phylum Chlorophyta yielded the largest number of orders identified in Microbialite, such as Sphaeropleales, Chlorellales and Chlorocystidales. On the other hand, the phylum Streptophyta in Mat and Water samples presented the largest number of orders, including Ophioglossales and Schizaeales for Mat, and Cupressales and Lamiales for Water samples. Associated with the primary producers were identified orders belonging to microorganism decomposers of organic matter such as Sphingobacteriales (Bacteroidetes phylum), identified only in Water samples. Orders Bacillales and Clostridiales, both of the Firmicutes phylum, identified in Water and Mat samples are two orders of halophilic bacteria capable of generating spores (e.g. López-López et al. 2010; Paredes-Sabja, Setlow and Sarker 2011). For the phylum Chloroflexi, two different orders of thermophilic anaerobes (Wagner and Wiegel 2008), Sphaerobacterales and Thermomicrobiales, were found in both Microbialite and Mat samples.

For Archaea the representative orders of the samples show the saline and alkaline environmental conditions; for instance, the order Natrialbales (obtained for all samples) is a group of haloalkaliphilic organisms that require not only high concentrations of NaCl, but also a high pH for its growth; it has been reported in soda lakes of Inner Mongolia, China (Xu et al. 2001) and hypersaline soils in Egypt (Hezayen et al. 2001).

The phylum Methanomicrobia was obtained only in the aquatic samples (Mat and Water) represented by orders such as Methanomicrobiales and Methanosarcinales; in particular this order has been recognized in hypersaline microbial mats of Baja California (Orphan et al. 2008). Members of this order can be metabolically versatile, generating methane from compounds such as acetate, hydrogen and methanol (Casaburi et al. 2016).

Though several orders were shared within samples, a principal component analyses at genus level suggested that each compartment, i.e. Microbialite, Mat and Water, had a distinctive bacterial and eukaryal community structure (Fig. 9C and D), while for the archaeal community (Fig. 9E) that pattern was not visible,

Table 6. Species for each domain identified within Rincon de Parangueo related to environmental DNA sequences isolated from sites with extreme life conditions such as high alkalinity, salinity and temperature.

Genus	Specie	Location	Reference
Bacteria			
Rhodobaca sp.	*Rhodobaca bogoriensis*	Water and sediments from Lake Bogoria, Kenya	Milford *et al.* 2000
	Rhodobaca barguzinensis	Lake Barguzin valley, Siberia	Boldareva *et al.* 2007
Nitriliruptor sp.	*Nitriliruptor alkaliphilus*	Sediments of the lake Kolunda Steppe, Russia	Sorokin *et al.* 2009
Nocardiopsis sp.	*Nocardiopsis valliformis*	Soil from from alkaline lake in Xinjiang, China	Yang *et al.* 2008
Psychroflexus sp.	*Psychroflexus salis*	Saline lake in Qinghai province, China	Zhong *et al.* 2016
	Psychroflexus planctonicus	Saline lake in Qinghai province, China	Zhong *et al.* 2016
Defluviimonas sp.	*Defluviimonas indica*	Hydrothermal sulfide chimney in the sea of southwestern India	Jiang *et al.* 2014
Microvirga sp.	*Microvirga subterranea*	Subsoil water in the geothermal aquifer, Great Artesian Basin, Australia	Kanso and Patel 2003
Elioraea sp.	*Elioraea tepidiphila*	Thermal source on the island of Sao Miguel, Azores	Albuquerque *et al.* 2008
Rubrobacter sp.	*Rubrobacter radiotolerans*	Thermal source in Sao Pedro do Sul, Portugal	Egas *et al.* 2014
Belliella sp.	*Belliella pelovolcani*	Volcanic mud in Wandan, Taiwan	Arun *et al.* 2009
Cecembia sp.	*Cecembia lonarensis*	Water samples from a haloalkaline lake in Maharashtra, India	Anil Kumar *et al.* 2012
Thermomicrobium sp.	*Thermomicrobium carboxidum*	Biofilms growing in Kilauea Volcano Hawaii, USA	King and King 2014
Salinarimonas sp.	*Salinarimonas ramus*	Saline soil at Shengli Oilfield, East China	Cai *et al.* 2011
Porphyrobacter sp.	*Porphyrobacter tepidarius*	Microbial mats from brackish thermal springs in Shidzuoka, Japan	Hanada *et al.* 1997
Eukarya			
Picocystis sp.	*Picocystis salinarum*	Saline lakes and hot springs of East Africa	Krienitz *et al.* 2012
Archaea			
Halosimplex sp.	*Halosimplex carlsbadense*	Salt crystals taken from Salado formation in southeastern New Mexico	Vreeland *et al.* 2002
Natronoarchaeum sp.	*Natronoarchaeum mannanilyticum*	Salt made from seawater salt making works in Niigata, Japan	Shimane *et al.* 2010
Natronolimnobius sp.	*Natronolimnobius innermongolicus*	Soda lakes in Inner Mongolia, China	Itoh *et al.* 2005
Natrialba sp.	*Natrialba aegyptiaca*	Hypersaline soils in Aswan, Egypt	Hezayen *et al.* 2001
Methanosalsum sp.	*Methanosalsum natronophilum*	Sediments of soda lakes in Kulunda Steppe, Russia	Sorokin *et al.* 2015
Methanolobus sp.	*Methanolobus oregonensis*	Saline soda lake in Lonar crater, India	Antony *et al.* 2012

possible due to the low diversity and the lack of identification of many phylotypes.

Finally, species isolated previously from active volcanic environments were reported in our results (Table 6). We consider that the presence of these species provides indirect evidence for the presence of groundwater inflow from a geothermal source. Although sequences belonging to these taxa represent low percentages of the overall community in RP, these organisms would maintain a competitive advantage for survival in this progressively more alkaline and saline environment.

CONCLUSION

RP was a maar with a perennial lake that produced an exceptional sequence of carbonate organo-sedimentary structures related to microbial activity during recent past. Partial desiccation of the lake in the past 38 years produced a dramatic change in the environmental parameters controlling the activity of the microbial communities. In this paper we used molecular methods to describe the three life domains living in three different niches of the crater, one in subaerial sediments and two inside highly alkaline remnant ponds. Several phylotypes of Bacteria and Eukarya were identified, contrasting with much fewer phylotypes of Archaea. Moreover, our results indicate an important unexplored biodiversity that needs to be explored in future studies. The phylotypes here identified within the crater were related to environmental genome sequences isolated from other sites with extreme life conditions such as high alkalinity (alkaliphiles), high salinity (halophilic) and high temperature (thermophiles). The most important phyla for the precipitation of carbonate minerals, Cyanobacteria, predominated in all samples analyzed. Partial desiccation of the paleo-lake resulted in a new subaerial environment for microbial communities. We described the presence of microbial mats living at the surface, pores and fractures of the porous unlithified carbonate, which represents a habitable substrate for holding microbial communities composed mainly of Cyanobacteria and Proteobacteria. Calcareous sediments provide an appropriate habitat for endolithic microbial communities since they have a very low density and are a highly porous soft rock, with high alkalinity. Here we suggest

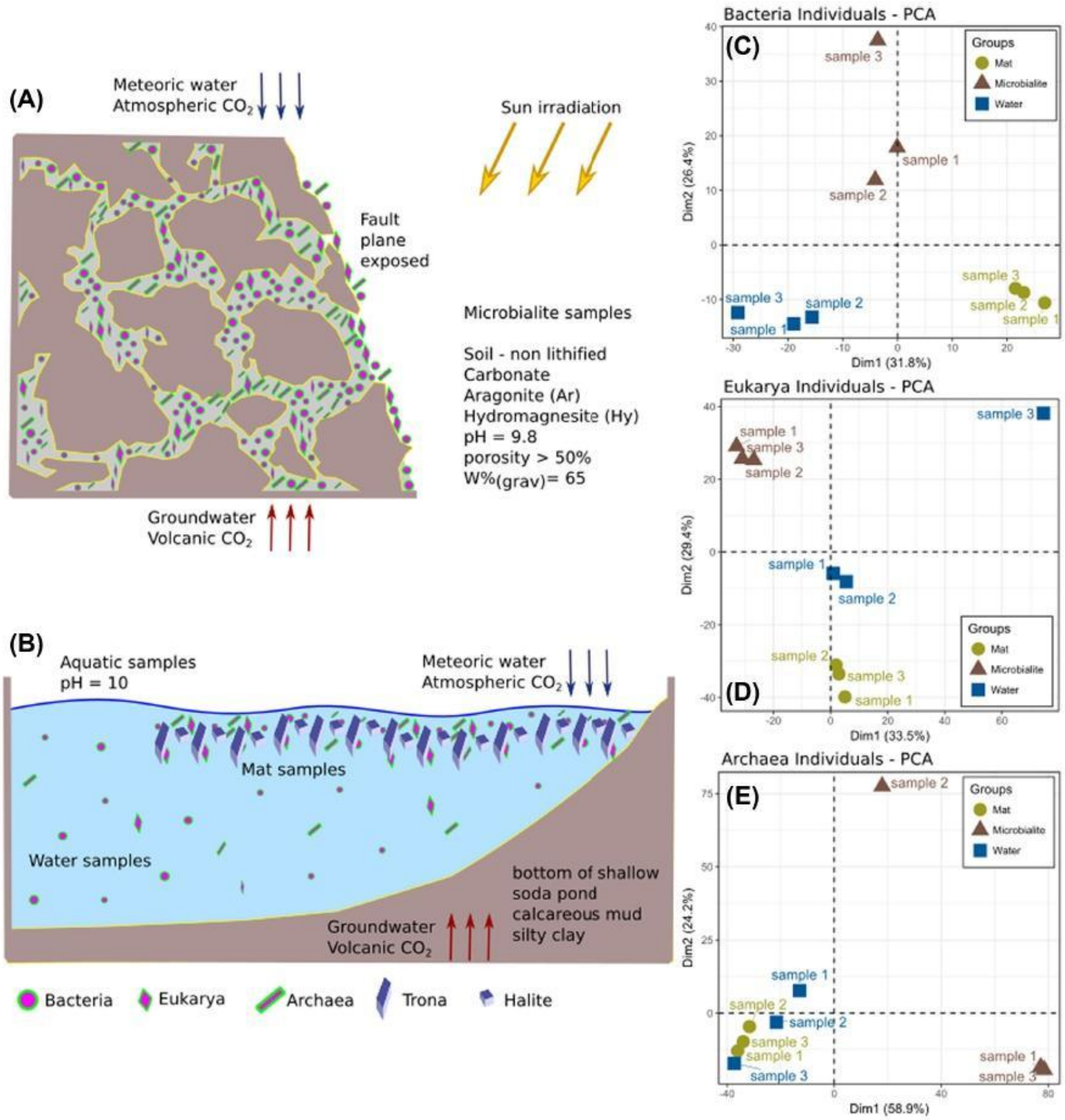

Figure 9. Distribution of microbial communities in the studied habitats subdivided into environmental compartments. (A) Porous unconsolidated sediments hosting microbial communities. The endolithic microbes took advantage of the characteristics of the sediments receptive for aquatic communities. Microbialite samples were collected in this compartment. (B) Submerged aquatic environment in a soda pond. We observed a clear division between sessile communities living on the pond floor and in microbial mats (Mat samples) (see Fig. 2B) and communities moving freely in water (Water samples). Graphic representation of principal component analyses (PCA) at the genus level showing the distribution of Bacteria (C), Eukarya (D) and Archaea (E) in relation to the environmental compartments.

that adaptation of the microbial community took advantage of the bioreceptive characteristics of the lacustrine sediments, i.e. high porosity and water retention in the soft and friable carbonates, and the endolithic way of life allowed the communities to avoid extreme evapotranspiration rates on the former lake bottom. Despite its small size, the remnant soda ponds studied here harbor a high microbiological component with a characteristic microbial association of Cyanobacteria, diatoms and algae, as well as other bacteria such as Proteobacteria, Chloroflexi, Actinobacteria, Firmicutes and Bacteroidetes. Any conservation effort of this perturbed ecosystem depends on the knowledge of the state and distribution of microorganism and the processes that sustain life in this environment.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC *Journal* online.

Authors' contributions

All authors discussed the results and commented on the manuscript. MC, JS-S, DC-F and AV-E designed the project and collected the soil samples. JS-S, CL-F and RJA-H performed the molecular biology analyses. MC, JJA-G, and DC-F described the geology of the site. MV, DC-F and GL carried out soil, SEM and XRD analyses. JS-S, CL-F, RJA-H and MC analyzed the metadata. MC and JS-S wrote the first draft of the manuscript.

Table 7. Microbial communities of the three life domains reported at order level for each sample inside Rincon de Parangueo.

Bacteria					
		Proteobacteria			
Sample	Cyanobacteria	Alphaproteobacteria	Betaproteobacteria	Deltaproteobacteria	Gammaproteobacteria
Microbialite	Chroococcales Nostocales Oscillatoriales Pleurocapsales	Caulobacterales Rhizobiales Rhodobacterales Rhodospirillales Sphingomonadales	Methylophilales	Bdellovibrionales	Cellvibrionales Chromatiales Methylococcales Nevskiales Oceanospirillales Pseudomonadales Xanthomonadales
Mat	Chroococcales Nostocales Oscillatoriales	Rhizobiales Rhodobacterales Rhodospirillales Sphingomonadales			Alteromonadales Cellvibrionales Chromatiales Oceanospirillales Thiotrichales Xanthomonadales
Water	Chroococcales Nostocales Oscillatoriales	Rhodobacterales Rhodospirillales Sphingomonadales			Alteromonadales Cellvibrionales Chromatiales Oceanospirillales Pseudomonadales Thiotrichales Xanthomonadales
	Actinobacteria	**Bacteroidetes**	**Firmicutes**	**Chloroflexi**	
Microbialite	Micrococcales Streptosporangiales Euzebyales Nitriliruptorales Rubrobacterales	Cytophagales Flavobacteriales	Bacillales	Anaerolineales Sphaerobacterales	
Mat		Cytophagales Flavobacteriales	Clostridiales	Anaerolineales Thermomicrobiales	
Water		Cytophagales Flavobacteriales Sphingobacteriales	Clostridiales	Anaerolineales	
Eukarya					
Sample	**Ascomycota**	**Basidiomycota**	**Chytridiomycota**	**Chlorophyta**	**Streptophyta**
Microbialite	Saccharomycetales			Chlamydomonadales Sphaeropleales Chlorellales Chlorocystidales Ulvales	
Mat			Spizellomycetales	Chlamydomonadales Marsupiomonadales Ulvales	Ophioglossales Schizaeales
Water		Tremellales		Chlamydomonadales Marsupiomonadales	Cupressales Lamiales
Archaea					
Sample	**Halobacteria**	**Methanomicrobia**			
Microbialite	Halobacteriales Natrialbales				
Mat	Halobacteriales Haloferacales Natrialbales	Methanomicrobiales Methanosarcinales			
Water	Halobacteriales Haloferacales Natrialbales	Methanomicrobiales Methanosarcinales			

ACKNOWLEDGEMENTS

We thank Ricardo Carrizosa and Sara Solís for help on the soil analysis, and Luis Aguilar, Alejandro de León, Carlos S. Flores and Jair García of the Laboratorio Nacional de Visualización Científica Avanzada.

FUNDING

This work was supported by Laboratorio de Mecánica de Geosistemas (LAMG, UNAM). JS-S received an MSc scholarship from Consejo Nacional de Ciencia y Tecnología (CONACyT).

Conflicts of interest. None declared.

REFERENCES

Albuquerque L, Rainey FA, Nobre MF *et al.* *Elioraea tepidiphila* gen. nov., sp. nov., a slightly thermophilic member of the Alphaproteobacteria. *Int J Syst Evol Microbiol* 2008;**773**: 778–58.

Anil Kumar P, Srinivas TN, Madhu S *et al.* *Cecembia lonarensis* gen. nov., sp. nov., a haloalkalitolerant bacterium of the family Cyclobacteriaceae, isolated from a haloalkaline lake and emended descriptions of the genera *Indiba cter*, *Nitritalea* and *Belliella*. *Int J Syst Evol Microbiol* 2012;**62**:2252–8.

Antony CP, Murrell JC, Shouche YS. Molecular diversity of methanogens and identification of *Methanolobus* sp. as active methylotrophic Archaea in Lonar crater lake sediments. *FEMS Microbiol Ecol* 2012;**81**:43–51.

Aranda-Gómez JJ, Cerca-Martínez M, Rocha-Treviño L *et al.* Structural evidence of active subsidence at the bottom of a 710 maar: Rincón de Parangueo, México, Special Publication: Monogenetic volcanism, Geological Society of London. In: Nemeth K, Carrasco-Nuñez G, Aranda-Gomez JJ, Smith IEM (eds). *Monogenetic Volcanism*. Geological Society, London, Special Publications, 2017, 446.

Aranda-Gómez JJ, Levresse G, Martínez JP *et al.* Active sinking at the bottom of the Rincón de Parangueo Maar (Guanajuato, México) and its probable relation with Subsidence faults at Salamanca and Celaya. *Boletín de La Sociedad Geológica Mexicana* 2013;**65**:69–188.

Aranda-Gómez JJ, Luhr FJ, Housh BT *et al.* The Yuriria – Valle de Santiago – Irapuato maar lineament (Guanajuato, México). In: Carrasco-Núñez G, Aranda-Gómez JJ, Ort MH, Silva-Corona JJ (eds.). *5th International Maar Conference*, Abstracts Volume: Juriquilla, Qro., México, Universidad Nacional Autónoma de México, Centro de Geociencias, 2014, 82–3.

Arun AB, Young CC, Chen WM *et al.* *Belliella pelovolcani* sp. nov., isolated from a mud- volcano in Taiwan. *Int J Syst Evol Microbiol* 2009;**59**:2534–7.

ASTM D422-63 (2007) e2, Standard Test Method for Particle-Size Analysis of Soils (Withdrawn 2016), ASTM International, West Conshohocken, PA, 2007, www.astm.org

ASTM D7263-09 (2018) e2, Standard Test Methods for Laboratory Determination of Density (Unit Weight) of Soil Specimens, ASTM International, West Conshohocken, PA, 2018, www.astm.org.

Berelson WM, Corsetti FA, Pepe-Ranney C *et al.* Hot spring siliceous stromatolites from Yellowstone National Park: Assessing growth rate and laminae formation. *Geobiology* 2011;**9**:411–24.

Bey BS, Fichot EB, Dayama G *et al.* Extraction of high molecular weight DNA from microbial mats. *BioTechniques* 2010;**49**:631–40.

Bokulich NA, Rideout JR, Kopylova E *et al.* A standardized, extensible framework for optimizing classification improves marker-gene taxonomic assignments. *PeerJ PrePrints* 2015;**3**:e934v2

Boldareva EN, Briantseva IA, Tsapin A *et al.* The new alkaliphilic bacteriochlorophyll a-containing bacterium *Roseinatronobacter monicus* sp nov from the hypersaline soda Mono Lake (California, United States). *Microbiology* 2007;**76**:95–106.

Cai M, Wang L, Cai H *et al.* *Salinarimonas ramus* sp. nov. and *Tessaracoccus oleiagri* sp. nov., isolated from a crude oil-contaminated saline soil. *Int J Syst Evol Microbiol* 2011;**61**:1767–75.

Casaburi G, Duscher AA, Reid RP *et al.* Characterization of the stromatolite microbiome from Little Darby Island, The Bahamas using predictive and whole shotgun metagenomic analysis. *Environ Microbiol* 2016;**18**:1452–69.

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

Cerca M, Rocha-Treviño L, Carreón-Freyre D *et al.* Physical experiments of land subsidence within a maar crater: insights for porosity variations and fracture localization. Proceedings of the International Association of Hydrological Sciences (PIAHS), IAHS, Ninth International Symposium on Land Subsidence (NISOLS), 2015, 285–90.

Chacón E, Aranda-Gómez JJ, Charles-Polo M *et al.* Biohermal thrombolites from a crater lake in Rincon de Parangeo, Central Mexico. *J South Amer Earth Sci* 2018;**85**:236–49.

Couradeau E, Benzerara K, Gérard E *et al.* Cyanobacterial calcification in modern microbialites at the submicrometer-scale. *Biogeosciences* 2013;**10**:5255–66.

Couradeau E, Benzerara K, Moreira D *et al.* Prokaryotic and eukaryotic community structure in field and cultured microbialites from the alkaline lake alchichica (Mexico). *PLoS One* 2011;**6**:e28767.

Edgar RC, Haas BJ, Clemente JC *et al.* UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;**27**:2194–200.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013;**10**:996–8.

Egas C, Barroso C, Froufe HJ *et al.* Complete genome sequence of the radiation-resistant bacterium *Rubrobacter radiotolerans* RSPS-4. *Stand Genomic Sci* 2014;**9**:1062–75.

Escolero-Fuentes ÓA, Alcocer-Durán J. Desecación de los lagos cráter del Valle de Santiago, Guanajuato. In: Jiménez B, Marín L (eds). *El agua en México vista desde la Academia*. Mexico City: 2004, 99–115.

Fan L, McElroy K, Thomas T. Reconstruction of ribosomal RNA Genes from Metagenomic Data. *PLoS One* 2012;**7**: e39948.

Grant W, Sorokin D. Distribution and diversity of soda lake alkaliphiles. In: Horikoshi K, Antranikian G, Bull AT, Robb FT, Stetter KO (eds). *Extremophiles Handbook*. Tokyo: Springer, 2011, 27–54.

Green J. Associations of zooplankton in six crater lakes in Arizona, Mexico and New Mexico. *J Zool* 1986;**208**:135–59.

Hanada S, Kawase Y, Hiraishi A *et al.* *Porphyrobacter tepidarius* sp. nov., a moderately thermophilic aerobic photosynthetic bacterium isolated from a hot spring. *Int J Syst Bacteriol* 1997;**47**:408–13.

Hezayen FF, Rehm BH, Tindall BJ *et al.* Transfer of *Natrialba asiatica* B1T to *Natrialba taiwanensis* sp. nov. and description of *Natrialba aegyptiaca* sp. nov., a novel extremely halophilic, aerobic, non-pigmented member of the Archaea from Egypt that produces extracellular poly (glutamic acid). *Int J Syst Evol Microbiol* 2001;**51**:1133

International Centre for Diffraction Data. 2005. *Powder diffraction file.* 12 Campus Blvd., Newtown Square, PA 19073, USA.

Itoh T, Yamaguchi T, Zhou P *et al.* *Natronolimnobius baerhuensis* gen. nov., sp. Nov. and *Natronolimnobius innermongolicus* sp. nov., novel haloalkaliphilic archaea isolated from soda lakes in Inner Mongolia, China. *Extremophiles* 2005;**9**: 111–6.

Jiang L, Xu H, Shao Z *et al.* *Defluviimonas indica* sp. nov., a marine bacterium isolated from a deep-sea hydrothermal vent environment. *Int J Syst Evol Microbiol* 2014;**64**:2084–8.

Kanso S, Patel BK. *Microvirga subterranea* gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. *Int J Syst Evol Microbiol* 2003;**53**:401–6.

Kazmierczak J, Kempe S, Kremer B *et al.* Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico. *Facies* 2011;**57**:543–70.

Kazmierczak J, Kempe S. Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga. *Naturwissenschaften* 2006;**93**:119–26.

Kempe S, Kazmierczak J, Reimer A *et al.* Microbialites and hydrochemistry of the crater lake of satonda-a status report. In: Reitner J, Neuweiler F, Gunkel F (eds). *Global and Regional Controls on Biogenic Sedimentation*. New York: Reef Evolution. Research Reports, 1996, 59–63.

Kienel U, Bowen SW, Byrne R *et al.* First lacustrine varve chronologies from Mexico: impact of droughts, ENSO and human activity since AD 1840 as recorded in maar sediments from Valle de Santiago. *J Paleolimnol* 2009;**42**: 587–609.

King CE, King GM. *Thermomicrobium carboxidum* sp. nov., and *Thermorudis peleae* gen. nov., sp. nov., carbon monoxide-oxidizing bacteria isolated from geothermally heated biofilms. *Int J Syst Evol Microbiol* 2014;**64**:2586–92.

Krienitz L, Bock C, Kotut K *et al.* *Picocystis salinarum* (Chlorophyta) in saline lakes and hot springs of East Africa. *Phycologia* 2012;**51**:22–32.

Lau E, Nash CZ, Vogler DR *et al.* Molecular diversity of cyanobacteria inhabiting coniform structures and surrounding mat in a Yellowstone hot spring. *Astrobiology* 2005;**5**:83–92.

Lay CY, Mykytczuk NC, Yergeau É *et al.* Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring. *Appl Environ Microbiol* 2013;**79**:3637–48.

López-García P, Kazmierczak J, Benzerara K *et al.* Bacterial diversity and carbonate precipitation in the giant microbialites from the highly alkaline Lake Van, Turkey. *Extremophiles* 2005;**9**:263–74.

López-López A, Yarza P, Richter M *et al.* Extremely halophilic microbial communities in anaerobic sediments from a solar saltern. *Environ Microbiol Rep* 2010;**2**:258–71.

Milford AD, Achenbach LA, Jung DO *et al.* *Rhodobaca bogoriensis* gen. nov. and sp. nov., an alkaliphilic purple nonsulfur bacterium from African Rift Valley soda lakes. *Arch Microbiol* 2000;**174**:18–27.

Mwirichia R, Cousin S, Muigai AW *et al.* Archaeal diversity in the haloalkaline Lake Elmenteita in Kenya. *Curr Microbiol* 2010;**60**:47–52.

NOM-021-SEMARNAT-2000. Norma Oficial Mexicana que establece las especificaciones de fertilidad, salinidad y clasificación de suelos, estudio, muestreo y análisis. Diario Oficial de la Federación, Secretaria de Gobernación, México. http://diariooficial.gob.mx/nota_detalle.php?codigo=717582&fecha=31/12/2002. (2 November 2018, date last accessed).

Ordóñez E. The Valle de Santiago volcanoes: México. *Memorias de la Sociedad Científica Antonio Alzate*. Mexico City: Sociedad Científica Antonio Alzate, 1900, 299–326.

Orphan VJ, Jahnke LL, Embaye T *et al.* Characterization and spatial distribution of methanogens and methanogenic biosignatures in hypersaline microbial mats of Baja California. *Geobiology* 2008;**6**:376–93.

Paredes-Sabja D, Setlow P, Sarker MR. Germination of spores of Bacillales and Clostridiales species: mechanisms and proteins involved. *Trends Microbiol* 2011;**19**:85–94.

Platt NH, Wright VP. Lacustrine Carbonates: facies models, facies distributions and hydrocarbon aspects. *In: Anadon P, Cabrera L, Kelts K (eds). Lacustrine Facies Analysis: International Association of Sedimentologists (I.A.S.) Special Publication, Blackwell Scientific Publications, Oxford* 1991;**13**:57–74.

Ramanan R, Kim BH, Cho DH *et al.* Algae-bacteria interactions: Evolution, ecology and emerging applications. *Biotechnol Adv* 2016;**34**:14–29.

Rocha-Treviño L. Análisis de la deformación y hundimiento activo causado por la desecación del lago del mar Rincón de Parangueo (Guanajuato, México) mediante modelos análogos y perfiles de Radar de Penetración Terrestre (RPT). Master's thesis. UNAM. Centro de Geociencias, Mexico 2015.

Shimane Y, Hatada Y, Minegishi H *et al.* *Natronoarchaeum mannanilyticum* gen. nov., sp. nov., an aerobic, extremely halophilic archaeon isolated from commercial salt. *Int J Syst Evol Microbiol* 2010;**60**:2529–34.

Sorokin DY, Abbas B, Merkel AY *et al.* *Methanosalsum natronophilum* sp. nov., and *Methanocalculus alkaliphilus* sp. nov., haloalkaliphilic methanogens from hypersaline soda lakes. *Int J Syst Evol Microbiol* 2015;**65**:3739–45.

Sorokin DY, van Pelt S, Tourova TP *et al.* *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. *Int J Syst Evol Microbiol* 2009;**59**:248–53.

Tavera R, Komárek J. Cyanoprokaryotes in the volcanic lake alchichica, puebla state, mexico. *Algol Stud* 1996;**83**:511–38.

Villarino E, Watson JR, Jönsson B *et al.* Large-scale ocean connectivity and planktonic body size. *Nature Commun* 2018;**9**:142.

Vreeland R, Straight S, Krammes J *et al.* *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. *Extremophiles* 2002;**6**: 445–52.

Wade BD, Garcia-Pichel F. Evaluation of DNA extraction methods for molecular analyses of microbial communities in modern calcareous microbialites. *Geomicrobiol J* 2003;**20**:549–61.

Wagner ID, Wiegel J. Diversity of thermophilic anaerobes. *Ann N Y Acad Sci* 2008;**1125**:1–43.

Wierzchos J, Ruggiero Di J, Vítek P *et al.* Adaptation strategies of endolithic chlorophototrophs to survive the hyperarid and extreme solar radiation environment of the Atacama Desert. *Front Microbiol* 2015;**6**:934.

Xu Y, Wang Z, Xue Y *et al*. *Natrialba hulunbeirensis* sp. nov. *and Natrialba chahannaoensis* sp. nov., novel haloalkaliphilic archaea from soda lakes in Inner Mongolia Autonomous Region, China. *Int J Syst Evol Microbiol* 2001;**51**: 1693–8.

Yang R, Zhang LP, Guo LG *et al*. *Nocardiopsis valliformis* sp. nov., an alkaliphilic actinomycete isolated from alkali lake soil in China. *Int J Syst Evol Microbiol* 2008;**58**:1542–6.

Zeyen N, Daval D, López-García P *et al*. Geochemical conditions allowing the formation of modern lacustrine microbialites. *Procedia Earth Planet Sci* 2017;**17**:380–3.

Zhang J, Kobert K, Flouri T *et al*. PEAR: A fast and accurate Illumina Paired-End reAd merger. *Bioinformatics* 2014;**30**:614–20.

Zhong ZP, Liu Y, Wang F *et al*. *Psychroflexus salis* sp. nov. and *Psychroflexus planctonicus* sp. nov., isolated from a salt lake. *Int J Syst Evol Microbiol* 2016;**66**:125–31.

REVISTA MEXICANA DE CIENCIAS GEOLÓGICAS v. 38, núm. 3, 2021, p. 178-192

Mobile mud layer underneath the desiccated maar lake of Rincón de Parangueo and insights into its microbial fingerprints

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ABSTRACT

We surveyed a subsurface layer of saturated mobile mud and its microbial fingerprints below the desiccated bottom of a maar lake at Rincón de Parangueo (RP), Mexico. A multi-scale approach was followed using geological fieldwork, coring of the sediments, ground penetrating radar survey, physico-chemical characterization including X-ray diffraction and scanning electron microscope, and high-throughput DNA sequencing methods. The mobile mud is an organic-rich silty clay, with high values of alkalinity, volumetric water content and conductivity. Mud mobility has been attributed to overpressure caused by disequilibrium compaction of sediments related to active subsidence and pore overpressure produced by an input of groundwater and gas content in the sediments and resulted in a diverse set of structures related to mud tectonics such as injection domes and fluid seeps through fractures. Extraction and sequencing of sedimentary environmental DNA in the mud layer were performed for Bacteria and Archaea. Despite the small number of samples obtained, the microbial fingerprint from the sedimentary environmental DNA at subsurface shares similarities with the microbial communities identified on the crater surface. Additionally, we identify the DNA of specific methanogenic microorganisms in the mud, such as Bathyarchaeia, Methanomassiliicoccales, and Methanobacteriales, and we speculate on their probable role in gas production and pore overpressure in the mud layer. The underground mud at Rincón de Parangueo represents a geologically dynamic environment with conditions that are favorable for the thriving of microbial communities.

Key words: saturated mud; microbiology; mud injection domes; maar cráter; lake.

RESUMEN

En este trabajo examinamos una capa subsuperficial de lodo saturado móvil y su huella microbiana (sedimentary environmental DNA) por debajo del fondo desecado del lago maar en Rincón de Parangueo (RP), México. Se siguió un enfoque de múltiples escalas, se integró trabajo de campo geológico, extracción de núcleos de sedimentos cercanos a la superficie, prospección por radar de penetración terrestre, caracterización fisicoquímica incluida la difracción de rayos X y microscopio electrónico de barrido y métodos de secuenciación de ADN de alto rendimiento. El lodo móvil está compuesto de arcilla limosa rica en materia orgánica total, con altos valores de alcalinidad, contenido volumétrico de agua y conductividad. La movilidad del lodo se atribuye a la sobrepresión causada por la compactación desequilibrada de los sedimentos, relacionada con la subsidencia activa en el cráter, y la sobrepresión de poro producida por una entrada de agua subterránea y gas en los sedimentos, lo que da como resultado un conjunto diverso de estructuras relacionadas con la tectónica de lodo, como domos de inyección de lodo y filtraciones de fluido a través de fracturas. La extracción y secuenciación de ADN ambiental en la capa de lodo se realizó para Bacteria y Archaea. A pesar de contar con un número pequeño de muestras de sedimentos, la huella microbiana obtenida en el subsuelo comparte similitudes con las secuencias identificadas en la superficie del cráter. Adicionalmente, en el lodo reportamos la presencia de microorganismos metanogénicos, tales como Bathyarchaeia, Methanomassiliicoccales y Methanobacteriales, y especulamos sobre su probable papel en la producción de gas y la sobrepresión del fluido de poro en la capa de lodo. El lodo subterráneo en Rincón de Parangueo representa un ambiente dinámico con condiciones favorables para el crecimiento de comunidades microbianas.

Palabras clave: lodo saturado; microbiología; domos de inyección de lodo; cráter maar; lago.

Sánchez-Sánchez, J., Cerca, M., Alcántara-Hernández, R.J, Aranda-Gómez, J.J., Carreón-Freyre, D., Lozano-Flores, C., Muñoz-Velasco, I., Levresse, G., Varela-Echavarría, A., 2021, Mobile mud layer underneath the desiccated maar lake of Rincón de Parangueo and insights into its microbial fingerprints: Revista Mexicana de Ciencias Geológicas, v. 38, núm. 3, p. 178-192.

DOI: http://dx.doi.org/10.22201/cgeo.20072902e.2021.3.1636

INTRODUCTION

The central part of the maar crater Rincón de Parangueo (RP) hosts a near-surface sedimentary sequence deposited in a closed lacustrine system (Kienel *et al.*, 2009; Aranda-Gómez *et al.*, 2013; 2017; Cerca *et al.*, 2015; Domínguez-Vázquez *et al.*, 2019; Sánchez-Sánchez *et al.*, 2019). Sedimentary processes within the lacustrine system were active until the 1980's decade when overexploitation of the regional aquifer initiated a gradual decline of the water level and triggered active land subsidence (Aranda-Gómez *et al.*, 2013; 2017). The morphology and sedimentation of the crater bottom have been profoundly affected since then by the rapid subsidence (*ca.* 1 m year^{-1}) and the large set of semicircular faults and fractures with throws mainly toward the center of the crater, forming a 15 m high topographic scarp (Rocha-Treviño, 2015; Aranda-Gómez *et al.*, 2017). Nowadays, most of the crater surface is characterized by a dry carbonate plain, and only small residual brine ponds are present in the depocenter of the crater. Brine ponds are fed either by meteoric water during the rainy season and/or by a local perched aquifer inside the crater (Aranda-Gómez *et al.*, 2013), and/or by hydraulic connection with the regional aquifer favored by the fragmentation of the host rock during the volcanic eruption (Aranda-Gómez *et al.*, 2013; Cerca *et al.*, 2015; Sánchez-Sánchez *et al.*, 2019). Despite the intense lake desiccation in this semiarid region, groundwater remains an important water contributor and the water table is maintained 1.2 m below the dry sedimentary cover. These abrupt changes in the environmental conditions allowed the presence, near the surface, of saturated mud that lost its layering structure and showed widespread evidence of mobilization and fluid migration manifested on the surface sediments. Structures observed include mud injection domes, brine pools, and gas seeps such as CO_2 and CH_4 (*e.g.*, Aranda-Gómez *et al.*, 2017; Paz *et al.*, 2020). Paz *et al.* (2020) suggested that the CH_4 emanating from the maar bottom has a biogenic origin. Through time the RP lacustrine system sustained the presence of microbial communities in an extreme environment (Levresse *et al.*, 2014; Chacón *et al.*, 2018; Sánchez-Sánchez *et al.*, 2019; Ibarra-Sánchez *et al.*, 2020). Sánchez-Sánchez *et al.* (2019) characterized the microbial component (Bacteria, Eukarya, and Archaea) in endolithic mats of calcareous sediments, and microbial mats and free-living microorganisms in the soda ponds in the crater. The phylotypes identified were related to the extreme environmental physicochemical characteristics (*e. g.*, alkalinity and salinity). The bacterial and archaeal community from a sediment layer (0–20 cm) along a central crater transect were also characterized by Ibarra-Sánchez *et al.* (2020). Both analyzes determined the presence of phylotypes including Cyanobacteria, Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota, Crenarchaeota and Euryarchaeota. However, our understanding of the taxonomic compilation of lacustrine communities in RP is presently limited to the crater surface. Saturated mud physicochemical and geological characteristics might control microbial populations on the subsurface. Gas producing microorganisms such as methanogenic Archaea might be present in the saturated mud providing a biological source for the methane observed on the surface. In this work, we detail the characteristics of the highly mobile and saturated subsurface muddy layer present in the area and the nature of the sedimentary environmental DNA recovered, based on a multi-scale approach involving geophysical datasets acquired by Ground Penetrating Radar (GPR), sediment physicochemical characterization, and microbial analyses by high throughput DNA sequencing methods.

Geological setting

Rincón de Parangueo (RP) is a relatively small Quaternary maar, a volcanic crater with a diameter of around 1.3 km, in the Michoacán-Guanajuato Volcanic Field at the central part of the Trans-Mexican Volcanic Belt (Figure 1a). The geology and stratigraphy of the RP maar and its surroundings have been previously presented by Aranda-Gómez *et al.* (2013; 2014; 2017). Here, we summarize some of the key points to give an oversight of the geological setting.

The Plio-Quaternary Michoacán – Guanajuato Monogenetic Volcanic Field (MGMVF), composed of more than 200 scoria cones, is located in the central part of the Trans-Mexican Volcanic Belt (TMVB). The northern part of the MGMVF is formed by continental shield volcanoes, scoria cones, and maars. RP maar is part of a volcanic complex (Valle de Santiago Volcanic Field; VSVF) formed by a continental lava shield, four maar-type volcanoes, and a lava dome, located near the town of Valle de Santiago (Aranda-Gómez *et al.*, 2014). RP represents the youngest eruption reported so far in the area, with a maximum eruption age of 137 ± 89 Ky (Aranda-Gómez *et al.*, 2017).

The precise onset time of the fine-grained, hydromagnesite- and organic-rich sedimentation in the RP lacustrine system is unknown but some constraints have been proposed. For instance, recent radiocarbon dating indicates an extrapolated age of approximately 21,000 years for the sediments collected at the base of a 14 m depth core collected in the center of maar (Domínguez-Vázquez *et al.*, 2019). Other available constraints on core samples suggest that the laminated sediments from the central part of the crater, collected at 60 to 70 cm in depth, record a time-lapse between AD 1839-1943 (Kienel *et al.*, 2009), and a wood nucleus on which a microbialite precipitated yielded a ^{14}C age of AD 1465 ± 33 (Levresse *et al.*, 2014).

The sediments, exposed exceptionally by the lake desiccation, display a simple concentric carbonate facies distribution in RP (Figure 1a) (Aranda-Gómez *et al.*, 2017; Sánchez-Sánchez *et al.*, 2019). The platform bench is a nearly flat or gently inclined plane with a sedimentary sequence characterized by the presence of abundant oncolites. Between the sublittoral and slope zones of the former lake there is a conspicuous annular alignment of domical biohermal thrombolites, coinciding with the present-day main annular fault scarp related to subsidence. The former slope at the lake's bottom is now a steep zone where tilted blocks bounded by tensional joints and high angle normal and reverse faults are observed. The pelagic zone of the lake basin covers most of the lakebed area. This innermost zone has a near-surface record of thinly nearly horizontally laminated sediments, formed by organic-rich and detritic-rich varves (Figure 1b). A section reported by Kienel *et al.* (2009) consisted of 8 cm diameter tubes in two cores, of which MXRP3-1 of around 106 cm in length records a continuous sedimentation disrupted in the upper 50 cm by mud breccia, associated with the more recent subsidence period. In these randomly oriented chaotic deposits, each clast keeps its own sedimentary laminae (Aranda-Gómez *et al.*, 2013; 2017) as depicted in Figures 1b and 2a, 2b. The underlying layered sediments are composed of a thin lamina of tan-colored carbonaceous micritic mud and silty-clayey sediments with a larger organic matter content (Figures 2b, 2c). Some silt and fine sand layers are also interbedded in the sequence (Kienel *et al.*, 2009). In addition, these authors reported the presence of "black intercalated layers" composed of silt and fine sand where the presence of green-yellow and brown-red organic-rich sludges were observed in the form of sheets or as disseminated materials, suggesting the possibility that organic matter is a local product of microorganisms. Below the laminated sediments (approx. 120 cm) there is an abrupt change in sediment properties, and we report here for the first time the presence of a highly compressible, massive, and saturated, organic, mobile, and not-laminated mud, their properties, and its microbial fingerprint. We discuss the implications that this mud layer has on the present-day morphology of the crater.

MATERIALS AND METHODS

Stratigraphy of the crater sediments and mud tectonics

Field work included the verification of evidence for the mud mobility. This included mapping the location, distribution and size of mud domes and other seepage features observed on the surface of the nearly dry lake inside the maar crater (Figure 3). For this study, stratigraphic logs obtained by coring down to 4 m depth in the sedimentary succession (Sediment samples in Figure 1a and 3a) were described. Two sediment samples from the cores, collected in the central part of the crater, were selected to determine the main physicochemical properties in a saturated paste prepared using distilled water (NOM-021-RECNAT-2000): gravimetric water content, organic matter content, pH and, electric conductivity (EC). The determinations were made at the Laboratorio de Mecánica de Geosistemas (LAMG), Universidad Nacional Autónoma de México (UNAM). Sampling was difficult by

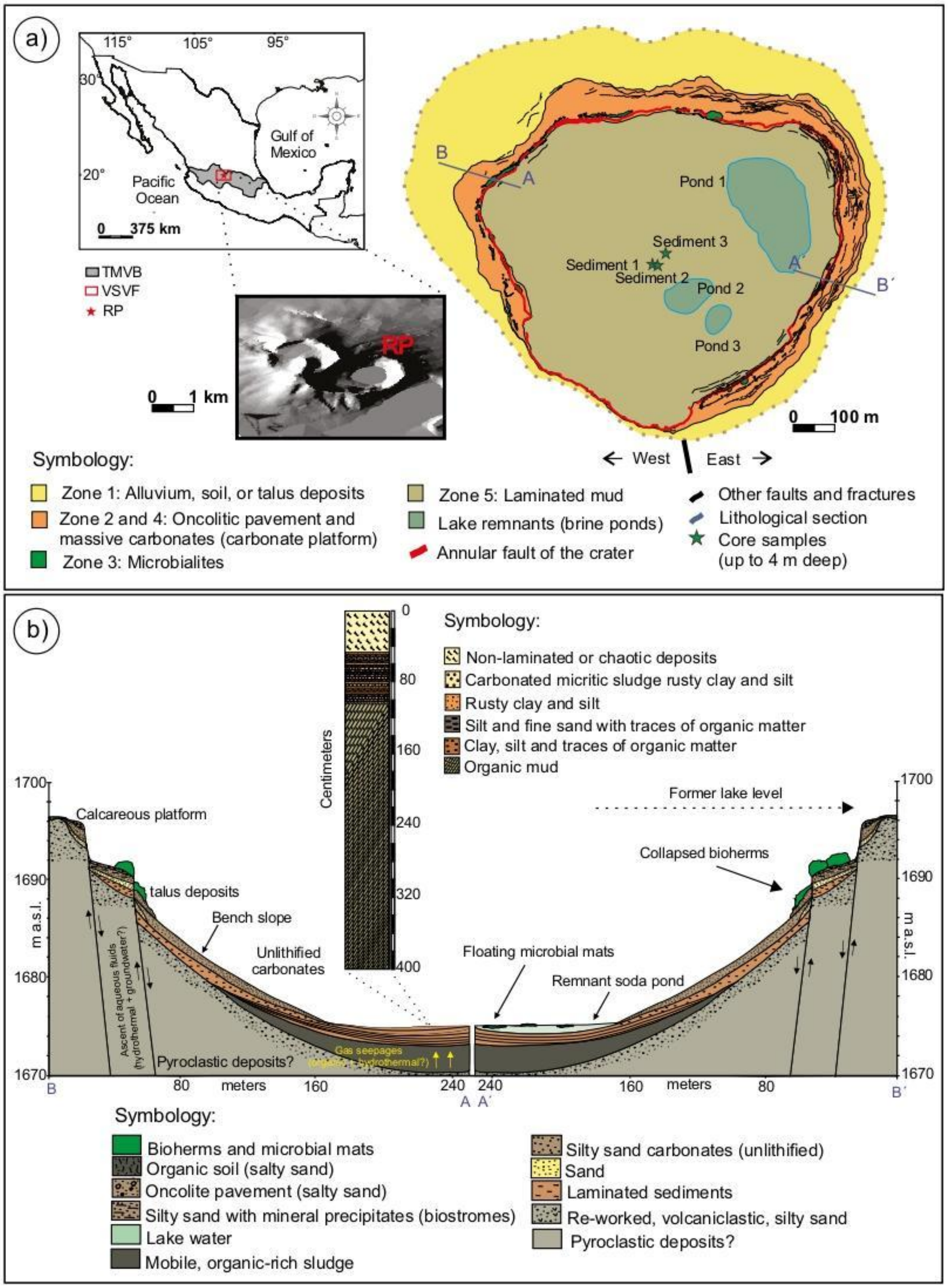

Figure. 1. a. Geographic location of the maar Rincón de Parangueo (RP), at the north-central part of the Trans Mexican Volcanic Belt (TMVB). The digital elevation map shows the morphology of RP and neighboring volcanoes. b. Generalized stratigraphic succession found at the sites where the Sediment 1, Sediment 2, and Sediment 3 cores were collected. Diagrammatic cross sections AB and A´B´. Note that the central portion of the former lake is omitted.

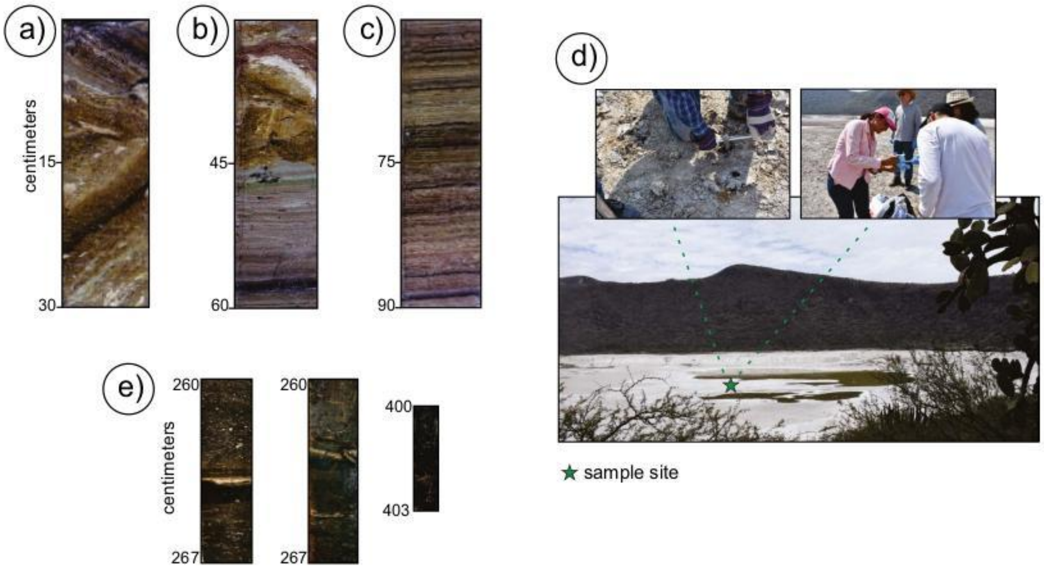

Figure. 2. Photographs a - c show the lithological and structural characteristics preserved in a mud core, down to 0.9 m depth. a. The first 45 cm of the lake sediments are composed of a chaotic microbreccia made by randomly oriented, laminated mud fragments set in a massive, structureless matrix. b and c. Laminated succession where individual lamina can be differentiated by color: the beige colored layers are composed of a carbonate sludge with micritic texture. The dark brown lamina consists of clay, silt, and traces of organic matter (after Kienel *et al.*, 2009). Black intercalated laminae are composed of silt and fine sand where the presence of thin sludge lamina, rich green-yellow or brown-red organic matter was observed. d. Some aspects of the process of sediment sampling with a mechanical corer. e. Two representative mud samples which were found below the laminated sequence at 260–267 cm and 400–403 cm, respectively. Recovery of deeper than 4 m mud samples is difficult because of the very low compaction of the saturated mud.

the instability of the mud surface and the impossibility of carrying a larger corer into the crater that is considered a protected natural park.

Ground Penetrating Radar (GPR) survey

GPR is a non-destructive geophysical prospecting technique that has been widely used for the identification of shallow subsurface geological structures corresponding to variations in the depositional environment, improving direct field observations. Changes in the radar wave propagation can be related to rock or sediment facies, changes involving variations in physical and mechanical properties such as grain size, density, compaction, the orientation of particles, the presence of faults and fractures, among others (Carreón-Freyre *et al.*, 2003). GPR profiles were recorded along the central part of the former lake for assessing the structure of the sediment succession accumulated at the bottom of the RP volcanic crater. The GPR survey included two profiles with a 200 MHz Antenna, each ~1000 m long, continuously recorded using a tachometer calibrated to collect 40 traces per meter. The path of the profile was controlled with GPS and detailed land morphology was included by using a high-resolution topographic survey. The position of selected sites along the profiles allowed to properly incorporate the topographic changes (dots along profiles A-A´ and B-B´ in Figure 3). Both complete profiles were preliminary presented in Cerca *et al.* (2014). For this work, we have processed and reinterpreted the GPR data for enhancing visualization of the mud intrusions geometries and the disruption of the stratigraphy at the central part of the maar-lake.

Geochemistry: major, minor, and trace element cations

Water samples from the mud brine were obtained by vacuum filtering of saturated mud collected at depths up to 4 meters, and analyzed at the Laboratorio de Geoquímica Ambiental, Centro de Geociencias, UNAM. The analysis of major, minor, and trace element cations was performed according to the EPA 200.7 method (EPA, 2001). The analysis was made in an Optical Emission Spectrophotometry with Inductively Coupled Thermo iCAP 6500 Duo Plasma, using an internal yttrium standard of 1 ppm to evaluate that the instrument's signal was within a ± 10 % error range. The calibration standards were divided into two curves. The major ion curve was prepared from 1000 ppm Inorganic Ventures solutions in seven different concentrations. The calibration curve for minor and trace cations was prepared from a high purity standard (ICP-200-5). The correlation coefficients for the calibration curves were greater than 0.9990, the recovery percentages were greater than 90 %, and were evaluated by means of laboratory control samples, prepared from certified standards of the high purity brand (QCS-26, standards Elemental mono and ICP-200-5).

Anion Analysis

Anion determination in groundwater obtained from the mud samples collected up to a 4 m depth was performed according to the EPA 300.1 method (Hautman and Munch, 1997), and analyzed at the Laboratorio de Geoquímica Ambiental of Centro de Geociencias, UNAM. The chemical analysis was performed using a Thermo-Dionex 1100 brand HPLC-IC with suppressor ASRS-Ultra 300 4-mm, column IonPac AS14A and electrochemical detector ED50. An 8.0 mM 1.0 mM Na_2CO_3 / $NaHCO_3$ solution was used as the mobile phase to carry the sample at a flow rate of 1 ml /min, with an injection volume of 50 µl. The preparation of the calibration curves was carried out with Inorganic Ventures IF-FAS-1A standards which consisted of seven concentrations, the control of the instrument drift was carried out by means of laboratory control samples prepared from a Seven Anion Standard of Dionex which was analyzed every 10 measurements. Both solutions used as reference standards have traceability to NIST. The correlation coefficient in the analytical curves used was greater than 0.9993 and the recovery rates of the control samples were greater than 90 %.

X-ray diffraction and scanning electron microscope observations

Scanning electron microscope (SEM) observations were performed in millimeter-sized mud fragments mounted on an aluminum plate covered with carbon conductive adhesive tape. The analysis was made

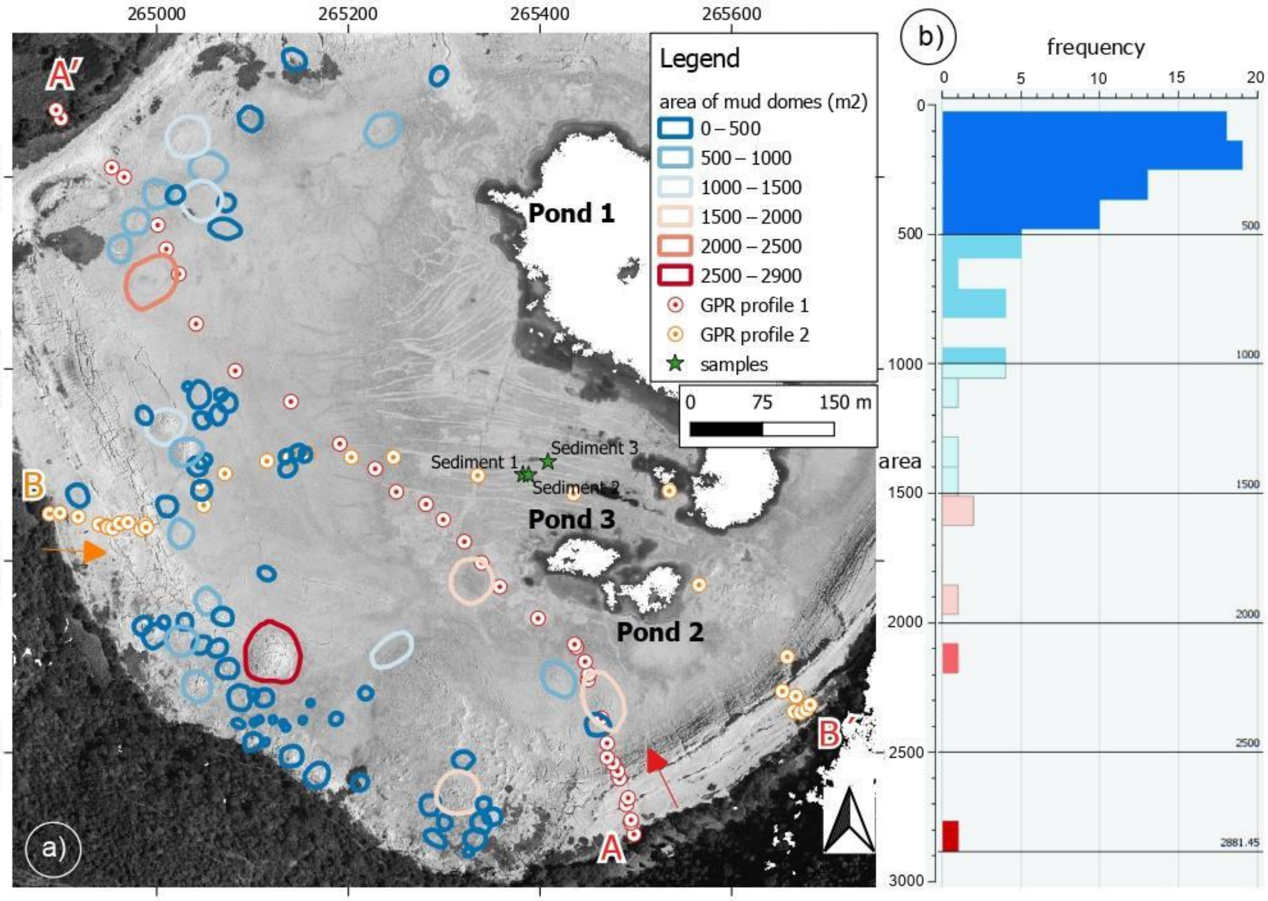

Figure. 3. a. Map showing 76 structures identified as injection mud domes on the surface in the western part of the crater, marked in different colors according to their size. White circles with dots in the center show the path followed to obtain the Ground Penetrating Radar profiles inside the former lakebed. White circles with orange dots are the locations measured used to construct the topography of profile A-A´ and white circles with red dots for profile B-B´. b. Spatial size-distribution and dome-size frequency of the mud domes.

at the Laboratorio de Geoquímica de Fluidos Corticales at Centro de Geociencias, UNAM. Samples were studied in a HITACHI TM1000 SEM at 15 kV with a backscattered detector. For X-ray diffraction (XRD), fractions of dry samples were disaggregated (to obtain fine grain <0.002 mm) in an agate mortar and were placed on aluminum plates. The samples were analyzed using a Miniflex Rigaku diffractometer using copper radiation Kα 1.5406 Å, between a 2θ angle of 5 to 80 degrees in steps of 0.02 degrees and 0.06 s for the measured spot. For crystalline phase identification, the PDF-2 database (Gates-Rector and Blanton, 2019) was used.

Sampling for sedimentary environmental DNA

The microbial fingerprint in the mud from sedimentary environmental DNA was characterized in aliquots taken from the mud samples collected in November 2017. Here, we use the term sedimentary-environmental DNA (sed-eDNA) for bulk DNA extracted from the mud layer as proposed by Ellegaard *et al.* (2020). Sampling was done with a mechanical corer at approximately 4 m depth below the desiccated bottom of the maar, in three different sampling stations in the central part of the crater (Figure 1a, 2d), named: Sediment 1 (20.430146°, -101.248621°), Sediment 2 (20.430142, -101.248565°), and Sediment 3 (20.430269°, -101.248373°). Mud aliquots were stored into 50 ml sterile conical tubes (Corning Company, USA), preserved on ice during transport, and stored at -20 °C until processing.

Nucleic acid extraction and data analysis

Genomic DNA extraction was done from 30 g of mud following the sample preparation protocol reported by Bey *et al.* (2010) and following the instructions accompanying the PowerSoil extraction kit DNA Isolation (MoBio). Extraction was performed at the Lab A-03 of Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, UNAM. DNA was amplified by using the primers 28F (GAGTTTGATCNTGGCTCAG) and 519R (GTNTTACNGCGGCKGCTG) for Bacteria, and Arch517F (GCYTAAAGSRNCCGTAGC) and 909R (TTTCAGYCTTGCGRCCGTAC) for Archaea (Lay *et al.*, 2013). Three samples (Sed 1, Sed 2 and Sed 3) were sent for Illumina MiSeq sequencing to RTL genomics company at Lubbock (TX, USA) (http://rtlgenomics.com). The 16S rRNA sequences were deposited in the NCBI database under the BioProject (PRJNA510628, BioSamples: SAMN14593312, SAMN14593313, SAMN14593314). Adapters and primers were removed using Trimmomatic v. 0.39 (Bolger *et al.*, 2014). Analysis and processing of 16S amplicons were made in R v. 3.6.3 (R core Team, 2020). Filtering and trimming by quality profiles, error estimation, dereplication process, merging of paired reads, remotion of chimerical sequences, and selection of amplicon sequence variants (ASVs) were carried out with DADA2 v. 1.14.1 (Callahan *et al.*, 2016). Taxonomy assignment was made against the SILVA SSU database v. 138 (Quast *et al.*, 2012) updated on august 15, 2020. Taxonomy and

ASVs tables, as well as associated samples of metadata, were converted into a phyloseq object (McMurdie and Holmes, 2013) for further analysis. Bar plots, richness analyses as well richness plots for each sample were made with phyloseq and ggplot2 v. 3.3.2 (Wickham, 2016).

RESULTS

Mud mobility observed in RP

Results derived from detailed structural mapping of the injected mud domes were combined with the analysis of the exposed stratigraphy variations and supported by the interpretation of GPR profiles. The map in Figure 3a shows 76 mud domes mapped on the surface in the western part of the crater, their spatial and size distribution, and the dome-size frequency (Figure 3b). The comparison of the stratigraphic sections with the GPR profiles show the relevant geological structures along the profiles (Figures 4a and 4b), including some of the faults at the main scarp bordering the sublittoral platform and the presence of a high conductivity zone associated with salt concentration in the central part of the lake floor (Figure 4c). A nearly horizontal reflector observed in both profiles is interpreted as the phreatic water level, marking the contact between relatively dry and brine-saturated mud. The depth of this reflector is about 2 m near the margins of the pelagic zone, and it decreases to 1.2 m near the residual brine pools

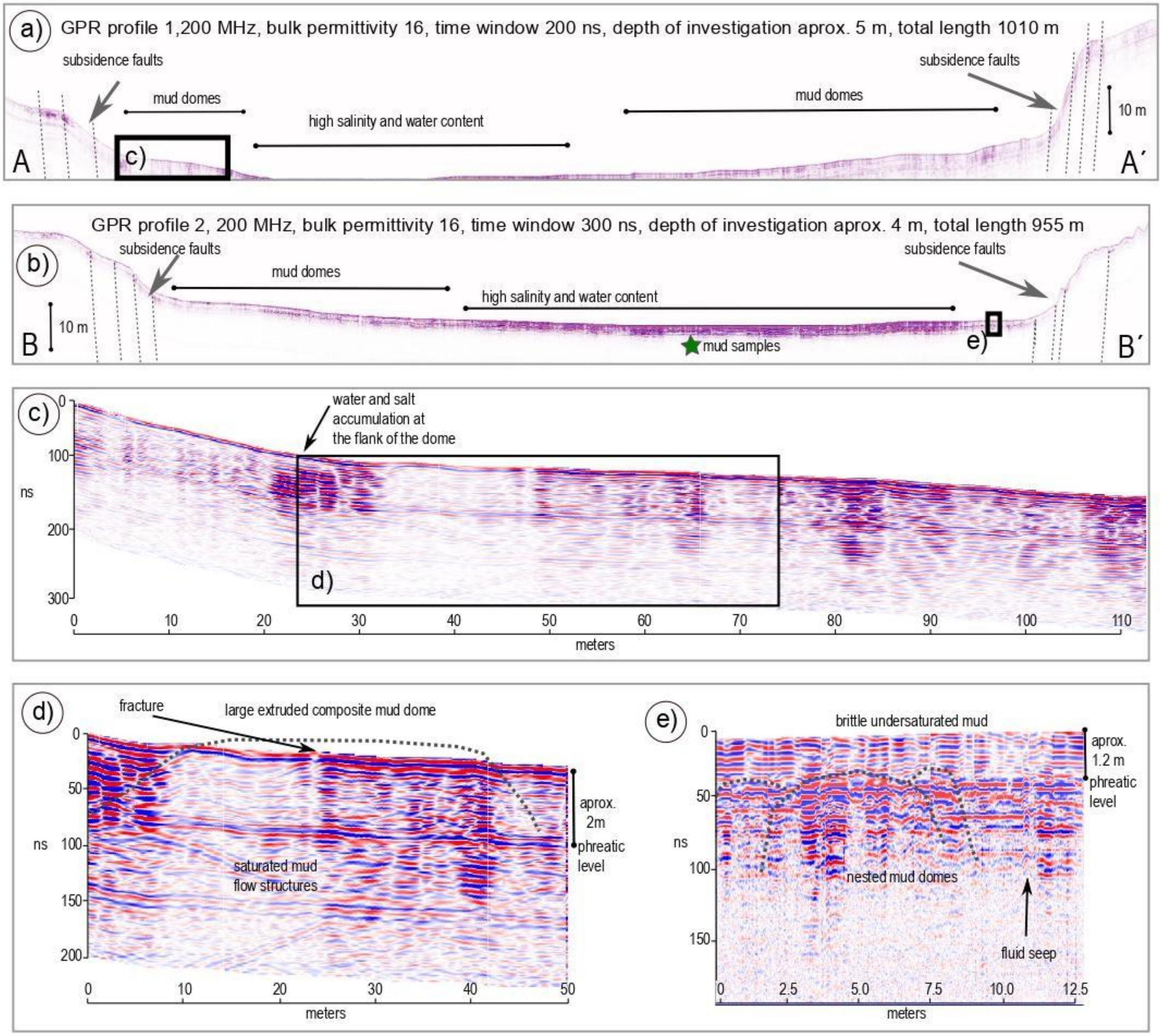

Figure. 4. Ground penetrating radar profiles of the dry lake bottom (see Figure 3 for location). a. Profile A-A´ shows the location of the annular ring faults and the presence of underground mud domes exposed on the surface. b. Profile B-B´. The orthogonal projection of the location of the mud sampling sites used for DNA analysis is shown by a green star. c. Closer view of profile A-A´ showing the geometry of the subsidence fault-scarp and the transitional changes toward the depocenter. d. Close up view of the domes. Discontinuous lines were added to enhance the interpreted structures. e. Small portion of the profile near the southern end of profile B-B´. Above the water table reflector (1.2 m), mud is relatively dry with a volumetric water content increasing gradually from the surface from 30 to 150 %. Saturated mud forms domal structures that reach from below to the plastic-brittle transition.

(Figures 4d and 4e). The change in the brine content of the mud also determines the mechanical behavior of the succession of sediments. Dry or unsaturated mud above the phreatic water level behaves as a brittle material and develops a network of dehydration fractures with a conspicuous polygonal array on the surface; whereas below the phreatic water level mud plasticity increases progressively until it displays a viscous fluid behavior (Aranda-Gómez *et al.*, 2017). Extrusive mud seen in the core of the domes, such as the example presented in Figure 4d, dry and maintain their structure; whereas the mud below the phreatic level can be remobilized and form a new intrusion nearby. This is the case of the zones with a high density of domes such as the SW part of the maar. A contrasting case is shown in Figure 4e, where the saturated mud accumulates at the phreatic water level and starts to uplift forming a dome shape at the base of the brittle layer. We consider this situation as evidence for underground fluid input to the mud. Brine-saturated mud can maintain high mobility as long as it keeps a high fluid (groundwater and/or gas) content (W_V around 200 %) and it becomes immobile when pore pressure drops, and the mud dome becomes dry.

Mud description

The massive subsurface mobile mud of RP (Figure 2e) is characterized by silt and clay grain size, high alkalinity (pH 10.38 –10.65), high volumetric water content (> 200 % W_V), high conductivity (23.29–51.4 mS/cm), organic-rich (> 6 %), and strong H_2S smell. For the brine the dominant anion is Cl^- and the dominant cation is Na^+ (Table 1). Low concentration of cations such as Ca^{+2} and Mg^{+2} identified in the samples is key to the chemical composition of the site because due to the shortage of these cations, anions such as Cl^- and CO_3^{2-} favor high alkalinity of the medium (*e.g.*, Jones *et al.*, 1998). Massive mud also has a high content of total organic matter of 6.77 %, which is evidenced by a characteristic black color, and might be related not only to the accumulation of plant- and animal-remains during a long depositional period but also to the biologic activity of the microbial community. SEM (Figures 5a and 5b) and DRX (Figures 5e and 5f) analyses of the mud show a highly porous structure with the presence of carbonates (aragonite: Ar; and hydromagnesite: Hy), salts (halite: Ha), minor amounts of clay minerals (montmorillonite), and detrital quartz and feldspars. At higher magnification, the mud contains subhedral crystals of halite (Figure 5c and 5d). SEM images in cryo-fixed samples of the mud revealed evidence of microorganisms (Figure 6) embedded mostly in a porous, muddy network, which acts as a substrate for their growth.

Microbial community composition observed in the mud brine

Sedimentary environmental DNA (Ellegard *et al.*, 2020) from three mud samples (Sed 1, Sed 2, and Sed 3) collected below the surface were analyzed for Bacteria and Archaea domains. The results include the DNA from the actual living organisms and the preserved DNA from the past when the original mud layer was deposited at the surface. For the Bacteria domain the three samples could be characterized, but for the Archaea domain only the samples Sediment 2 and Sediment 3 had sequences that could be identified. Table 2 shows the concentrations obtained for each sample and their degree of purity (ratio 260/280). The highest yield was observed for Sediment 2 from which 32.41 ng of DNA was obtained per 30 g of sample processed; the lowest yield was obtained for Sediment 3 with 16.67 ng of DNA per 30 g.

The number of filtered DNA sequences obtained for each of the samples is shown in Table 3. The minimum number of sequences obtained for a sample was about 5500, but most samples had at least 6000 sequences. The rarefaction curves were asymptotic, indicating that analyzing more sequences would yield only a limited number of more ASVs. The number of bacterial and archaeal ASVs ranged from 99 to 182. The 16S rRNA phylogenetic analysis revealed a diverse microbial component. The bacterial assemblage was dominated by Cyanobacteria (20.2–69.1 %), Firmicutes (4.4–48.4 %), Proteobacteria (15–30.6 %), Bacteroidota (9.2–15.7 %) and, to a lesser extent, Actinobacteriota, Verrucomicrobiota, and Chloroflexi (Figure 7a). For samples Sediment 2 and Sediment 3, all the bacterial phyla were recognized but for sample Sediment 1, 0.54 % of the sequences could not be classified. At order level, Synechococcales (11.1–48.5 %), Cyanobacteriales (8.5–46.5 %), Lachnospirales (1.3–38.3 %), Bacteroidales (9.2–15.7 %), Rhodobacterales (9.1–13.4 %), Phormidesmiales (1.9–11.4 %) were the better represented orders (Figure 7b). Alpha diversity metrics for the bacterial communities included observed richness (class level), Shannon-Weiner, and Simpson indices (Table 4 and Figure 8a).

For the Archaea domain, the most abundant microorganisms are related to Crenarchaeota (50–75.7 %), Halobacterota (13.6–27.2 %), Hadarchaeota (8.5–8.6 %), Thermoplasmatota (1.1–7.2 %), Euryarchaeota (0.5–1.3 %), and as minor components Nanoarchaeota and Asgardarchaeota. A small number of sequences were not classified (0.18–0.69 %; Figure 7c). At order level (Figure 7d), Nitrososphaerales (38.6–45.8 %), Halobacterales (10.9–26.4 %), and Hadarchaeia_or (8.5–8.6 %) sequences were obtained in high percentages. Additionally, Woesearchaeales, Thermoplasmatota_or, Methanomicrobiales, Methanomassiliicoccales, Methanobacteriales, and ANME-1b were identified in minor abundance. Observed richness, Shannon-Weiner, and Simpson indices values from archaeal communities recovered at subsurface in the RP lacustrine system are reported in Table 4 and Figure 8b.

Table 1. Analysis of cations and anions in the brine (obtained by vacuum filtering) from two mud samples collected at 4 meters depth.

	Sample 1	Sample 2
Na (mg/L)	16263	6396
K (mg/L)	1733	766
P (mg/L)	14.93	4.41
Ca (mg/L)	3.42	2.91
Mg (mg/L)	34	27
S (mg/L)	123	207
K (mg/L)	1733	766
F^- (mg/L)	39	12.50
Cl^- (mg/L)	7564	2874
SO_4^{2-} (mg/L)	771	114

DISCUSSION

Fluid mobility

Layers of lacustrine, organic rich, high porosity, massive clayey sediments in the crater of RP host mud mobilization and disrupt the original stratification of the ancient lake. This results in a highly dynamic geological environment. Disruption of the original stratification by the mud mobility has profound implications to the stability of the sedimentary record of RP that needs to be considered in other environmental studies that require a stable sedimentary layering. The high volumetric water content of these sediments (> 200 % W_V) and deformation of the lakebed causes slow pore fluid dissipation and mud overpressure (*e.g.*, Revil, 2002). Eventually, mud ascends and is ejected locally in mud domes. More than 76 domes or small mud intrusions were found distributed mainly on the surface at the western portion of the subsiding bottom of the former lake at RP. Their size and ubiquitous presence indicates forced mud ascent to the surface from a shallow underground source. Differences observed in shapes of the

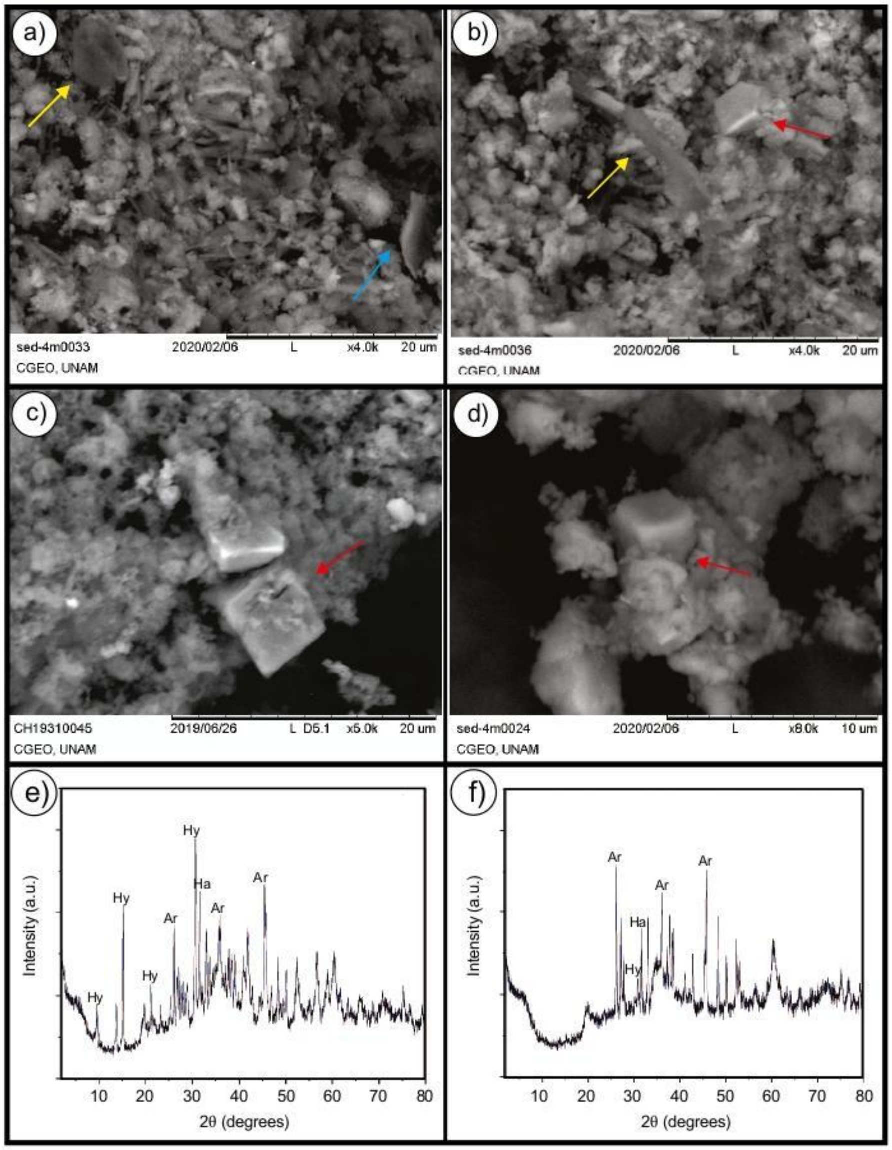

Figure. 5. SEM images and DRX analyses in selected mud samples collected at approx. 4 m below the surface. a. The image shows heterogeneous morphologies in the sediment and a highly porous structure. Clays are indicated with a blue arrow and a biological structure interpreted as a cell is also depicted (yellow arrow). b. Mineral and salt crystals (red arrow) occur near biological structures (yellow arrow). e, f. X-ray diffraction pattern of sediments showing peaks characteristic of aragonite (Ar), hydromagnesite (Hy) and Halite (Ha) which were observed in the sample, marked by red arrows in c and d.

structures can be attributed to mud viscosity, differences in the brine and gas content and pore pressure of the mobile mud, and fracturing patterns in the unsaturated cap layer. When mud ascends to the surface it dries in a short period of time and the structures are preserved in the driest zone of the crater. Upward movement of mud is currently active as it has been observed the growth of larger domes and the emergence of new, smaller structures. Two main mechanisms responsible for the creation of fluid overpressure in RP mud are considered: a) disequilibrium compaction and b) gas input/generation (*e.g.*, Wangen, 2001).

Disequilibrium compaction

Disequilibrium compaction results when mud is unable to drain fluids in response to loading or compressive stresses. Disequilibrium compaction forces mud to flow upwards. Structures that formed in close relation to mud displacement can be seen at the margins of the former lake including ductile deformation structures such as folds and the mud injections. The main geometrical and kinematic aspects of this deformation for the margin of the crater were explored and described in detail by Aranda-Gómez *et al.* (2017). In the case of RP, the injection domes formed by disequilibrium compaction show three different associations regarding their origin (Aranda-Gómez *et al.*, 2017; Figure 3): a) Domes associated with compressive stresses generated by volume loss during fault displacement, these structures are generated by gravitational gliding/spreading and are between the largest domes in RP. The likely mechanism for the formation of these domes is overpressure formed at the diffuse detachment level that is associated with the gliding structures near the margin of the crater (Figure 4). b) Domes caused by local overpressure triggered by load associated with small landslides; these structures are formed in areas partially surrounded by megabreccias associated with landslides in areas where

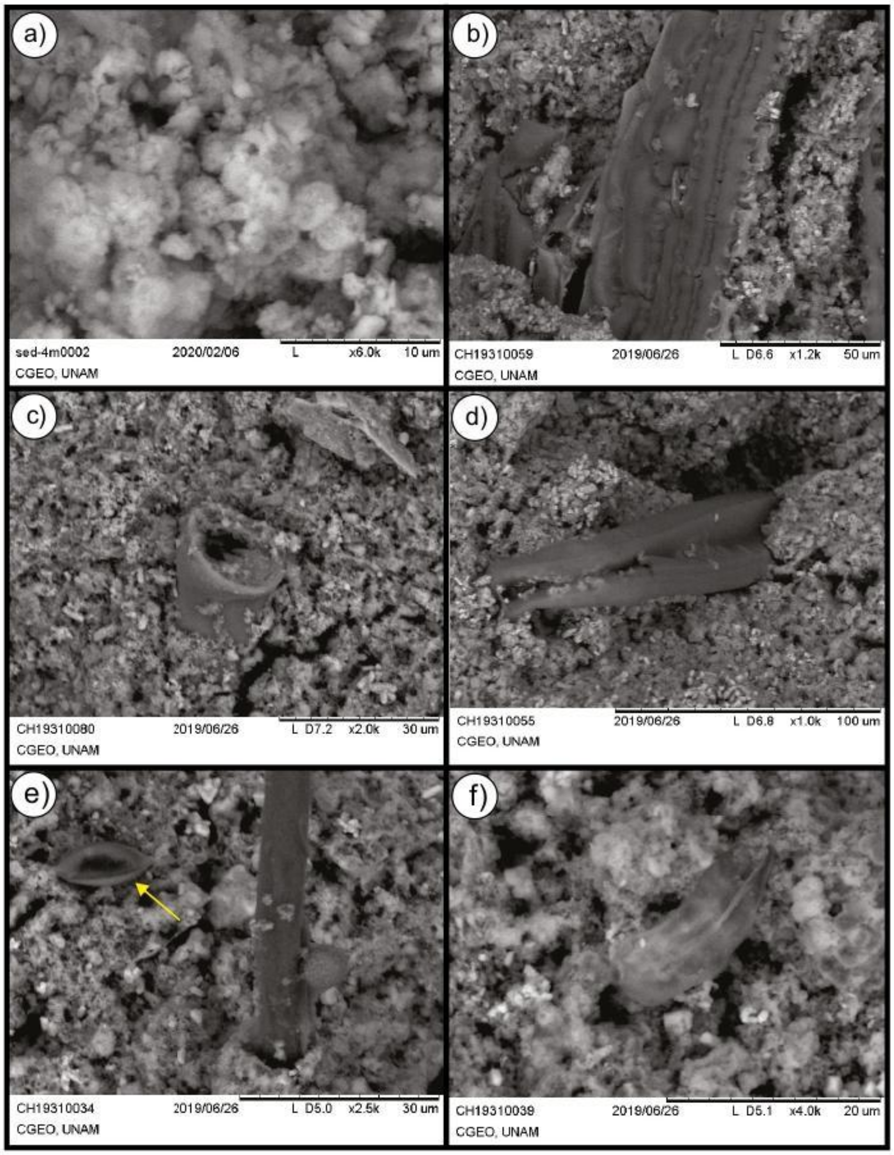

Figure. 6. SEM images of organic structures with different morphologies in the mud samples collected at 4 m depth. In all the photomicrographs sediment has a high porosity. a. Coccoid morphologies. b. A plant. c, d. Different biological morphologies embedded in the high porosity sediment. e. Two different biological morphologies, one of them similar to a diatom (Bacillariophyta: yellow arrow). f. A microorganism probably belonging to the Rotifera phylum.

the calcareous platform is nearly absent. The weight of the landslide deposit can produce overpressure in the underlying wet mud triggering mud injection (Figures 3 and 4); c) Domes and mud extrusion in portions of extensional faults/fractures. Massive mud concentrations with a characteristic "popcorn" weathering texture in relatively small, nearly circular areas suggest the presence of domes and/or "central vents" in areas at the carbonate platform scarp or in the pelagic zone. In cross-section wet mud appears to be injected in tensional fractures/faults. These structures were interpreted in the GPR profiles as domes and mud uplifting associated with gas seeps (Figure 4e).

Mud mobility possibly related to groundwater input and degassing in RP

Fluid mobility and mud extrusion can also be associated with the volumetric expansion accompanying the phase change associated with gas generation in the pore system or from groundwater input (*e.g.*, Revil, 2002). RP has previously been shown to emit fluids and gases. The present-day manifestations include soil degassing and pipe-like structures (Paz *et al.*, 2020). Bubbling can be observed in remnant brine ponds. A recent study by Paz *et al.* (2020) has verified the emanation of gases such as CO_2 with an estimated total flow of 10.6 ton·d^{-1} and 6.3 ton·d^{-1} of CH_4 in RP. These authors suggest a strong biogenic contribution in the origin of methane, while CO_2 is probably a mixing among volcanic and biologic sources mainly within sediments. Gas production may be a consequence of a prolonged phase of low volcanic activity (*e.g.*, Loher *et al.*, 2018) and/or result of underground biological activity. Small amounts of methane can produce expansion and drive extrusion (*e.g.*, Brown, 1990).

A close spatial association of large domical biohermal thrombolites and the annular fault (*i.e.*, the diatreme - country rock boundary; Lorenz, 1986) suggest that this structure might have controlled groundwater flow into the paleolake before desiccation (Aranda-

Table 2. Total DNA obtained from 30 g of mud in the processed samples Sediment 1, Sediment 2, and Sediment 3.

Sample	Concentration (ng/μl)	Total DNA (ng)	Volume (μl)	Relation 260/280
Sediment 1	0.80	22.33	27	1.67
Sediment 2	1.16	32.41	27	1.75
Sediment 3	0.60	16.67	27	1.65

Table 3. Number of total sequences obtained from the sedimentary environmental DNA recovered of the highly mobile and saturated subsurface in the RP lacustrine system.

Sample	Bacteria	Archaea
Sediment 1	5408	-
Sediment 2	12736	10089
Sediment 3	7700	6335

Gómez *et al*., 2017; Sánchez-Sánchez *et al*., 2019). Further evidence of this channelized, preferential groundwater flow includes the presence of chimney-like structures and the thrombolytic texture of most microbialites (Sánchez-Sánchez *et al*., 2019), which also suggests an upward groundwater flow during the lake period that might have helped to maintain a stable water level in the lake. The presence of springs associated with groundwater inflow was observed during the lake period in the past century (Escolero-Fuentes and Alcocer-Durán, 2004). We interpret this evidence as an indication of active input of fluids into the mud layer.

In the GPR profiles dome-shaped geometries were observed below the desiccated crust at the center of the crater, in areas not directly associated with the disequilibrium compaction in the margins. In this zone the surface dry mud crust forms a brittle cover atop the wet mud (Figure 4d and 4e). In the central part of the crater, we measured about 1.2 m of dry brittle mud three months after the end of the rainy season of 2018. The vertical transition between dry and wet mud in RP is diffuse because pore pressure increases with depth and almost certainly has a seasonal depth variation. In this setting, particularly at the western side of the lake bottom, wet mud has formed several diapir-like mud injections whose basal diameter varies from several tens of meters to less than one meter in diameter. These structures interpreted from the GPR profiles present surface evidence of fluid leakage as well as mud extrusion, indicated by the presence of a popcorn texture in the mud that reached the surface.

Insights from the microbial communities DNA fingerprint documented in the mud

This is the first study of sedimentary environmental DNA for identifying the bacterial and archaeal communities in the near-surface sediments of Rincón de Parangueo maar. Our results can provide an insight into the identities of members of the bacterial and archaeal communities in the three samples obtained in the crater discharging methane. As a prospective study, our results have limitations, one is the small number of samples collected (three) that prevent a complete statistical analysis among them. Despite this limitation, the high-quality data of the Bacteria and Archaea phylotypes allow us to calculate alpha diversity indices for each sample, making it possible to differentiate diversity values from previous studies in the area. Alpha diversity and observed ASVs of the bacterial and archaeal community measured in mud samples were lower than those reported by Ibarra-Sánchez *et al*. (2020) in superficial sediment samples. Despite physico-chemical parameters like grain size, alkalinity and conductivity were similar in both investigations, this downward trend in diversity values is consistent with the results, since it is known that the relative abundance of 16S rRNA genes from Archaea and Bacteria generally decreases with depth of sediment (Hoshino and Inagaki, 2019). The results reveal a connection of the occurrence of specific microbes with the geological environment, can be particularly valuable for understanding community composition in future works, and give useful information on the relative importance of specific environmental drivers. Our interpretation is thus dependent not only on the DNA results but also on understanding contemporary geological and ecological controls as well as the sedimentation environment and its context.

Additionally, sedimentary environmental DNA does not distinguish from living organisms or those derived from genetic archives of past environments preserved in dead cells, resting stages, or as an extracellular fraction in the mud (Torti *et al*., 2015; Vuillemin *et al*., 2017; Giguet-Covex *et al*., 2019). In RP, the persistence of extracellular DNA may be influenced by adsorbing to soil minerals. Silt and clay minerals could protect extracellular DNA from nuclease mediated enzymatic hydrolysation and by adsorbing DNases and nucleases, thereby reducing the potential for enzymatic DNA restriction (Levy-Booth *et al*., 2007). Montmorillonite, a clay mineral identified in the mud samples (Armienta *et al*., 2008; Aranda-Gómez *et al*., 2013), has a higher DNA binding capacity than other minerals (*e.g.*, kaolinite) (Pietramellara *et al*., 2001). The adsorption of DNA to clay minerals is enhanced by divalent cations like Mg^{2+} and Ca^{2+}, also described for the samples, forming bridges between DNA and clay minerals above pH 5 (Levy-Booth *et al*., 2007). Mineral mud properties allow the conservation of extracellular DNA at high pH (> 10) in RP. Thus, the sedimentary environmental DNA provides simultaneous information on multiple taxa potentially composed of phylotypes that live or lived in the sediments, probably within the last hundreds of years.

Our results suggest that the overall DNA fingerprint obtained shares similarities with the DNA of the microbial communities identified previously on the crater surface at the phylum level (Sánchez-Sánchez *et al*., 2019; Ibarra-Sánchez *et al*., 2020). Bacteria phyla such as Cyanobacteria and Proteobacteria were abundant in two mud samples (this work, Sediment 1 and Sediment 3) as well as in the surface samples (Sánchez-Sánchez *et al*., 2019; Ibarra-Sánchez *et al*., 2020). Phyla identified in the mud such as Firmicutes, Actinobacteriota, Bacteroidota, Verrucomicrobiota, and Chloroflexi were also previously identified on the surface. These bacterial phyla dominate often in sediment samples of saline lakes (Vuillemin *et al*., 2018) and play an important role in biological cycles at these habitats. For instance, members of Proteobacteria, Firmicutes, Actinobacteriota, and Bacteroidota are heterotrophic organisms, so they are involved in processes of organic matter transformation (Sorokin *et al*., 2014; Ibarra-Sánchez *et al*., 2020). Phylotypes belonging to Firmicutes can fix nitrogen and members of Deltaproteobacteria and Firmicutes participate in the sulfur cycle (Sorokin *et al*., 2014).

The archaeal DNA obtained at depth in RP was classified into seven phyla: Crenarchaeota, Halobacterota, Hadarchaeota, Thermoplasmatota, Euryarchaeota, Nanoarchaeota, and Asgardarchaeota. Crenarchaeota was the most abundant phylum found in the mud at depth, in contrast to surface samples where the dominant phylum was Euryarchaeota (Sánchez-Sánchez *et al*., 2019; Ibarra-Sánchez *et al*., 2020). Crenarchaeota are globally ubiquitous, and members of this phylum are often the most abundant Archaea in various environments including marine sediments (Kubo *et al*., 2012) and salt marsh sediments (Seyler *et al*., 2014). Candidatus_Nitrososphaera, the dominant genus identified in mud samples for the Crenarchaeota phylum, is a moderately thermophilic microorganism (46 °C) with demonstrated

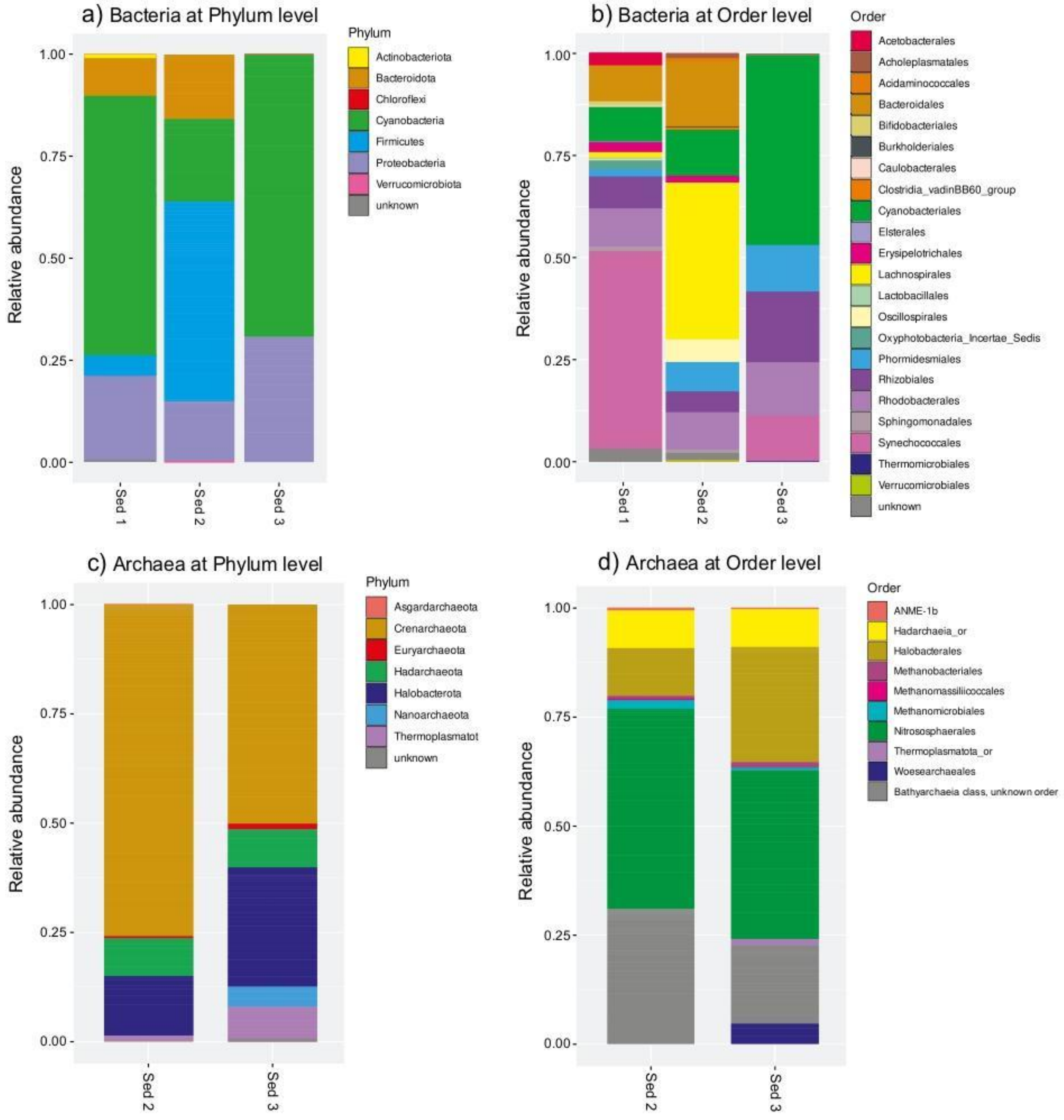

Figure. 7. Phylogenetic distribution of bacterial and archaeal 16S rRNA sequences in sediment samples from Rincón de Parangueo. a, b. Phylogenetic distribution of Bacteria at phylum and order level, showing the relative abundance for samples Sediment 1, Sediment 2 and Sediment 3. c, d. Phylogenetic distribution of Archaea at phylum and order level, for samples Sediment 2, and Sediment 3.

capacity for ammonia oxidation (Hatzenpichler *et al.*, 2008). These archaeal genera have been found to be one of the dominant phylotype in hypersaline sediments from the saline-alkaline soils of the former lake Texcoco (Navarro-Noya *et al.*, 2015) and, also was found by Ibarra-Sánchez *et al.* (2020) as the dominant genera in superficial sediments samples (0–20 cm) in RP. Halobacterota, the second archaeal phylum most represented, are obligately halophilic (Papke *et al.*, 2011), which explains their high relative abundance in the alkaline saline sediment of RP. Some habitats for these microorganisms are widespread around the world in salt lakes, hypersaline soils, underground salt deposits, and evaporation ponds (Oren *et al.*, 1995; Arahal *et al.*, 1996; Wainø *et al.*, 2000; Mesbah *et al.*, 2007; Papke *et al.*, 2011; Kambura *et al.*, 2016; Han *et al.*, 2017).

Methanogenic microorganisms and biogenic methane production in the sediments

Phylotypes of methanogenic Archaea identified in RP comprise high percentages (13.2–32.1 %) of the total archaeal DNA, and include four phyla: Crenarchaeota, Halobacterota, Thermoplasmatota, and Euryarchaeota (Lyu *et al.*, 2018). Archaea methanogenic microorganisms are strictly anaerobic and convert several substrates (*i.e.*, H_2 + CO_2, methyl compounds, and acetate) into methane gas in order to obtain energy. Methanogens groups occupy geothermal CO_2 and H_2 as the predominant substrates for methane production in places that emit gases, such as hot springs and volcanic fissures (Whitman *et al.*, 2006), making them valuable for assessing volcanic degassing. Some of the organisms reported here have been found in deep sediments and

Table 4. Observed richness, Shannon-Weiner and Simpson diversity index values from the sedimentary environmental DNA recovered at subsurface in the RP lacustrine system.

Sample	**Alpha diversity**		
	Observed richness	Shannon index (H′)	Simpson index
Sediment 1 Bacteria	132	4.443	0.985
Sediment 2 Bacteria	84	4.066	0.976
Sediment 3 Bacteria	40	3.100	0.942
Sediment 2 Archaea	93	4.142	0.980
Sediment 3 Archaea	92	4.346	0.984

soils elsewhere (Paul *et al.*, 2012). The Bathyarchaeia class, member of Crenarchaeota, was previously reported in the deep sediment biosphere of lakes (Thomas *et al.*, 2020) and subseafloor sediments (Kirkpatrick *et al.*, 2019). Methane production by Bathyarchaeia was hypothesized by the metabolic reconstruction of its genome, the key genes associated with archaeal methane metabolism including methyl–coenzyme M reductase complex suggests their potential for diverse methyl compound utilization for gas production (Evans *et al.*, 2015). The order Methanomicrobiales of Halobacterota, is a hydrogenotrophic methanogen exclusively restricted to CO_2 as a substrate for methanogenesis with the principal electron donor being H_2 (Costa and Leigh, 2014). It has been previously reported in hot springs of the Central-Eastern Tibet (Huang *et al.*, 2011), wetland soil (Tian *et al.*, 2010), in two alkaline Indian hot springs (Panda *et al.*, 2016), and soda lake sediments (Antony *et al.*, 2012). The Methanomicrobiales and Methanomassiliicoccales orders of Thermoplasmatota use an external H_2 source to reduce methyl-compounds into methane (Borrel *et al.*, 2014). They were originally recognized in digestive tracts of animals (Poulsen *et al.*, 2013) but also have been reported in natural wetlands, rice paddy fields, subseafloor and freshwater sediment (Borrel *et al.*, 2013). Methanobacteriales is another hydrogenotrophic methanogen order found in this study belonging to the Euryarchaeota phylum (Buan, 2018). This order has been identified in sediments of fluvial (Chaudhary *et al.*, 2017), lake (Yang *et al.*, 2017), and mangrove environments (Li *et al.*, 2016).

Based on the 16S rRNA results RP mud contains microorganisms with the potential to produce methane. Methylotrophic methanogens, the most represented methanogenic microorganisms in the sediment samples, are common in hypersaline and marine sediments (Lyu *et al.*, 2018). Obligately methylotrophic methanogens have an ecological advantage over other groups of methanogens since they utilize compounds such as methanol and methylated amines, avoid competition with sulfate-reducing Bacteria also present in this type of environments (Antony *et al.*, 2012). Methanogenic Archaea use organic solutes for osmotic stabilization in hypersaline environments at a high energetic cost, methylotrophic methanogenesis produces more energy than methanogenesis from acetate and CO_2 reduction (Oren, 2001). The greatest amount of energy generated by this type of methanogenesis may provide a possible explanation for why methanogenesis on methylated compounds is more feasible at the high salt concentrations in the RP sediments. On the other hand, hydrogenotrophic methanogenesis suggested by the identification of Methanomicrobiales and Methanobacteriales phylotypes could be explained by the presence of post eruptive gases still released in the RP crater. The huge amounts of CO_2 gas verified by Paz *et al.* (2020) could be being reduced by methanogenic Archaea for methane formation.

Mud in RP could be considered as analogue habitats of fluidized mobile muds in submarine mud volcanoes, since they consist of a mixture of fine-grained, fluid-rich mobile sediment (*i.e.*, Pachiadaki *et al.*, 2011). Active fluid seepage and gas bubble release are common in other similar environments such as submarine mud volcanoes driven by overpressured subsurface sediment found at tectonically active and passive continental margins (Lazar *et al.*, 2011). Mud volcanoes emitting biogenic methane have been characterized by previous molecular genetic analyses, detecting the three methanogenic pathways (methylotrophic, hydrogenotrophic and acetoclastic) (*e.g.*, Lazar *et al.*, 2011; Lazar *et al.*, 2012). These former authors report that methylotrophic methanogenesis was the most active process in a mud volcano where hypersaline conditions persist.

CONCLUSION

We report the presence of a layer of mobile mud below the surface of the crater RP. Mud is organic rich, high porosity, with high volumetric water (> 200 % W_V), massive, clayey, and has a sedimentary origin. This mud layer hosts present-day mobilization triggered by two combined processes, disequilibrium compaction and pore over-

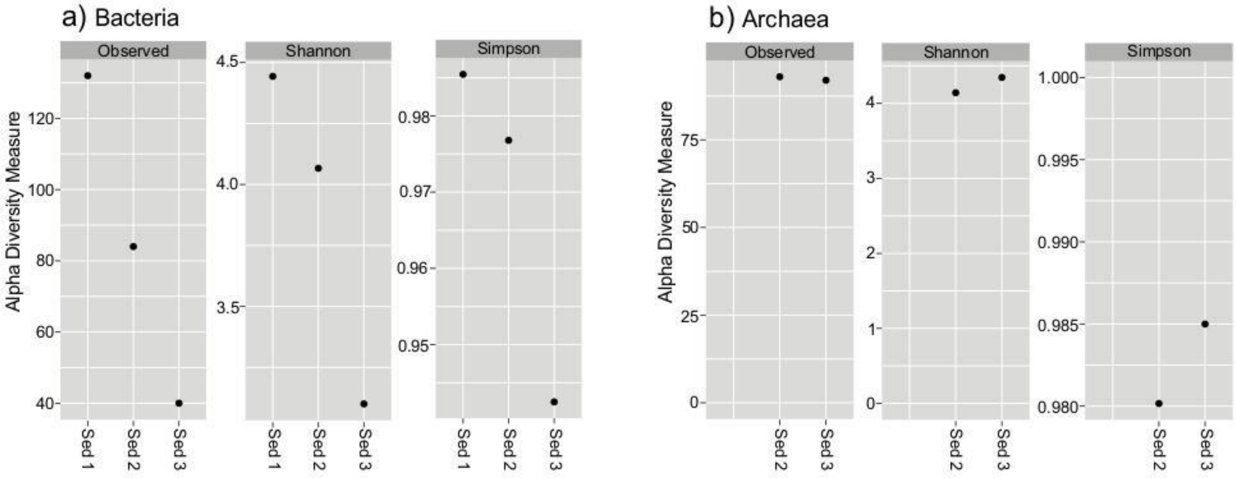

Figure. 8. Observed counts and alpha diversity measured by the Shannon and Simpson indices. a. Observed counts and alpha diversity for Bacteria in samples Sediment 1, Sediment 2, and Sediment 3. b. Observed counts and alpha diversity for Archaea in samples Sediment 2 and Sediment 3.

pressure enhanced by an input of groundwater and gas content in the sediments. Mud mobilization disrupted the stratification of the ancient lake and produced mud tectonic structures such as injection mud domes, recorded in field and in GPR profiles. Mud ascends from a shallow underground source to the surface and results in more than 76 domes or small mud intrusions distributed mainly at the western portion of the former lake at RP.

The microbial communities' fingerprint, or sedimentary environmental DNA, documented in the mud provide an insight into the identities of members of the bacterial and archaeal communities in three mud samples, and allow to discuss the probable role played by biogenic methane in mud mobilization observed on the surface. We emphasize that given the small number of samples available for this work, the details of the methanogenic activity in the RP sediments needs to be corroborated with further and more detailed studies. We hypothesize that pore pressure in the mud may periodically be increased as a consequence of microbe's activity; for instance, the biogenic degradation of organic matter (*i.e.*, methanogenesis). We identified methanogenic microorganisms in the DNA fingerprint. The phylotypes Bathyarchaeia, Methanomicrobiales, Methanomassiliicoccales, and Methanobacteriales were recognized in the mud samples, giving way to the possibility of biogenic gas production by them. Analogue habitats of fluidized or mobile muds are those observed in brine-sediments (mud volcanoes) driven by overpressured subsurface sediment where methanogenic microorganisms have an active influence in the methane fluxes. Coupling the geological and microbiotic factors can provide a holistic view of this dynamic extreme environment.

ACKNOWLEDGMENTS

We thank Sara Solís Valdez, Marina Vega, Ricardo Carrizosa and María Carolina Muñoz Torres for help on the soil and water analysis, and Luis Aguilar, Alejandro de León, Carlos S. Flores and Jair García of the Laboratorio Nacional de Visualización Científica Avanzada (UNAM) for assistance in the bioinformatic analysis. Barbara Moguel is thanked for help on the DNA analysis. We thank Dante Morán-Zenteno and two anonymous reviewers for their revision that helped to improve this work. This work was supported by projects of the Laboratorio de Mecánica de Geosistemas (LAMG, UNAM). Sánchez-Sánchez received a PhD scholarship from Consejo Nacional de Ciencia y Tecnología (CONACyT).

REFERENCES

Antony, C.P., Murrell, J.C., Shouche, Y.S., 2012, Molecular diversity of methanogens and identification of *Methanolobus* sp. as active methylotrophic Archaea in Lonar crater lake sediments: FEMS Microbiology Ecology, 81, 43-51, https://doi.org/10.1111/j.1574-6941.2011.01274.x

Arahal, D.R., Dewhirst, F.E., Paster, B.J., Volcani, B.E., Ventosa, A., 1996, Phylogenetic analyses of some extremely halophilic archaea isolated from Dead Sea water, determined on the basis of their 16S rRNA sequences: Applied and Environmental Microbiology, 62(10), 3779-3786.

Aranda-Gómez, J.J., Cerca, M., Rocha-Treviño, L., Pacheco, J., Levresse, G., Ramos-Leal, J.A., Yutsis, V., Arzate-Flores, J.A., 2014, Structural analysis of subsidence-related deformation at the bottom of Rincón de Parangueo maar, México, *in* Proceedings IAVCEI-5th International Maar Conference: Querétaro, Mexico, Centro de Geociencias, Universidad Nacional Autónoma de México, 174-175.

Aranda-Gómez, J.J., Cerca, M., Rocha-Treviño, L., Carrera-Hernández, J.J., Levresse, G., Pacheco, J., Yutsis, V., Arzate-Flores, J.A., Chacón, E., Beraldi-Campesi, H., 2017, Structural evidence of enhanced active subsidence at the bottom of a maar: Rincón de Parangueo, México: Geological Society, London, Special Publications, 446(1), 225-254.

Aranda-Gómez, J.J., Levresse, G., Martínez, J.P., Ramos-Leal, J.A., Carrasco-Núñez, G., Chacón-Baca, E., González-Naranjo, G., Chávez-Cabello, G., Vega-González, M., Origel, G., 2013, Active sinking at the bottom of the Rincón de Parangueo maar (Guanajuato, México) and its probable relation with subsidence faults at Salamanca and Celaya: Boletín de la Sociedad Geológica Mexicana, 65(1), 169-188.

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(2), 249-258.

Bey, B.S., Fichot, E.B., Dayama, G., Decho, A.W., Norman, R.S., 2010, Extraction of high molecular weight DNA from microbial mats: Biotechniques, 49(3), 631-640.

Bolger, A.M., Lohse, M., Usadel, B., 2014, Trimmomatic: a flexible trimmer for Illumina sequence data: Bioinformatics, 30, 2114-2120, https://doi.org/10.1093/bioinformatics/btu170

Borrel, G., O'Toole, P.W., Harris, H.M., Peyret, P., Brugere, J.-F., Gribaldo, S., 2013, Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis: Genome Biology and Evolution, 5(10), 1769-1780.

Borrel, G., Parisot, N., Harris, H.M., Peyretaillade, E., Gaci, N., Tottey, W., Bardot, O., Raymann, K., Gribaldo, S., Peyret, P., 2014, Comparative genomics highlights the unique biology of Methanomassiliicoccales, a Thermoplasmatales-related seventh order of methanogenic archaea that encodes pyrrolysine: BMC Genomics, 15(1), 1-24, https://doi.org/10.1186/1471-2164-15-679

Brown, K.M., 1990, The nature and hydrogeologic significance of mud diapirs and diatremes for accretionary systems: Journal of Geophysical Research: Solid Earth, 95(B6), 8969-8982.

Buan, N.R., 2018, Methanogens: Pushing the boundaries of biology: Emerging Topics in Life Sciences, 2(4), 629-646, https://doi.org/10.1042/ETLS20180031

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, https://doi.org/10.1038/nmeth.3869

Carreón-Freyre, D., Cerca, M., Hernández-Marín, M., 2003, Correlation of near-surface stratigraphy and physical properties of clayey sediments from Chalco basin, México, using ground penetrating radar: Journal of Applied Geophysics, 53(2-3), 121-136, https://doi.org/10.1016/S0926-9851(03)00042-9

Cerca, M., Carreón-Freyre, D., Aranda-Gómez, J.J., Rocha-Treviño, L., 2014, GPR profiles for characterizing subsidence deformation in lake sediments within a maar cráter, *in* Proceedings of the 15th International Conference on Ground Penetrating Radar: Brussels, Belgium, IEEE Xplore, 274-278, doi: 10.1109/ICGPR.2014.6970428

Cerca, M., Rocha, L., Carreón-Freyre, D., Aranda, J., 2015, Physical experiments of land subsidence within a maar crater: Insights for porosity variations and fracture localization: Proceedings of the International Association of Hydrological Sciences, 372, 285-290, https://doi.org/10.5194/piahs-372-285-2015

Chacón, E., Aranda-Gómez, J.J., Charles-Polo, M., Sánchez-Ramos, M.A., Rivera-Muñoz, E.M., Levresse, G., Millán-Malo, B., 2018, Biohermal thrombolites of the crater lake Rincón de Parangueo in central México: Journal of South American Earth Sciences, 85, 236-49, https://doi.org/10.1016/j.jsames.2018.04.013

Chaudhary, P.P., Rulík, M., Blaser, M., 2017, Is the methanogenic community reflecting the methane emissions of river sediments? comparison of two study sites: Microbiology Open, 6(4), e00454, https://doi.org/10.1002/mbo3.454

Core, Team., 2020, R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria, computer software availale at https://www.R-project.org/

Costa, K.C., Leigh, J.A., 2014, Metabolic versatility in methanogens: Current Opinion in Biotechnology, 29, 70-75, https://doi.org/10.1016/j.copbio.2014.02.012

Domínguez-Vázquez, G., Osuna-Vallejo, V., Castro-López, V., Israde-Alcántara, I., Bischoff, J.A., 2019, Changes in vegetation structure during the Pleistocene–Holocene transition in Guanajuato, central México: Vegetation History and Archaeobotany, 28(1), 81-91, https://doi.org/10.1007/s00334-018-0685-8

Ellegaard, M., Clokie, M.R.J., Czypionka, T., 2020, Dead or alive: sediment DNA archives as tools for tracking aquatic evolution and adaptation: Commun Biol, 3 (1), 1-11, https://doi.org/10.1038/s42003-020-0899-z

Escolero-Fuentes, Ó, A., Alcocer-Durán, J., 2004, Desecación de los lagos cráter del Valle de Santiago, Guanajuato, *in* Jiménez, B., Marín, L., (eds.), El Agua en México vista desde la Academia: México City, Academia Mexicana de Ciencias, 99-115.

Evans, P.N., Parks, D.H., Chadwick, G.L., Robbins, S.J., Orphan, V.J., Golding, S.D., Tyson, G.W., 2015, Methane metabolism in the archaeal phylum bathyarchaeota revealed by genome-centric metagenomics: Science, 350(6259), 434-438, https://doi.org/10.1126/science.aac7745

Gates-Rector, S., Blanton, T., 2019, The powder diffraction file: a quality materials characterization database: Powder Diffraction, 34(4), 352-360.

Giguet-Covex, C., Ficetola, G.F., Walsh, K., Poulenard, J., Bajard, M., Fouinat, L., Sabatier, P., Gielly, L., Messager, E., Develle, A.L., 2019, New insights on lake sediment DNA from the catchment: Importance of taphonomic and analytical issues on the record quality: Scientific Reports, 9(1), 1-21, https://doi.org/10.1038/s41598-019-50339-1

Han, R., Zhang, X., Liu, J., Long, Q., Chen, L., Liu, D., Zhu, D., 2017, Microbial community structure and diversity within hypersaline Keke salt lake environments: Canadian Journal of Microbiology, 63(11), 895-908, https://doi.org/10.1139/cjm-2016-0773

Hatzenpichler, R., Lebedeva, E.V., Spieck, E., Stoecker, K., Richter, A., Daims, H., Wagner, M., 2008, A moderately thermophilic ammonia-oxidizing Crenarchaeote from a hot spring: Proceedings of the National Academy of Sciences, 105(6), 2134-2139, https://doi.org/10.1073/pnas.0708857105

Hautman, D.P., Munch, D.J., 1997, Method 300.1 determination of inorganic anions in drinking water by ion chromatography: Cincinnati, OH, US Environmental Protection Agency, 1-36.

Hoshino, T., Inagaki, F., 2019, Abundance and distribution of Archaea in the subseafloor sedimentary biosphere: The ISME Journal, 13(1), 227-231.

Huang, Q., Dong, C.Z., Dong, R.M., Jiang, H., Wang, S., Wang, G., Fang, B., Ding, X., Niu, L., Li, X., 2011, Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China: Extremophiles, 15(5), 549, DOI: 10.1007/s00792-011-0386-z

Ibarra-Sánchez, C.L., Prince, L., Aguirre-Noyola, J.L., Sánchez-Cerda, K.E., Navaro-Noya, Y.E., Luna-Guido, M., Conde-Barajas, E., Dendooven, L., Gomez-Acata, E.S., 2020, The microbial community in an alkaline saline sediment of a former maar lake bed: Journal of Soils and Sediments, 20(1), 542-555, https://doi.org/10.1007/s11368-019-02371-x

Jones, B.E., Grant, W.D., Duckworth, A.W., Owenson, G.G., 1998, Microbial diversity of soda lakes: Extremophiles, 2(3), 191-200.

Kambura, A.K., Mwirichia, R.K., Kasili, R.W., Karanja, E.N., Makonde, H.M., Boga, H.I., 2016, Bacteria and Archaea diversity within the hot springs of Lake Magadi and Little Magadi in Kenya: BMC Microbiology, 16(1), 136, https://doi.org/10.1186/s12866-016-0748-x

Kienel, U., Bowen, S.W., Byrne, R., Park, J., Böhnel, H., Dulski, P., Luhr, J.F., Siebert, L., Haug, G.H., Negendank, J.F., 2009, First lacustrine varve chronologies from México: Impact of droughts, ENSO and human activity since AD 1840 as recorded in maar sediments from Valle de Santiago: Journal of Paleolimnology, 42(4), 587-609.

Kirkpatrick, J.B., Walsh, E.A., D'Hondt, S., 2019, Microbial selection and survival in subseafloor sediment: Frontiers in Microbiology, 10, 956, https://doi.org/10.3389/fmicb.2019.00956

Kubo, K., Lloyd, K.G., Biddle, J.F., Amann, R., Teske, A., Knittel, K., 2012, Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments: The ISME Journal, 6(10), 1949-1965, https://doi.org/10.1038/ismej.2012.37

Lay, C. Y., Mykytczuk, N. C., Yergeau, É., Lamarche-Gagnon, G., Greer, C. W., Whyte, L, G., 2013, Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring: Applied and Environmental Microbiology, 79(12), 3637-3648.

Lazar, C. S., John Parkes, R., Cragg, B. A., l'Haridon, S., Toffin, L., 2012, Methanogenic activity and diversity in the centre of the Amsterdam Mud Volcano, Eastern Mediterranean Sea: FEMS Microbiology Ecology, 81(1), 243-254.

Lazar, C. S., Parkes, R. J., Cragg, B. A., l'Haridon, S., Toffin, L., 2011, Methanogenic diversity and activity in hypersaline sediments of the centre of the Napoli mud volcano, Eastern Mediterranean Sea: Environmental Microbiology, 13(8), 2078-2091.

Levy-Booth, D.J., Campbell, R.G., Gulden, R.H., Hart, M.M., Powell, J.R., Klironomos, J.N., Dunfield, K.E., 2007, Cycling of extracellular DNA in the soil environment: Soil Biology and Biochemistry, 39(12), 2977-2991.

Levresse, G., Aranda-Gómez, J.J., Bard R.C., Cerca, M., Rocha-Treviño, L., Pacheco-Martínez, J., Ramos-Leal, J.A., Yutsis, V., Arzate-Flores, J., 2014, COB isotopic evolution of lacustrine microbialites of Rincón de Parangueo maar, México, *in* Proceedings IAVCEI-5th International Maar Conference: Querétaro, Mexico, Centro de Geociencias, Universidad Nacional Autónoma de México, 132-133.

Li, W., Guan, W., Chen, H., Liao, B., Hu, J., Peng, C., Rui, J., Tian, J., Zhu, D., He, Y., 2016, Archaeal communities in the sediments of different mangrove stands at Dongzhaigang, China: Journal of Soils and Sediments, 16(7), 1995-2004, https://doi.org/10.1007/s11368-016-1427-0

Loher, M., Pape, T., Marcon, Y., Römer, M., Wintersteller, P., Praeg, D., Torres, M., Sahling, H., Bohrmann, G., 2018, Mud extrusion and ring-fault gas seepage–upward branching fluid discharge at a deep-sea mud volcano: Scientific Reports, 8(1), 1-11, https://doi.org/10.1038/s41598-018-24689-1

Lorenz, V., 1986, On the growth of maars and diatremes and its relevance to the formation of tuff rings: Bulletin of Volcanology, 48(5), 265-274, https://doi.org/10.1007/BF01081755

Lyu, Z., Shao, N., Akinyemi, T., Whitman, W.B., 2018, Methanogenesis: Current Biology, 28(13), R727-R732, DOI: 10.1016/j.cub.2018.05.021

McMurdie, P.J., Holmes, S., 2013, phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data: PloS One, 8, e61217, https://doi.org/10.1371/journal.pone.0061217

Mesbah, N.M., Abou-El-Ela, S.H., Wiegel, J., 2007, Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi and Natrun, Egypt: Microbial Ecology, 54(4), 598-617, https://doi.org/10.1007/s00248-006-9193-y

Navarro-Noya, Y.E., Valenzuela-Encinas, C., Sandoval-Yuriar, A., Jiménez-Bueno, N.G., Marsch, R., Dendooven, L., 2015, Archaeal communities in a heterogeneous hypersaline-alkaline soil: Archaea, 2015, 646820.

NOM-021-RECNAT-2000, Norma Oficial Mexicana que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudio, muestreo y análisis: México, D.F., Mexico, Diario Oficial de la Federación, 2002, 1-22.

Oren, A., 2001, The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems, *in* Melack, M., Jellison, R., Herbst, D.B., (eds.), Saline Lakes: Springer, 61-72, https://doi.org/10.1007/978-94-017-2934-5_6

Oren, A., Gurevich, P., Gemmell, R.T., Teske, A., 1995, Halobaculum gomorrense gen. Nov., sp. Nov., a novel extremely halophilic archaeon from the Dead Sea: International Journal of Systematic and Evolutionary Microbiology, 45(4), 747-754, https://doi.org/10.1099/00207713-45-4-747

Pachiadaki, M. G., Kallionaki, A., Dählmann, A., De Lange, G. J., Kormas, K. A., 2011, Diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep-sea mud volcano: Microbial Ecology, 62(3), 655-668.

Panda, A.K., Bisht, S.S., De Mandal, S., Kumar, N.S., 2016, Bacterial and archeal community composition in hot springs from Indo-Burma region, North-east India: AMB Express, 6(1), 111, https://doi.org/10.1186/s13568-016-0284-y

Papke, R.T., White, E., Reddy, P., Weigel, G., Kamekura, M., Minegishi, H., Usami, R., Ventosa, A., 2011, A multilocus sequence analysis approach to the phylogeny and taxonomy of the Halobacteriales: International Journal of Systematic and Evolutionary Microbiology, 61(12), 2984-2995, https://doi.org/10.1099/ijs.0.029298-0

Paul, K., Nonoh, J.O., Mikulski, L., Brune, A., 2012, "Methanoplasmatales", Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens: Applied and Environmental Microbiology, 78(23), 8245-8253.

Paz, M.P.J., Inguaggiato, C., Levresse, G., Robidoux, P., Granados, H.D., Tassi, F., 2020, Total CO$_2$ output and carbon origin discharged from Rincón de Parangueo maar (México): Journal of Geochemical Exploration, 106558, https://doi.org/10.1016/j.gexplo.2020.106558

Pietramellara, G., Franchi, M., Gallori, E., Nannipieri, P., 2001, Effect of molecular characteristics of DNA on its adsorption and binding on homoionic montmorillonite and kaolinite: Biology and Fertility of Soils, 33, 402-409.

Poulsen, M., Schwab, C., Jensen, B.B., Engberg, R.M., Spang, A., Canibe, N., Højberg, O., Milinovich, G., Fragner, L., Schleper, C., 2013, Erratum: Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen: Nature Communications, 4(1), 1-2, https://doi.org/10.1038/ncomms2432

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, 4, D590-D596, https://doi.org/10.1093/nar/gks1219

Revil, A., 2002, Genesis of mud volcanoes in sedimentary basins: A solitary wave-based mechanism: Geophysical Research Letters, 29(12), 15-1-15-4, https://doi.org/10.1029/2001GL014465

Rocha-Treviño, L., 2015, Análisis de la deformación y hundimiento activo causado por la desecación del lago del maar Rincón de Parangueo (Guanajuato, México) mediante modelos análogos y perfiles de Radar de Penetración Terrestre (RPT): Querétaro, Qro. Mexico, Universidad Nacional Autónoma de México, Master's thesis, 87 pp, https://repositorio.unam.mx/contenidos/65241

Sánchez-Sánchez, J., Cerca, M., Alcántara-Hernández, R.J., Lozano-Flores, C., Carreón-Freyre, D., Levresse, G., Vega, M., Varela-Echavarría, A., Aranda-Gómez, J.J., 2019, Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico: FEMS Microbiology Ecology, 95(5), fiz051, https://doi.org/10.1093/femsec/fiz051

Seyler, L.M., McGuinness, L.M., Kerkhof, L.J., 2014, Crenarchaeal heterotrophy in salt marsh sediments: The ISME Journal, 8(7), 1534-1543, https://doi.org/10.1038/ismej.2014.15

Sorokin, D. Y., Berben, T., Melton, E. D., Overmars, L., Vavourakis, C. D., Muyzer, G., 2014, Microbial diversity and biogeochemical cycling in soda lakes: Extremophiles, 18(5), 791-809, https://doi.org/10.1007/s00792-014-0670-9

Thomas, C., Francke, A., Vogel, H., Wagner, B., Ariztegui, D., 2020, Weak influence of paleoenvironmental conditions on the subsurface biosphere of Lake Ohrid over the last 515 ka: Microorganisms, 8(11), 1736, https://doi.org/10.3390/microorganisms8111736

Tian, J., Wang, Y., Dong, X., 2010, Methanoculleus hydrogenitrophicus sp. Nov., a methanogenic archaeon isolated from wetland soil: International Journal of Systematic and Evolutionary Microbiology, 60(9), 2165-2169, https://doi.org/10.1099/ijs.0.019273-0

Torti, A., Lever, M.A., Jørgensen, B.B., 2015, Origin, dynamics, and implications of extracellular DNA pools in marine sediments: Marine Genomics, 24, 185-196, https://doi.org/10.1016/j.margen.2015.08.007

EPA (US Environmental Protection Agency), 2001, EPA-821-R-01-010, Method 200.7: Trace elements in water, solids, and biosolids by inductively coupled plasma-atomic emission spectrometry, Revision 5.0: Washington, D.C., US Environmental Protection Agency, Office of Science and Technology, 68 pp.

Vuillemin, A., Ariztegui, D., Horn, F., Kallmeyer, J., Orsi, W.D., Team, P.S., 2018, Microbial community composition along a 50 000-year lacustrine sediment sequence: FEMS Microbiology Ecology, 94(4), fiy029, https://doi.org/10.1093/femsec/fiy029

Vuillemin, A., Horn, F., Alawi, M., Henny, C., Wagner, D., Crowe, S. A., Kallmeyer, J., 2017, Preservation and significance of extracellular DNA in ferruginous sediments from Lake Towuti, Indonesia: Frontiers in Microbiology, 8, 1440, https://doi.org/10.3389/fmicb.2017.01440

Wainø, M., Tindall, B., Ingvorsen, K., 2000, Halorhabdus utahensis gen. Nov., sp. Nov., an aerobic, extremely halophilic member of the Archaea from Great Salt lake, Utah: International Journal of Systematic and Evolutionary Microbiology, 50(1), 183-190, https://doi.org/10.1099/00207713-50-1-183

Wangen, M., 2001, A quantitative comparison of some mechanisms generating overpressure in sedimentary basins: Tectonophysics, 334(3-4), 211-234, https://doi.org/10.1016/S0040-1951(01)00064-6

Whitman, W.B., Bowen, T.L., Boone, D.R., 2006, The methanogenic bacteria: Prokaryotes, 3(9), 165-207.

Wickham, H., 2016, ggplot2: Elegant Graphics for Data Analysis: New York, Springer-Verlag, 260 pp.

Yang, Y., Li, N., Wang, W., Li, B., Xie, S., Liu, Y., 2017, Vertical profiles of sediment methanogenic potential and communities in two plateau freshwater lakes: Biogeosciences, 14(2), 341-351, https://doi.org/10.5194/bg-14-341-2017

Manuscript received: may 24, 2021
Corrected manuscript received: october 18, 2021
Manuscript accepted: october 20, 2021

Received: 13 September 2022 | Revised: 7 September 2023 | Accepted: 9 October 2023
DOI: 10.1111/fwb.14191

Freshwater Biology WILEY

ORIGINAL ARTICLE

Geomicrobiology of the Rincon de Parangueo maar crater: Exploring the link between an evolving extreme environment and its potential metabolic diversity

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Funding information
Nacional de Ciencia y Tecnología (CONACyT), Grant/Award Number: 365865 and 707035

Abstract

1. Rincon de Parangueo (RP) is a Quaternary maar crater located in the Michoacan-Guanajuato Volcanic Field in central Mexico. Like other volcanic craters in the region, the central part was occupied by an endorheic lacustrine system. As a consequence of extensive groundwater extraction, the perennial lake started a gradual desiccation process and now the remnant ponds host a highly saline–alkaline ecosystem. The stratigraphic record indicates an exceptional and long-term sedimentation of carbonate microbialites. Previous studies based on the 16S rRNA gene have shown that microbial communities have a widespread presence in the crater at the microbialites, the remaining saline ponds, superficial soils and below the surface. To understand the possible role of the microbial communities that generate these biogeological structures, novel analytical methods to resolve the amplicon datasets and their microbial metabolic potential were used.
2. We describe in detail the relationship between the microbial communities, the evolution of physicochemical parameters, and carbonate morphology, in four micro-environments. A 16S rRNA gene sequencing dataset from previous publications was re-analysed using an ASV-based bioinformatic pipeline to identify new insights into the microbial assemblage. Finally, a taxon-based metabolic profile was used to predict the potential metabolic contribution of the prokaryotic community.
3. Results indicated a refinement in terms of community composition using the ASV-based bioinformatic methods. Functional profiling through 16S rRNA gene-based analysis suggested metabolisms associated with carbonate precipitation, indicating a broad potential for microbially mediated carbonate precipitation (e.g., carbon fixation and photosynthesis). The possibility of biogenic methane gas production by methanogenic microorganisms was recognised, supporting the estimated flow of methane on the surface soils.

4. The lacustrine evolution over the last years and the extreme physicochemical characteristics of RP have an impact on the microbial community structure. Prokaryotic community and metabolic potential results from RP coincide with the diversity and abundance of microbial communities and functional metabolisms reported from other microbialite-forming lakes along the Trans Mexican Volcanic Belt. The identification of important possible phylotypes involved in carbonate precipitation might be an important factor in considering RP microbialites as active calcifying entities.
5. These findings provide novel insights into the potential key role of metabolic pathways driving the process of carbonate precipitation inside RP and evidence of its importance as a microbial biodiversity hotspot in Mexico.

KEYWORDS
biodiversity, geomicrobiology, maar lake Rincon de Parangueo, metabarcoding, physicochemical lithological parameters

1 | INTRODUCTION

Saline–alkaline lakes found in arid or semi-arid subtropical latitudes inlands of the continents (Grant & Jones, 2016) host communities of microorganisms that are of ecological importance. Many of these lakes originally formed in endorheic basins and were exposed to high evaporation rates (Jones et al., 1998) are threatened by desiccation. As water levels decrease, the geochemical and physical characteristics impose extreme living conditions, such as low concentrations of calcium and magnesium, high concentrations of sodium chloride and other dissolved salts (>35 ppt), and a high pH (>10), which allows them to dissolve carbonate ions in the waters (Vavourakis et al., 2018). Despite the challenging conditions, these environments harbour highly diverse communities of haloalkaliphilic microorganisms, most of them prokaryotes, that have adapted to survive and grow (Aguirre-Garrido et al., 2016; Cellamare et al., 2018; Rees et al., 2004). Through time, some alkaline lakes hosted exceptional cases of organo-sedimentary structures, or microbialites, formed by microbially mediated mineral precipitation (Couradeau et al., 2011; Gérard et al., 2018; Iniesto et al., 2021). Microbialite formation most likely results from the interaction between microorganisms with great taxonomic and metabolic diversity, under the influence of environmental parameters such as temperature, alkalinity, salinity, sediment composition, and geological parameters including discharge of volcanic gases (Boros & Kolpakova, 2018; Couradeau et al., 2011). The most important microbial metabolisms reported to be involved in carbonate precipitation in microbialites are oxygenic photosynthesis (Couradeau et al., 2011; Iniesto et al., 2021; Saghaï et al., 2015), anoxygenic photosynthesis (Bundeleva et al., 2012), sulfate reduction (Bontognali et al., 2008), nitrate-driven sulfide oxidation, sulfate-driven anaerobic oxidation of methane (Himmler et al., 2018; Michaelis et al., 2002) and anaerobic respiration (Hollander et al., 2007). These metabolisms lead to local carbonate supersaturation by increasing the pH and/or alkalinity in the medium. However, other metabolisms such as sulfide oxidation, aerobic respiration, or fermentation inhibit carbonate precipitation by acidification of the medium (Saghaï et al., 2015).

Previous works conducted in Mexico have focused on perennial lakes inside volcanic craters (Aguirre-Garrido et al., 2016; Couradeau et al., 2011; Gérard et al., 2013; Iniesto et al., 2021; Valdespino-Castillo et al., 2018; Zeyen et al., 2017). Here, we assess the potential metabolic functions of the microbial community of the Rincon de Parangueo (RP) maar crater. Groundwater overexploitation in the regional aquifer has transformed the perennial alkaline lake in the central part of its crater into a subaerial saline–alkaline desert soil with small-sized remnant brine ponds, in the last 40 years. The exposed sedimentary stratigraphic record indicates an exceptional and long-term formation of lacustrine carbonated microbialites that include morphologies such as biostromes, stromatolites, thrombolites, oncolites and bioherms (Chacón et al., 2018; Sánchez-Sánchez et al., 2019). The extraordinary growth of microbialites in RP can only be compared in Mexico with the still active Alchichica lake (Águila et al., 2021; Couradeau et al., 2011; Kaźmierczak et al., 2011; Valdespino-Castillo et al., 2014).

The sediments and brine ponds of RP provide ideal extreme environmental conditions where “polyextremophiles”––microbes adapted to live and thrive within a combination of unusual environmental stressors (Albarracín et al., 2016)––have been described (Gómez-Acata et al., 2021; Ibarra-Sánchez et al., 2020; Pérez-Bernal et al., 2017, 2020; Sánchez-Sánchez et al., 2019, 2021). Microorganisms adapted to live in RP crater deal with high dissolved salt concentrations (salinity) and high pH values (alkalinity). Recognising the microbial community and, particularly, its potential metabolic diversity can help us to understand carbonate precipitation processes, and their relationship with the special geological conditions and the harsh physicochemical environment of the lakebed inside RP. The areas with growth of microbial communities reported previously by Sánchez-Sánchez et al. (2019, 2021) are described in detail herein: (1) main topographic scarp with microbialite growth, (2) microbial mats and scatter cells in shallow waters (plankton) of the hypersaline ponds at the crater bottom and, (3) lacustrine sediments in the central part of the crater. Also, the high-throughput 16S rRNA gene sequencing dataset generated by Sánchez-Sánchez et al. (2019, 2021) was re-evaluated to improve its quality. Updated databases and pipelines commonly used as standard for microbial amplicon data (ASV and DADA2 based approach) were used. Finally, an indirect metabolic approach employing iVikodak software was implemented to obtain the potential metabolic capacities of their prokaryotic community.

1.1 | Regional geology and maar lake evolution

Rincon de Parangueo (20°25′ N, 101°14′ W; Figure 1) is a volcanic crater, specifically a maar formed by a phreatomagmatic explosion, located near the town of Valle de Santiago, Guanajuato, at an altitude of 1705 m. Regionally, it is part of the north-central portion of the Trans-Mexican Volcanic Belt (TMVB), the active volcanic arc in central Mexico (Ferrari et al., 2012), wherein magmatic activity is related to the subduction along the Middle American Trench of the Rivera and Cocos plates under the North American plate (Aranda-Gómez & Carrasco-Núñez, 2014). Locally, RP belongs to the northern part of the Plio-Quaternary Michoacan–Guanajuato monogenetic Volcanic Field (MGMVF; Aranda-Gómez & Carrasco-Núñez, 2014). RP maar represents the youngest eruption reported in the area, inferred to have occurred less than 0.137 million years ago based on cross-cutting and stratigraphic relationships with the nearest craters (Aranda-Gómez et al., 2017).

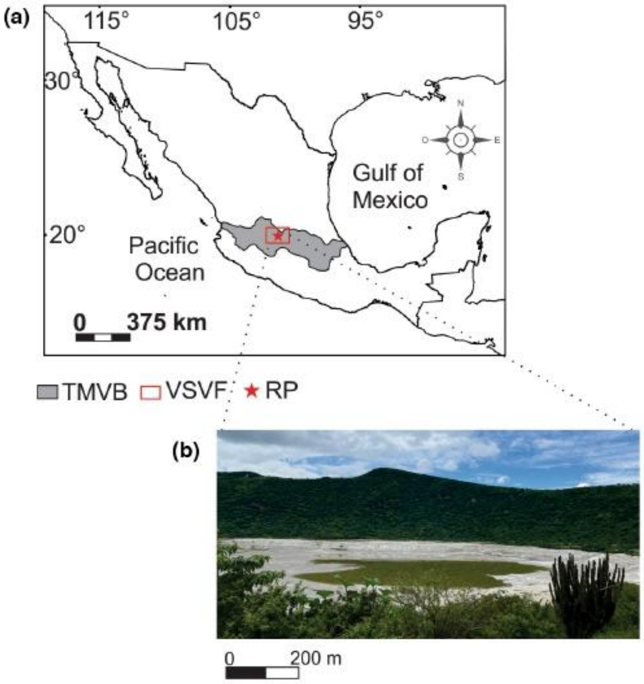

FIGURE 1 (a) Location of Rincon de Parangueo (RP) in the north-central portion of the Trans-Mexican Volcanic Belt (TMVB) in the northern part of the Plio-Quaternary Michoacan–Guanajuato monogenetic Volcanic Field (MGMVF) as part of the volcanic complex (Valle de Santiago Volcanic Field; VSVF); (b) photograph of the central part of the crater showing the remnant brine ponds.

The central part of the RP crater hosted a perennial lacustrine system until the 1980's. Water scarcity for the irrigation of croplands in this semiarid region has intensified groundwater extraction and the reduction of the regional aquifer by 2.5 m/year, triggering a gradual desiccation of Lake Parangueo (Aranda-Gómez et al., 2013; Rocha-Treviño, 2015). The original lake level was 9 m deep; however, in 1995 the level had lowered to 7.5 m deep and nowadays the lake is almost dry with remnant ponds less than 0.5 m deep (Alcocer, 2007; Aranda-Gómez et al., 2017). The progressive desiccation of the RP crater lake has been discussed previously (Alcocer, 2007; Alcocer et al., 2000; Aranda-Gómez et al., 2013; Escolero & Alcocer, 2004). Currently, RP functions as a playa-lake, increasing or decreasing in size over the year, with at least one remaining pond located in the crater during the drought season, and sometimes three ponds during the rainy season. However, it is noted that these remnant ponds probably also are fed by an aquifer perched inside the maar and/or by the hydraulic connection with the regional aquifer, suggesting a special relationship between hydrological and volcano-tectonic conditions (Aranda-Gómez et al., 2013; Cerca et al., 2015). Because of desiccation, the lake sediments in the central part have suffered land subsidence accompanied by soil fracturing and the formation of fault scarps (Aranda-Gómez & Carrasco-Núñez, 2014). The most remarkable topographic structure at the bottom of RP maar is a high fault scarp parallel to the former lake coast with a height of 15 m (Aranda-Gómez et al., 2013, 2017; Rocha-Treviño, 2015).

2 | METHODS

2.1 | Physicochemical characteristics of the lithological record, carbonate morphology, and evolution of physicochemical parameters inside RP

Microbialite morphology mapping and field recognition of carbonate deposits built on previous microbialite description (Chacón et al., 2018), geological mapping (Aranda-Gómez et al., 2017), and aerial images taken with adequate resolution (Carrera-Hernández et al., 2016; Figure 2). Also, we review the physical and morphological characteristics of the lacustrine sedimentary sequence in the central part of the RP crater after desiccation of the perennial lake. The stratigraphy of lacustrine sediments in the central part of the crater was described previously by Kienel et al. (2009), Domínguez-Vázquez et al. (2019) and Sánchez-Sánchez et al. (2021). These data were corroborated with a new stratigraphic log obtained by coring down to 4 m depth in the sediments at the site described by these works (asterisk in Figure 2). For this new profile, *in situ* measurement of pH, conductivity, temperature and oxidation–reduction potential was made with two testers Combo® HI98130 and HI98121 in saturated paste every 20 cm, and a detailed description of the layers

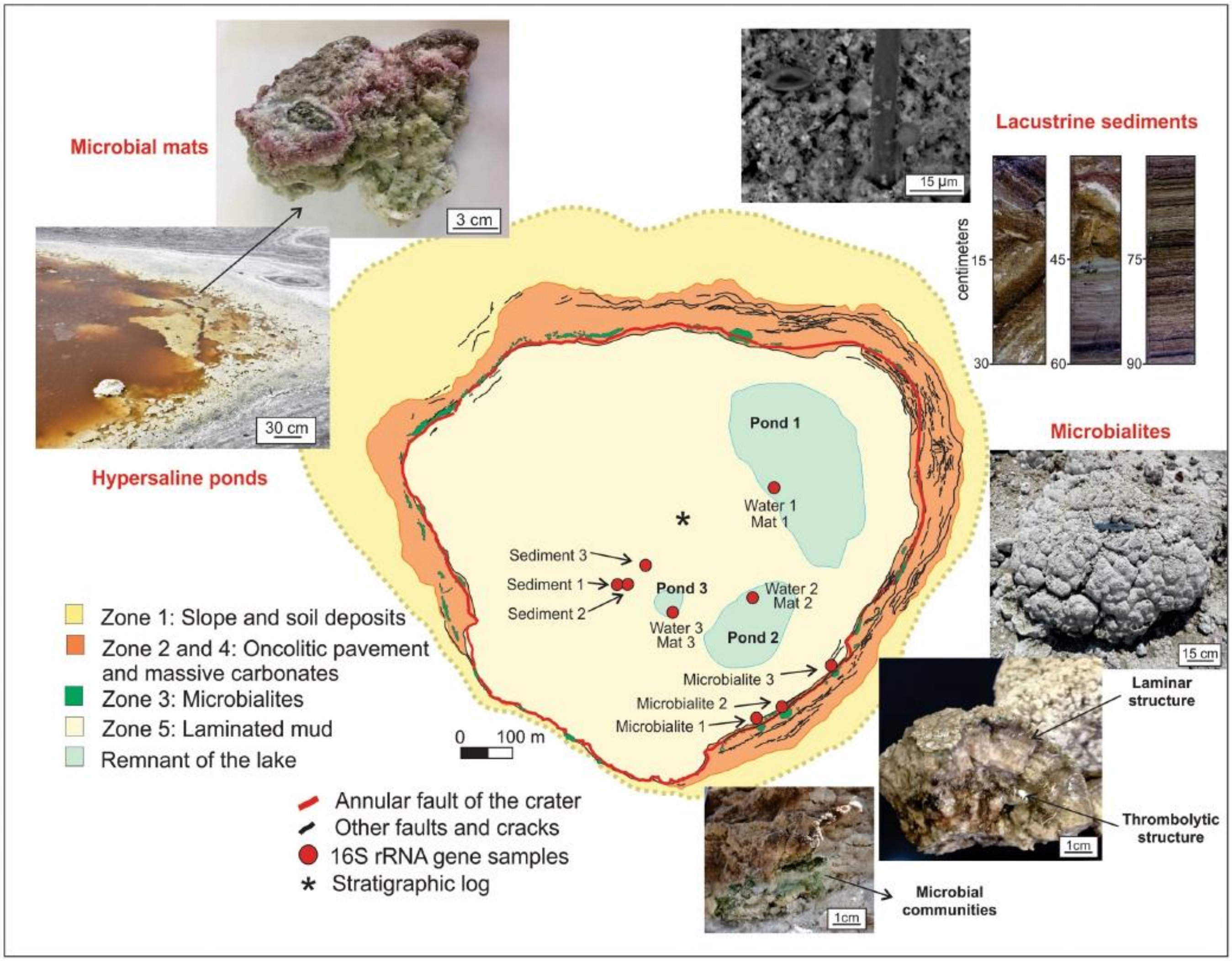

FIGURE 2 Environmental map and micro-ecosystem representation of the RP maar. The colours represent five zones with concentric distribution of carbonate sediments, remnant alkaline ponds, annular fault, and other faults and cracks. Within the laminate mud zone (5) and the annular fault, the red dots represent the sampling points for Sediment, Water, Mat and Microbialite samples. Photographs of the micro-ecosystems to enlarge their characteristics (red fonts) with microbialite internal structure shown by black arrows. The location of the new stratigraphic log obtained by coring down to 4 m depth in the sediments is represented by an asterisk.

was made on photographs. A compilation of data (pH, temperature and conductivity) obtained from previous works through the last 40 years was integrated with new data obtained in the dry season of the year 2022 (this work) to obtain a picture of the evolution of these parameters inside the RP lacustrine environment.

2.2 | Analysis of sequencing data

For this work, we re-analyse a total of 662,035 reads from the 16S rDNA V1–V3 hypervariable region for Bacteria and the V4–V5 hypervariable region for Archaea from 12 samples reported previously by Sánchez-Sánchez et al. (2019, 2021). Three samples correspond to microbialites collected from the main topographic scarp in the margin of the crater (Microbialite 1, 2, 3); three from the remnant water ponds (Water 1, 2, 3); three from microbial mats floating on the ponds surface (Mat 1, 2, 3); and three from the central lacustrine sediments at 4 m depth (Sediment 1, 2, 3). Metagenomic DNA was extracted with the sample preparation protocol reported and the Power Soil extraction kit DNA Isolation (MoBio Laboratories, Inc.). Approximately 30 g of material/biomass were used for Microbialite, Mat, and Sediment samples; 500 ml for Water 2 and 3; and 3500 ml for Water 1. DNA was amplified using the primers 28F ”GAGTTTGATCNTGGCTCAG“ and 519R ”GTNTTACNGCGGCKGCTG” for Bacteria, and Arch517F ”GCYTAAAGSRNCCGTAGC” and 909R ”TTTCAGYCTTGCGRCCGTAC” for Archaea. Amplicons were paired-end sequenced within a MiSeq Illumina platform (Illumina, Inc.). The amplicon sequencing reads can be accessed through the NCBI database (http://www.ncbi.nlm.nih.gov/biosample) under the BioProject PRJNA510628 (see Table S1).

For our analysis, we integrate the amplicon sequencing reads from the 12 samples of previous works and analyse them with an

ASV-based pipeline by Divisive Amplicon Denoising Algorithm 2 (DADA2) version 1.14.1 (Callahan et al., 2016). Studies that rely on ASVs use statistical models intended to remove the errors associated with sequencing, returning individual and unique sequences that represent individual taxa (Kerrigan & D'Hondt, 2022; Prodan et al., 2020). Therefore, ASV approaches can provide a significant advantage since a single base difference in the sequence will result in a unique ASV, and a more precise identification of the diversity of a given sample (Jeske & Gallert, 2022).

Analysis of the sequencing data using the ASV-based pipeline included the following steps: adapters and primers were removed using Trimmomatic version0.39 (Bolger et al., 2014); and filtering and trimming by quality profiles, error estimation, dereplication process, merging of paired reads, chimerical sequences removal and selection of amplicon sequence variants (ASVs) were carried out with DADA2 version 1.14.1 (Callahan et al., 2016). Taxonomy assignment was generated based on the SILVA SSU database version138 (Quast et al., 2012), updated on 15 August 2020. Taxonomy, ASV table and sample metadata from Bacteria and Archaea sequences were grouped and converted into a phyloseq object (McMurdie & Holmes, 2013) for further analysis. Bar plots and diversity indexes were made with *phyloseq* and *ggplot2* version 3.3.2 packages (Wickham, 2016) in an R version 3.6.3 environment (R Core Team, 2013). The correlation matrix using Bray–Curtis distances considering ASV presence/absence and frequency was built to apply a non-metric multidimensional scaling (NMDS) ordination analysis with the *vegan* version 2.5-7 package in the R environment (Oksanen et al., 2021). To statistically compare the microbial community composition among groups an ADONIS analysis was performed based on NMDS ordination and metadata matrix, followed by the betadisper analysis in *vegan* version 2.5-7 (Oksanen et al., 2021). Ecological network analysis (ENA) was built based on phyloseq object and Bray–Curtis dissimilarity, plots for each sample were done with the *igraph* package (Csardi & Nepusz, 2006) and *Cytoscape* 3.9.1 (Otasek et al., 2019) to integrate the model and visualise the plot, both in the R version 3.6.3 environment. Metabolic inferred functions of microbial communities were predicted after prokaryotic groups were obtained, by modelling genes from the 16S rDNA survey derived from the generated ASVs and its reference genome database, using the local mapper of iVikodak (Nagpal et al., 2019). Bar plots were made based on the iVikodak gene infer annotation against the KEGG database.

3 | RESULTS

3.1 | Lacustrine sedimentary system and physicochemical properties

The near-surface lacustrine stratigraphy of RP is dominated by carbonated deposits with a sedimentary fabric that reflects the influence of benthic microbial communities through time—thus, microbial metabolisms drive microbialite formation through authigenic cementation (Figure 3).

At the shallow margins of the palaeolake, a massive carbonate deposit with low detrital contents and abundant embedded oncolites forms a platform geomorphology. Oncolites, generally spherical, consist of clastic or wood nuclei and are arranged as a cobblestone pavement. Oncolytic pavement extended along the border of the crater, showing some erosion. Each oncolite was approximately 3–8 cm in diameter and had a stromatolitic crust of approximately 1 cm and an internal thrombolytic structure (Figure 3d). Other microbialites associated with logs and branch fragments exhibited a mostly elongated form, their size depending on the original length of the plant fragments falling into the lake (Figure 3e,f).

Clusters of biohermal microbialites, either with a defined domical or irregular shape and an average size of approximately 1 m in width and 1.8 m in length, were observed near the slope change of the carbonate platform (Figure 3a,b). They formed a discontinuous ring around the crater. Internally, both bioherms and oncolites had a thrombolytic mesostructure covered by a thin layer of stromatolitic mesostructure (Figure 3c). Hydromagnesite [$Mg_5(CO_3)_4(OH)_2(H_2O)_4$] predominates in the thrombolytic matrix, whilst aragonite [$CaCO_3$] is the predominant mineral phase within the stromatolitic crusts (Chacón et al., 2018; Sánchez-Sánchez et al., 2019). The slope stratigraphy was represented by low-density fine-grained massive carbonate (biostromes) over 3 m in thickness.

The pelagic central zone of the lake was filled with lacustrine clayey carbonaceous sediments which were also biologically induced/produced (Kienel et al., 2009; Sánchez-Sánchez et al., 2021). We collected a new 4-m-depth core at the centre of the crater (asterisk, Figure 2) in 2022, coinciding in location with cores recovered in previous studies (Domínguez-Vázquez et al., 2019; Kienel et al., 2009; Sánchez-Sánchez et al., 2021). The core comprised a sequence of laminated (layering thickness c. 1–10 mm) and unlaminated sediments, mostly of fine silt and clay grain size with varied coloration. The visual lithological analysis of the sedimentation showed some variations through depth and groups of layers with similar characteristics could be differentiated (Figures 4 and 5). The upper part of the core consisted of beige layers mostly of carbonaceous micrite with a thickness of approximately 45 cm with sparse carbonate nodules. Layers of olive or reddish organic-rich muds with variable thickness occurred in the sequence. Highly altered reddish and thin layers observed in the upper part of the sequence might correspond to the tephra layers reported previously by Kienel et al. (2009). Below 45 cm, the sediment sequence alternated between coarsely laminated and massive dark sediments with high organic matter content and few carbonate nodules (Figure 4).

The physicochemical properties measured *in situ* for the sequence of lacustrine sediments are shown in Figure 5. The maximum temperature measured in the core was 26.8°C (320 cm) and the lowest was 23.1°C (140 cm). Constant temperatures were recorded between 0 and 80 cm (~25°C); however, between 100 and 200 cm there was a moderate decrease, and between 220 and 400 cm an increase with depth. Two high-temperature peaks were recorded at

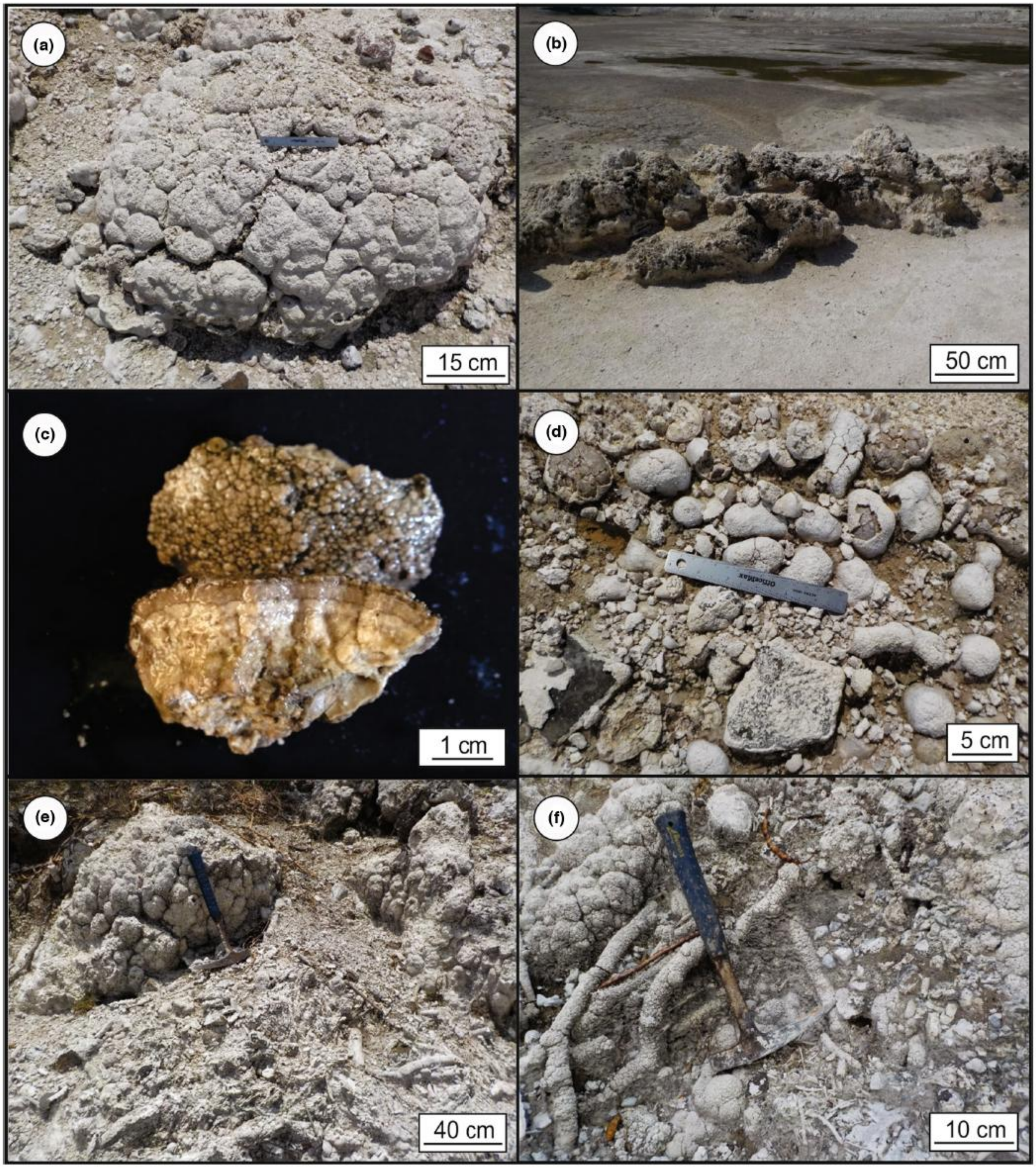

FIGURE 3 Microbialite facies at RP maar: (a, b) domical and irregular bioherms on the main topographic scarp; (c) stromatolitic-thrombolytic mesostructure at bioherms; (d) oncolytic pavement extended along the border of the crater; and (e, f) microbialites associated with logs and branch fragments.

depths of 180 (26.5°C) and 340 (26.8°C) cm. The maximum pH value was 11.36 at 220 cm and the minimum 10.11 at 180 cm, whereas lower pH values were recorded at 200 (10.11) and 220 (10.25) cm. Conductivity ranged between 15,100 and 22,260 μS/cm. The redox potential increased with depth from −254 to −210 mV in the interval between 0 and 220 cm, and decreased from −280 to −368 mV in the depth interval between 220 and 400 cm.

Measurements of pH, salinity, conductivity and temperature have been documented over time in RP in different types of samples, mainly sediment, water and microbialites by several

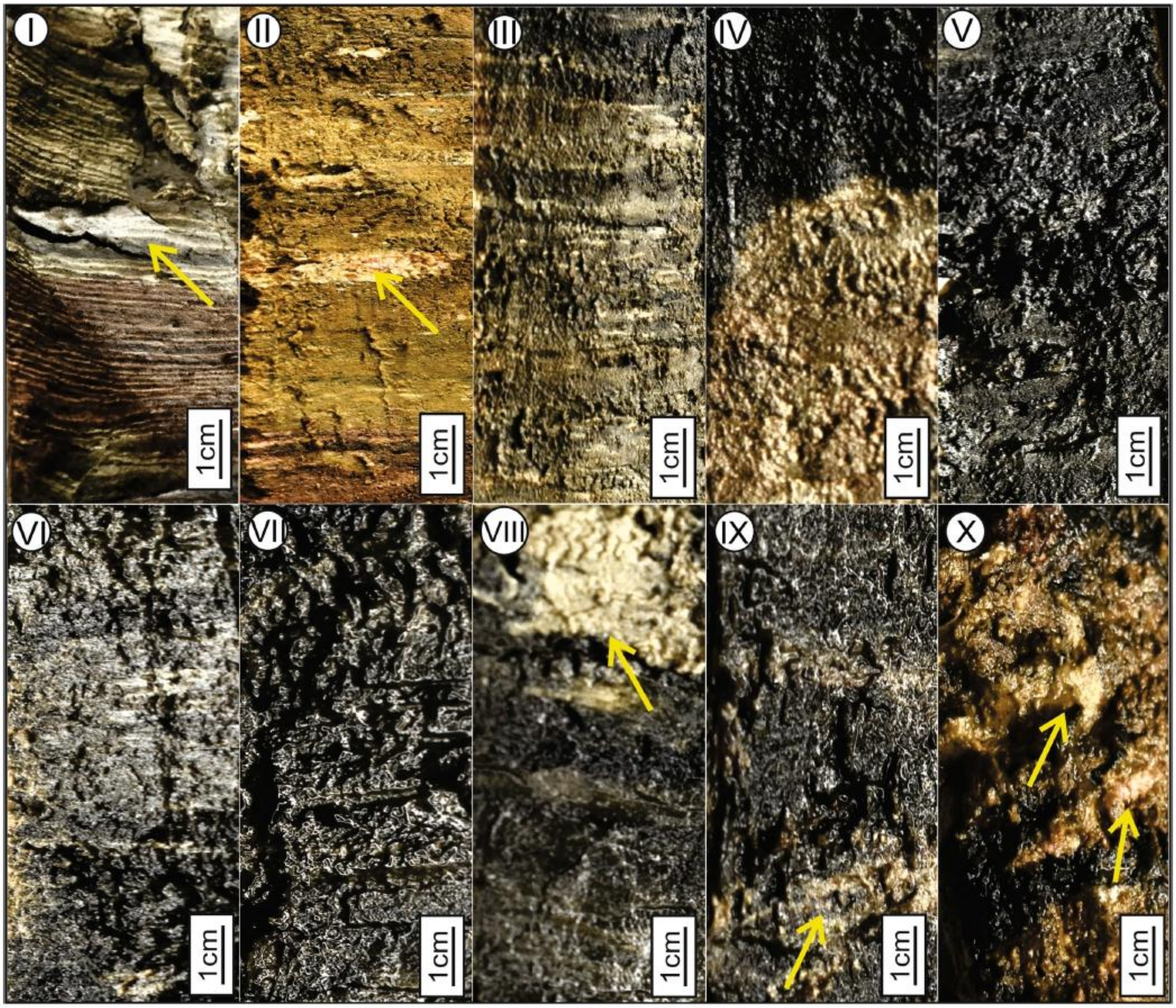

FIGURE 4 Visual analysis of the lithology of the sedimentation revealing variations through depth (depth of each picture indicated in Figure 5). The upper part of the core consists of a beige randomly arranged of laminated sediments of c. 70 cm with carbonate nodules present (I–IV). Massive organic mud (IV–VI). Layers of olive or reddish organic-rich muds occur, with different thicknesses (VI–IX). Carbonate nodules were abundant in the lower part of the sequence (X). Most of the sediment sequences are alternately coarse laminated and massive dark sediments with high organic matter content and few carbonate nodules (yellow arrows).

authors (Armienta et al., 2008; Cortés López, 2016; Escolero & Alcocer, 2004; Green, 1986; Ibarra-Sánchez et al., 2020; Rivera Martínez, 2015; Sánchez-Sánchez et al., 2019, 2021; Valerdi Negreros, 2015). A summary of the available data and the new values measured in 2022 is presented in Table 1, where differences between years and seasons were recognised. The parameter with the greatest variation was conductivity. In 1982 the water lake conductivity was 25,000 μS/cm (Green, 1986). Since then, there has been a gradual increase: by 1995 the conductivity of the water reached 70,000–80,000 μS/cm and by 1999, 165,000 μS/cm (Armienta et al., 2008). Notably, the conductivity values measured in this work for the sediments are similar to those measured by Green (1986). The pH value tended to increase through the years: the minimum was 9.4 in 2016 and the maximum 11.3 in 2022, both values measured in sediments. Temperature had a maximum of 37.2°C for microbialite samples in 2013 and a minimum of 19°C for water in 1995, and for 2022 the highest temperature measured was 26.8°C in sediments.

3.2 | An overview of the samples: Bioinformatic pre-process and richness diversity

The application of the ASV-based bioinformatic method (DADA2) generated 364,038 reads from the 16S rDNA V1–V3 hypervariable region for Bacteria, and 297,997 reads from the V4–V5 hypervariable region for Archaea (total dataset 662,035 reads). The filtered dataset obtained through the ASV-based pipeline comprised a total

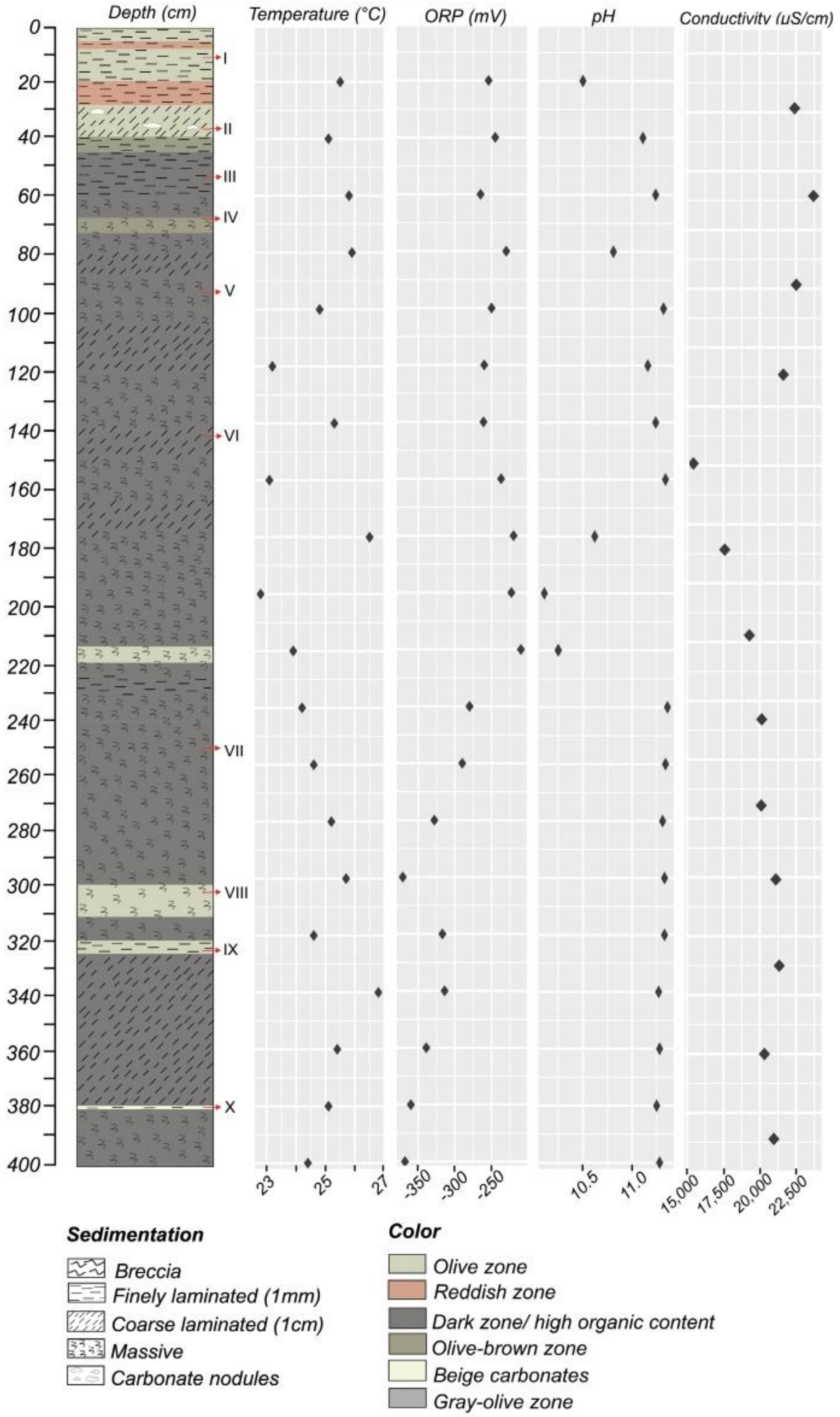

FIGURE 5 Stratigraphic column and physicochemical characteristics from a stratigraphic log obtained by coring down to 4m depth in the central part of the RP crater.

of 443,085 reads with 437 unique sequences or ASVs (Table 2), after data trimming and quality filtering with at least 28,000 sequences per sample. By contrast, the previous OTU-based pipeline resulted in a total of 346,784 filtered sequences with 254 operational taxonomic units at a 97% identity threshold that resulted in 22,000 sequences as a minimum per sample (Table 3). Sediment 1 lacked archaeal sequences (Table S3) and was not considered in the posterior analysis.

Figure 6 shows the ASV-based beta and alpha diversity metrics, including observed counts, Shannon–Wiener and Simpson indices for whole-community diversity (Bacteria and Archaea) per sample.

The highest diversity was observed in Mat, Microbialite and Water, in contrast to Sediment samples which showed the lowest diversity (Figure 6a). Hierarchical clustering and visualisation on a dendrogram (Bray–Curtis dissimilarity) showed the genetic distance relationships among sites, revealing greater genetic similarity

TABLE 1 Compilation of previous and new physicochemical data from Water, Sediment and Microbialite samples in RP.

						Time of the year		Type of sample			
Date	T (°C)	pH	EC (μS/cm)	Depth (m)	Volumetric water content (%)	Dry season	Rainy season	Water	Sediment	Microbialite	References
1982	30		25,000	>9				✓			Escolero and Alcocer (2004), Green (1986)
1995	19–23	9.8	70,000–80,000	7.5				✓			Alcocer (2017), Escolero and Alcocer (2004)
1999	18.1–23.6	10–10.2	165,000	5		✓		✓			Armienta et al. (2008)
2012	25	11.16	122,000				✓	✓			Valerdi Negreros (2015)
2013	37.2			<0.03	2.2	✓				✓	Cortés López (2016)
2013	21.9			<0.03	14.3		✓			✓	Cortés López (2016)
2013	22.3–24.4	10.05	33,300	0.03–0.05			✓		✓		Rivera Martínez (2015)
2014	33.4	10.50		0.03–0.05			✓		✓		Rivera Martínez (2015)
2015		10.2–10.3	29,800-74,400	0.2		✓			✓		Ibarra-Sánchez et al. (2020)
2016		9.4–9.7		<1	50.5–75.1		✓		✓		Sánchez-Sánchez et al. (2019)
2019		10.38–10.65	23,200-51,400	4	>200		✓		✓		Sánchez-Sánchez et al. (2021)
2022	24.8	11.1		<0.5		✓		✓			This study
2022	23.1–26.8	10.1–11.3		0–4		✓			✓		This study
2022	26.6	9.8		<0.02		✓				✓	This study

TABLE 2 Number of reads after filtered data, OTU or ASV derived from the two different sequencing data pipelines.

	Reads (Illumina MiSeq)	Reads after filtered data	OTU or ASV	Database
Previous analysis	662,035	346,784	254 OTU	RTL genomics database (NCBI database)
Current analysis	662,035	443,085	437 ASV	SILVA SSU database v.138

TABLE 3 Number of total sequences obtained from Microbialite, Mat, Sediment and Water samples derived from the two different sequencing data pipelines.

		ASV-based pipeline	OTU-based pipeline
Sample	Input	Filtered	Filtered
Microbialite 1	42,681	28,793	26,441
Microbialite 2	108,579	74,206	51,546
Microbialite 3	83,068	53,652	48,049
Mat 1	45,735	30,853	24,149
Mat 2	62,751	46,520	34,176
Mat 3	56,511	42,530	26,303
Sediment 1	32,732	19,322	–
Sediment 2	47,271	33,133	–
Sediment 3	33,820	22,711	–
Water 1	40,400	28,584	22,211
Water 2	40,912	29,673	22,499
Water 3	67,575	33,108	49,142
Total	662,035	443,085	

within samples in the same micro-ecosystem (Figure 6b). Only sample Microbialite 2 was grouped with Sediment samples. Nonmetric multidimensional scaling in two dimensions (NMDS) also showed a stronger clustering of micro-ecosystem samples over their taxonomic fingerprints (Figure 7b). The ADONIS analysis showed statistically significant differences among micro-ecosystems, evidenced by a p-value <0.001. Pairwise comparisons provided a more detailed insight into the differences between the groups. A statistically significant difference in the observed and permuted p-value was recognised in Mat–Water ($p \le 0.05$) and Mat–Sediment ($p < 0.1$).

3.3 | Microbiota biodiversity

The ASV-based analysis of the 16S rRNA gene dataset revealed the relative abundance of Bacteria and Archaea at different taxonomic levels (Figure 7a, phylum level). Most of the filtered sequences were classified within the archaeal domain (Table S3); phylotypes were classified at the phyla Nanoarchaeota (3.67%–87.24%), Thermoplasmatota (0.27%–70.26%), Crenarchaeota (0.67%–67.69%) and Halobacterota (0.23%–23.29%). Microbialite samples were dominated by sequences affiliated with Thermoplasmatota (7.23%–70.26%) and Crenarchaeota (1.55%–67.69%). For Water and Mat samples, the most abundant phylum was Nanoarchaeota (19.88%–87.24% and 76.14%–80.84%, respectively). Sediment samples 2 and 3 were dominated by Crenarchaeota (39.91%–55.38%), yet Sediment Sample 1 yielded only bacterial sequences and was removed from the analysis.

The rest of the sequences were annotated as bacterial phylotypes that included Cyanobacteria (2.00%–26.98%) and Proteobacteria (3.58%–21.04%) across sites, except Cyanobacteria for sample Water 3, which was not present. Cyanobacteria dominated in Microbialite (10.91%–26.98%) and Sediment samples (5.25%–14.06%), with the anomalous sample Sediment 1 (63.74%), whereas Proteobacteria was more abundant in Mat (9.44%–14.29%) and Water samples (3.58%–9.93%). Other less representative Bacteria phyla within samples included Firmicutes (0.02%–12.57%), Bacteroidota (0.22%–9.22%), Patescibacteria (0.47%–1.03%) and Chloroflexi (0.02%–0.06%) (Figure 7a).

As expected by using an updated database, most of the previously reported unclassified sequences (Sánchez-Sánchez et al., 2019) were classified. New archaeal and bacterial phyla were recognised including Asgardarchaeota, Euryarchaeota, Thermoplasmatota, Hadarchaeota, Halobacterota, Crenarchaeota, Nanoarchaeota and Patescibacteria.

A greater diversity of archaeal and bacterial phylotypes at lower taxonomic levels were detected compared with the previous analysis. In the case of Archaea, only two classes (i.e., Methanomicrobia and Halobacteria) had been reported before. In this new approach, the new identified classes of Archaea included Nitrososphaeria, Nanoarchaeia, Thermoplasmata, Methanobacteria, Methanosarcinia, Hadarchaeia, ANME-1 and Thermococci. In the case of Bacteria, newly recognised classes included Phycisphaerae, Parcubacteria and Gracilibacteria.

At a finer phylogenetic scale, the largest bacterial diversity was particularly manifested in the cyanobacterial and proteobacterial

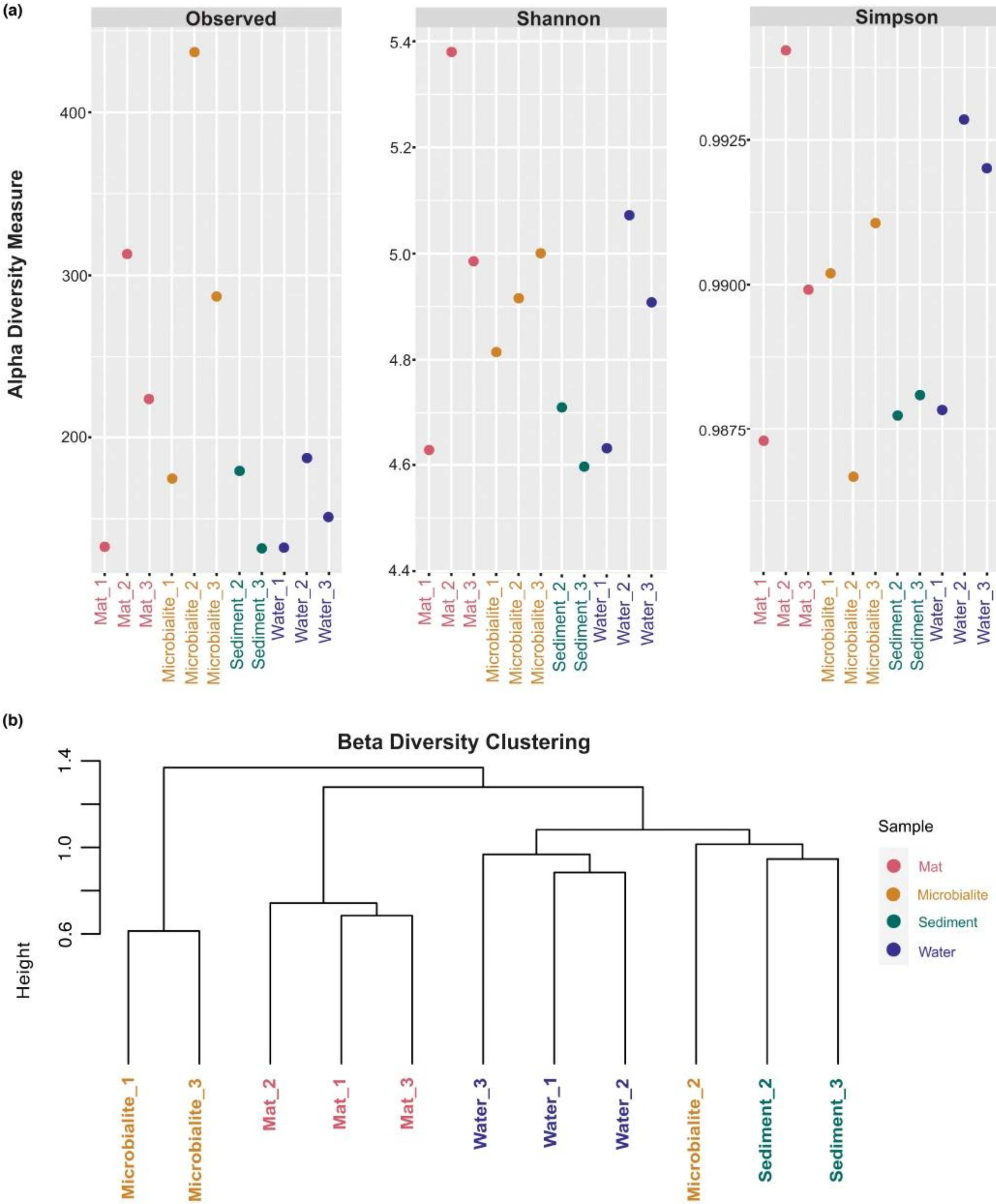

FIGURE 6 (a) Dispersion plots of observed richness, Shannon–Weiner and Simpson diversity index values from the micro-biodiversity thriving in micro-ecosystems inside RP. (b) Beta diversity clustering between communities from RP, using Bray–Curtis dissimilarity.

phylotypes (Table S4). Cyanobacteriales sequences were affiliated to the genera *Nodularia, Nostoc, Geitlerinema, Synechocystis, Symphothece, Gloeocapsa, Pleurocapsa, Chroococcopsis* and *Anabaena*. Rhodobacterales and Rhizobiales were the most diverse proteobacterial groups particularly in Microbialite samples. Rhodobacterales diversity was represented by the

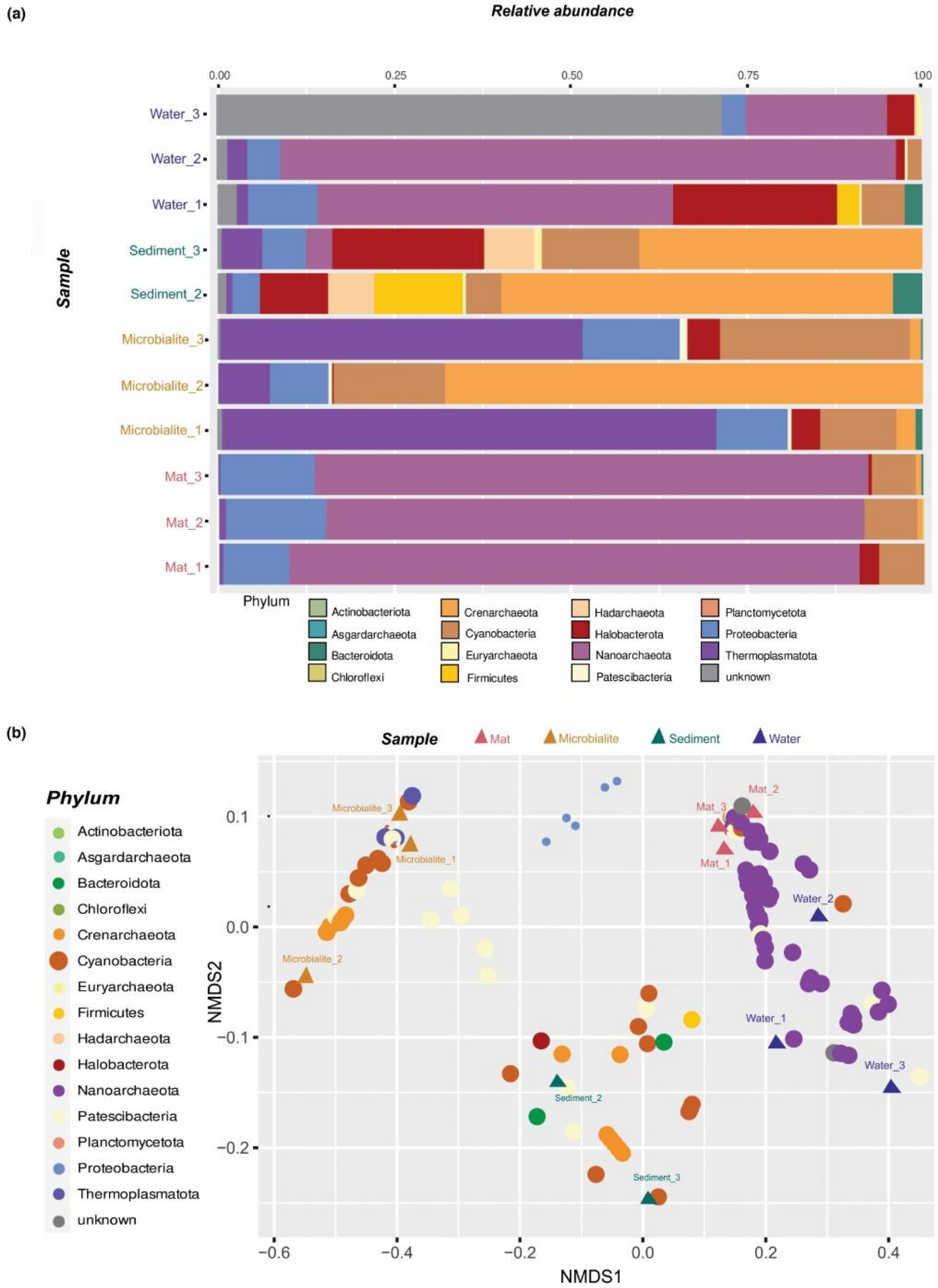
(a)
Relative abundance
0.00
0.25
0.50
0.75
1.00
Sample
Water_3
Water_2
Water_1
Sediment_3
Sediment_2
Microbialite_3
Microbialite_2
Microbialite_1
Mat_3
Mat_2
Mat_1
Phylum
Actinobacteriota
Asgardarchaeota
Bacteroidota
Chloroflexi
Crenarchaeota
Cyanobacteria
Euryarchaeota
Firmicutes
Hadarchaeota
Halobacterota
Nanoarchaeota
Patescibacteria
Planctomycetota
Proteobacteria
Thermoplasmatota
unknown
(b)
Sample
Mat
Microbialite
Sediment
Water
Phylum
Actinobacteriota
Asgardarchaeota
Bacteroidota
Chloroflexi
Crenarchaeota
Cyanobacteria
Euryarchaeota
Firmicutes
Hadarchaeota
Halobacterota
Nanoarchaeota
Patescibacteria
Planctomycetota
Proteobacteria
Thermoplasmatota
unknown
Microbialite_3
Microbialite_1
Microbialite_2
Mat_2
Mat_3
Mat_1
Water_2
Water_1
Water_3
Sediment_2
Sediment_3
NMDS2
0.1
0.0
−0.1
−0.2
NMDS1
−0.6
−0.4
−0.2
0.0
0.2
0.4

FIGURE 7 (a) Relative abundances of the obtained phylotypes of Bacteria and Archaea. Bar chart shows a high abundance of Nanoarchaeota in two types of samples (Water and Mat). Microbialite is represented by a high percentage of Crenarchaeota and Thermoplasmatota, unlike the Sediment sample, which is defined mostly by Crenarchaeota and Cyanobacteria. (b) NMDS biplot showing the specific taxa distribution on the samples.

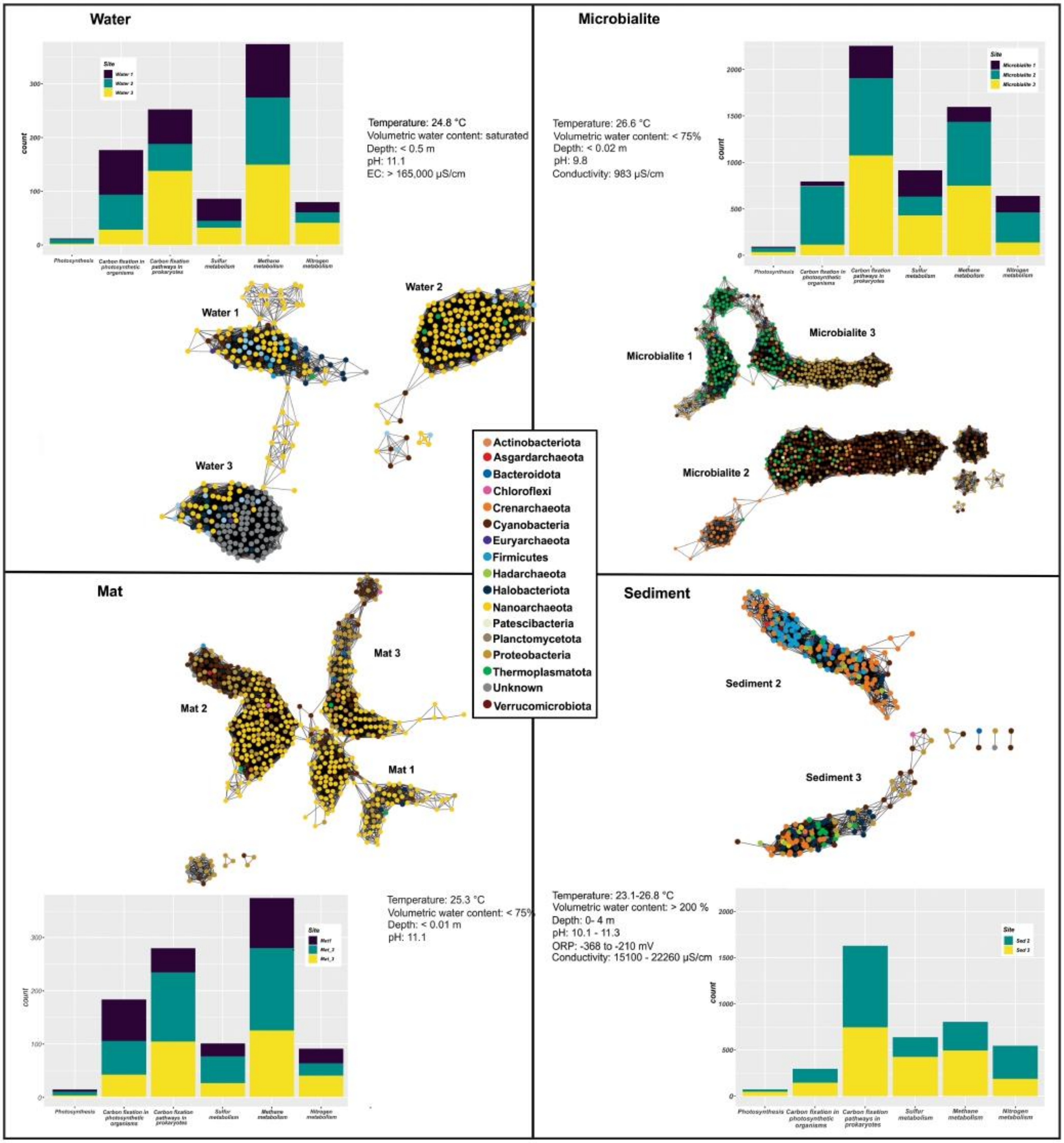

FIGURE 8 Reconstructed ecological network analysis based on 16S rRNA gene sequencing data and taxon-based metabolic profiling of prokaryotic communities across micro-environments inside RP. Different colour nodes represent ASVs affiliated with different phyla. The links between nodes that are more related to one another are shown by lines.

genera *Roseinatronobacter, Rhodobaca, Rhodobaculum, Gemmobacter, Roseibaca, Natronohydrobacter, Plastorhodobacter, Seohaeicola, Falsirhodobacter, Gemmobacter, Yoonia-Loktanella, Paenirhodobacter, Tabrizicola* and *Haematobacter*. Rhizobiales was characterised by the genera *Chelativorans, Tepidamorphus, Salinarimonas, Pelagibacterium, Hoeflea, Aliihoeflea, Pseudaminobacter, Oricola, Aquamicrobium, Mesorhizobium* and *Pseudohoeflea*.

The largest archaeal diversity was recognised in the Halobacterales order in all samples, but notably in Sediment samples (Table S4). The genera *Halohasta, Natronorubrum, Halalkalicoccus, Saliphagus, Halobiforma, Natronococcus, Halopiger, Natronomonas* and *Natronolimnobius* were identified.

The resulting undirected random graphs of the ecological network analysis (ENA) for each micro-ecosystem are presented in Figure 8. These results indicate the probability of linear connection between pairs of phyla (filled circles) based on the phyloseq object. A shorter distance suggests a stronger connection. Expectedly, shorter paths between phyla of each sample represent how strongly the sequences obtained belong to their communities. However, small groups of phyla exhibited no connection with the communities. By contrast, weak connections among samples suggest taxonomic compartmentalisation likely to have been caused by the interaction with the external environment.

3.4 | Metabolic potential characterisation of microbial communities

The implementation of the iVikodak algorithm resulted in the prediction of potential metabolic profiles based on the dominant microbial taxa (Figure 8).

Carbon fixation metabolism in prokaryotes was found particularly in Sediment and Microbialite samples. This metabolism was represented by methanogenic microorganisms, as well as oxygenic and anoxygenic phototrophs. Two carbon fixation pathways were suggested by the presence of specific microorganisms: (a) the reductive pentose phosphate pathway (Calvin cycle) represented by photosynthetic organisms (i.e., Cyanobacteria: *Synechococcus, Synechocystis, Anabaena* and *Nostoc*; Proteobacteria: *Mesorhizobium*) and (b) the reductive acetyl-CoA pathway (Wood–Ljungdahl pathway) represented by methane-forming microorganisms such as *Methanobacterium, Methanocalculus* and *Methanosalsum*, and anoxygenic Proteobacteria (Acetobacteraceae).

The iVikodak results suggested methane metabolism as the most abundant pathway in Water and Mat samples and the second most abundant in Sediment and Microbialite samples (Figure 8). Methane metabolism in Water and Mat samples could be abundant owing to the anoxic conditions, which can be attributed to restricted water exchange, rapid O_2 consumption and volcanic conditions (gas input to the surface from a shallow underground source). In Sediment samples, the anaerobic oxidation of methane (AOM) was represented by archaeal anaerobic methane oxidisers (ANMEs) from the phylogenetic group ANME-1 subgroup ANME-1b. Methanogenic Archaea from the genera *Candidatus_Methanofastidiosum, Methanobacterium, Methanocalculus, Methanosalsum* and the family Methanomassiliicoccaceae were recognised in all samples, except in Mat samples.

Sulfur metabolism was represented by sulfur-oxidising bacteria in the majority of Mat and Microbialite samples. The genera *Rhodobaca* and *Roseinatronobacter*, obligately heterotrophic alkaliphiles that oxidise sulfur compounds to sulfate, were recognised. The potential sulfide-oxidising capacity of the Thermoplasmatota family also was found mainly in Microbialite samples.

Nitrogen fixation was represented by Cyanobacteria, including the heterocyst-forming genera Nodularia, *Nostoc* and *Anabaena*, as well as the non-heterocystous *Geitlerinema*. The ammonium-oxidising archaea *Candidatus_Nitrososphaera* and *Candidatus_Nitrocosmicus* also were detected.

Photosynthesis represented by oxygenic and anoxygenic phototrophs was the least abundant pathway in the samples. A variety of bacterial lineages that potentially perform oxygenic and anoxygenic photosynthesis (e.g., *Nodosilinea, Nodularia, Nostoc, Geitlerinema, Rhodobaca, Rhodobacter, Rhodobaculum, Hoeflea*) were identified in all samples.

4 | DISCUSSION

4.1 | General microbial diversity across samples

DNA metabarcoding has become an indispensable tool to evaluate the microbial diversity of various environments. Advances in several possible analyses of amplicon sequence datasets have resulted in the challenge of selecting the best-suited method (e.g., Joos et al., 2020). In this work, we selected to update the biological interpretation of metabarcoding data using error model-based methods (ASVs) (e.g., Joos et al., 2020) and the most up-to-date 16S rRNA gene database. The bioinformatic processing pipeline and analysis tools improved the results significantly. The choice of 16S rRNA gene database was significant as new phylotypes have been identified since the SILVA database was reported as one of the most accurate 16S rRNA gene databases (Abellan-Schneyder et al., 2021).

Hierarchical clustering dendrogram and NMDS based on Bray–Curtis beta diversity demonstrated that samples fall into the four defined categories of micro-ecosystems. This classification allows comparisons between micro-ecosystems concerning bacterial and archaeal biodiversity, proposing a local community structuring. The whole community (Archaea and Bacteria) alpha diversity metrics, indicated that Mat, Water and Microbialite samples displayed higher diversity than that of Sediment (Figure 6a).

In contrast with alpha diversity metrics reported by Ibarra-Sánchez et al. (2020) in superficial RP sediment samples, deep sediments revealed lower diversity metrics (Table 4). This downward tendency in alpha diversity values is consistent with the results since the relative abundance of 16S rRNA genes generally decreases with the depth and burial time (Hoshino & Inagaki, 2019).

Microbialite alpha diversity values for RP were lower ($H' = 4.909$) than those reported in previous analyses from microbialites distributed throughout Mexico, including desert ponds, coastal lagoons and Alchichica crater-lake (Centeno et al., 2012). Discrepancies in alpha diversity values are probably related to the collection site, microbialite stage and microbialite age, among others. The low diversity in RP microbialites could be related to an adaptive process of microbiota to the lake desiccation and an increase in alkalinity and salinity levels through the last years.

The RP microecosystems offer favourable conditions for extremophile microorganisms associated with the relatively high abundance of Archaea phylotypes. Specially, Nanoarchaeota abundant in Water (Figures 7a and 8), are symbiont/parasites with a reduced genome broadly distributed in global geothermal systems (St. John et al., 2019), hot springs (Munson-McGee et al., 2015) and mesophilic hypersaline environments (Casanueva et al., 2008). Lower Nanoarchaeota abundance and high numbers of unknown phylotypes were found in sample Water 3. The low diversity of this sample might be a consequence of a low water level in the pond and higher salinity (see Table S1), probably favouring rare or endemic taxa.

TABLE 4 Observed richness, Shannon–Wiener and Simpson diversity index values from the micro-biodiversity thriving in micro-ecosystems inside RP.

	Observed	Shannon	Simpson
Bacteria			
Microbialite 1	64	3.499	0.959
Microbialite 2	337	5.349	0.993
Microbialite 3	205	4.441	0.980
Mat 1	22	2.690	0.908
Mat 2	136	4.391	0.983
Mat 3	95	4.123	0.979
Sediment 1	—	—	—
Sediment 2	84	4.066	0.976
Sediment 3	40	3.100	0.942
Water 1	33	3.336	0.960
Water 2	17	2.244	0.865
Water 3	11	2.211	0.878
Archaea			
Microbialite 1	111	4.502	0.987
Microbialite 2	100	4.027	0.977
Microbialite 3	81	4.224	0.983
Mat 1	111	4.472	0.985
Mat 2	177	4.980	0.991
Mat 3	129	4.580	0.985
Sediment 1	—	—	—
Sediment 2	93	4.142	0.980
Sediment 3	92	4.346	0.984
Water 1	98	4.322	0.983
Water 2	170	5.013	0.992
Water 3	54	3.914	0.978

Crenarchaeota was the most abundant phylum found in sediment samples (Figures 7a and 8). Members of this phylum are commonly reported as the most abundant Archaea in various environments including marine (Kubo et al., 2012) and salt marsh sediments (Seyler et al., 2014). Other identified archaeal phyla such as Asgardarchaeota have been frequently associated with low-oxygen aquatic sedimentary saline environments (Banciu et al., 2022), supporting their high abundance in our sediment samples.

Additional archaeal phylotypes identified within RP samples such as Thermoplasmata, an uncultivated archaea group found in great abundance in Microbialite samples (Figures 7a and 8), has been reported in microbial mats associated with microbialite formation (Schneider et al., 2013) and hydrothermal springs in the Yellowstone National Park (Schouten et al., 2007). New methanogenic Archaea, not previously observed in the dataset, were discovered in Sediment samples; among them were Methanobacteria, a hydrogenotrophic methanogen, isolated from marine sediments (Shlimon et al., 2004), anoxic sediments (Schirmack et al., 2014) and alkaline lakes (Worakit et al., 1986). In addition, we report *Methanosarcina*, the most metabolically diverse group of methanogenic Archaea, also identified in marine sediments with high methane content (Sowers et al., 1984).

The bacterial community dominated by Cyanobacteria, Proteobacteria and Firmicutes represents a general footprint, not only for lacustrine and marine microbialites (Bishop et al., 2020; Bosak et al., 2012), but also for many non-lithifying microbial mats (Iniesto et al., 2021; Johnson et al., 2018). Cyanobacteria were the most represented bacterial group, in agreement with previous observations in saline–alkaline lakes (Andreote et al., 2018). The other less representative Bacteria phyla (Bacteroidota, Patescibacteria and Chloroflexi) have been reported previously in microbialites (Couradeau et al., 2011), sediment samples of saline lakes (Vuillemin et al., 2018) and hypersaline microbial mats (Boidi et al., 2022; Vignale et al., 2021).

4.2 | Indirect metabolic potential based on taxonomic analysis

As an indirect method applied to predict metabolisms of microbial communities, the results need to be interpreted carefully. This means results describing functional pathways are inferred from taxonomy based on the relative abundance of ASVs and not on direct metabolic activity (Nagpal et al., 2019). Despite this limitation, the iVikodak approach has been demonstrated to accurately predict the functional metabolisms of microbial communities in microbialite-forming mats (Nguyen et al., 2022), and other microbial mats from geothermal springs (Coman et al., 2015). Our metabolic potential results were mostly consistent with those of a recent work (Iniesto et al., 2021) that recognised taxon-based functional metabolisms from microbialites in other active lakes of the TMVB. Furthermore, specific studies conducted in RP (Cortés López, 2016; Gómez-Acata et al., 2021; Hernández Zavala, 2016; Ibarra-Sánchez et al., 2020; Mundo Velázquez, 2020; Pérez-Bernal et al., 2017, 2020) strongly support our prediction of the metabolic functions for carbon fixation, photosynthesis and methanogenesis.

Carbon fixation pathways were detected in all analysed samples and were the most representative metabolism in Sediment and Microbialite samples, suggesting that the autotrophic incorporation of inorganic carbon plays a significant role in these micro-environments. In particular, the Calvin cycle and Wood–Ljungdahl pathway appear to be the dominant autotrophic process owing to the presence of photosynthetic (oxygenic and anoxygenic) and methanogenic microorganisms.

Metabolic prediction suggested a high potential for photosynthetic metabolism represented by Cyanobacteria and Proteobacteria phylotypes in all samples. Cyanobacteria have been proposed as an important microorganism in microbialite genesis by local supersaturation of the solution with carbonate phases by increasing pH and/or alkalinity resulting in carbonate precipitation. The production of exopolymeric substances (EPS) by Cyanobacteria can trap cations (e.g., Mg^{2+} or Ca^{2+}) and with the later degradation of the EPS, fraction cations are released increasing the cation concentration necessary for the formation of microbialites (Couradeau et al., 2011; Farias et al., 2014). Cyanobacteria (e.g., Pleurocapsales) induced the formation of aragonite in Alchichica microbialites (Gérard et al., 2013) and have been associated with the cauliflower crust exhibited by microbialites from Lake Dziani Dzaha (Gérard et al., 2018). Likewise in RP, Pleurocapsa might be an important contributor to the precipitation of aragonite and influence the structure of the deposits. Photosynthetic Alphaproteobacteria (Rhizobiales, Rhodobacterales, Sphingomonadales, Caulobacterales) identified in RP samples also have been related to microbialite formation (Iniesto et al., 2021; Saghaï et al., 2015).

Methanogenesis in saline–alkaline lakes occurs as one of the terminal processes during the microbial decomposition of organic matter under anaerobic conditions (Nolla-Ardèvol et al., 2012) and has been reported as an important part of the microbial carbon cycle in soda lakes (Sorokin et al., 2015). Several orders of methanogenic Archaea were recognised within RP microbial communities, giving way to the possibility of biogenic methane gas production as proposed by Paz-Jacome et al. (2020), especially in RP sediments. Furthermore, the anaerobic oxidation of methane (AOM) carried out by anaerobic methane-oxidising Archaea (ANME) could be an important step in the methane cycle inside RP because they were found mainly in Sediment samples. However, anaerobic methanotrophs were not identified in RP sediment, suggesting that the oxidation of methane is an aerobic process at this location.

Metabolic prediction suggested nitrogen and sulfur metabolisms. Ammonium oxidation, N_2 fixation and nitrite reduction have been reported previously as predominant metabolic functions on surface soils of RP by Ibarra-Sánchez et al. (2020). Nitrogen fixation by Cyanobacteria (Nostocales, Chroococcales and Oscillatoriales) also has been described in microbialites from Lake Alchichica (Falcón et al., 2002), and other microbialite and mat-forming consortia from different aquatic environments in Mexico (Alcántara-Hernández et al., 2017; Beltrán et al., 2012). In addition, Rhodobacteriales and Rhizobiales the microorganisms related to denitrification in this study have been reported in Alchichica microbialites (Alcántara-Hernández et al., 2017).

Potential sulfate-reducing bacteria were not identified in RP samples. Potential sulfate-reducing Deltaproteobacteria were reported at low abundances (<0.01%) in microbialites from Cayo Sabinal, Cuba (Valdespino-Castillo et al., 2018). However, they were not detected in the metagenomes of Lake Alchichica (Saghaï et al., 2016) and Shark Bay (Ruvindy et al., 2016). These results suggest that although sulfate reduction has been linked to the precipitation of carbonates in modern microbialites, low abundance or absence of sulfate-reducing bacteria seems to be a trend in modern microbialites in many cases. The absence of sulfate-reducing microorganisms in our dataset does not completely exclude their presence; it also might be related to endemicity since sulfate-reducing strains have been isolated recently from superficial sediments at the shoreline RP ponds (Pérez-Bernal et al., 2017, 2020).

4.3 | Correlation between phyla, metabolic functions and physicochemical properties

Our findings suggest the metabolic versatility of the evaluated extremophilic communities for developing strategies for survival and growth in the challenging environment of RP. The nature of the sample (Water, Mat, Microbialite or Sediment)––thus, the interplay between physicochemical parameters and geological conditions––are crucial factors shaping the microbial community composition and therefore metabolic functions.

The reconstructed ecological network analysis based on 16S rRNA gene sequencing data (ENA analysis) suggests a connection between the different micro-environments based on the presence of shared taxa (Figure 8). For instance, phylotypes such as Cyanobacteria, Proteobacteria and Halobacteriota, are commonly associated with saline–alkaline lakes (Gérard et al., 2013, 2018). Together, the predominant and the less abundant phyla contribute collectively to the metabolic potential. From the predominant phylotypes, only Nanoarcheota does not contribute to the metabolic functions because it is known to have a highly reduced genome lacking most biosynthetic capacity (Munson-McGee et al., 2015).

Methane metabolism and carbon fixation are further discussed as they are two critical metabolic functions identified in this inactive but still degassing volcanic crater. The flux gas output measured for methane is between 2.1 and 1577 g/m^2/day, whereas for carbon dioxide it is 10.6 ton/day, reported by Paz-Jacome et al. (2020). Notably, inside the soda ponds, it is common to see isolated gas bubbles suggesting that gases are continuously expelled to the surface. Methanogenic groups may take advantage of the geothermal carbon dioxide input, using it as the predominant substrate for methane production, underlining the abundant methane metabolism recognised within samples.

The carbon fixation metabolism is supported by the secondary consumption of the released magmatic carbon dioxide by carbonatogenic phylotypes. The Na^+–HCO_3^- rich water in the ponds may be produced/enhanced by a combination of the silicate hydrolysis of volcanic rocks and the high partial input of magmatic carbon dioxide (e.g., Aranda-Gómez et al., 2013; Paz-Jacome et al., 2020). These conditions could explain the elevated carbon fixation metabolism recognised in this environment. Faults and fractures distributed in the crater may act as preferential conducts for degassing. The dominant phyla in Microbialite samples are represented by Thermoplasmatota, a phylotype associated with hydrothermal springs (Schouten et al., 2007), emphasising their significance in the present-day degassing of the crater. Microbialite samples coincide with the trace of the main fault scarp, and preferential gas input is channelised through the annular fault inducing biogenic precipitation of carbonates by Cyanobacteria and Proteobacteria phylotypes.

5 | CONCLUSION

Analysis of the microbial communities of RP demonstrated that the 16S rRNA sequences define the four categories of micro-ecosystems. The compartmentalization in RP is likely to have been driven by changes in the physicochemical properties of the ecosystem caused by a drastic decrease in water level in the last 40 years. As a result, the area has been exposed to subaerial conditions transforming the lake into a soda desert soil with high salinity and alkalinity remnant ponds. Despite the extreme physicochemical characteristics, microbial communities thrive owing to a long-term history of biologically induced/produced carbonates. The microecosystems in RP offer favourable conditions for extremophile Archaea phylotypes such as Asgardarchaeota, Thermoplasmatota, Hadarchaeota, Halobacterota and Nanoarchaeota.

Assessment using an updated bioinformatic processing pipeline and 16S rRNA database resulted in improved results on community composition. The taxon-based prediction of the metabolic functions supports the potential of microbially mediated carbonate precipitation. In particular, the biogeochemical cycle involved in carbonate precipitation is a key factor in considering RP microbialites as active calcifying entities. Their diversity in textures, sizes and shapes is a consequence of the complexity of this geological scenery and the genetic potential of the microbial community involved in carbonatogenesis.

A correlation between phyla, metabolic functions and physicochemical properties was suggested for each micro-ecosystem. The metabolic functions are similar between micro-ecosystems and to those found in other microbialite-forming lakes along the TMVB, suggesting that the environment has conserved a long-term condition. Our analysis suggests that methanogenesis is an important metabolic process and could be directly related to sediment degassing within the crater.

As representatives of the oldest macroscopic evidence for life on Earth, microbialites constitute excellent models for understanding not only microbial evolution, but also elemental cycling in the biosphere. RP microbialites develop at the intersection of geological, physicochemical and anthropogenic factors. Their unique microbial composition and their vast metabolic potential represent an environmental resource that needs to be studied, recognised for their importance, and conserved as biodiversity hotspots. Further works in RP must consider developing urgent strategies to mitigate the human impacts and rational water use in the surrounding areas.

AUTHOR CONTRIBUTIONS

Conceptualization, data interpretation, writing, developing methods, sample collection: MC, JS-S, BBM; data analysis: JS-S, BBM, RJA-H, and IM-V; preparation of figures and tables: JS-S and IM-V; description of the sediments and geology: MC, JS-S, DC-F, and GL; Drafting of the first manuscript: MC, JS-S, BBM. All the authors reviewed and made criticism for the final manuscript.

ACKNOWLEDGEMENTS

We thank Sara Solís Valdez, Ricardo J. Carrizosa Elizondo, María Carolina Muñoz Torres and Vania Ferrer for help on the soil and water analysis, and Luis Aguilar, Alejandro de León, Carlos S. Flores and Jair Santiago Garcia Sotelo of the Laboratorio Nacional de Visualización Científica Avanzada (UNAM) for assistance in the bioinformatic analysis. We express our appreciation and gratitude to two anonymous reviewers for their thoughtful, insightful, and scholarly evaluation of this manuscript.

FUNDING INFORMATION

This work was supported by funds coming from extraordinary projects of the Laboratorio de Mecánica de Geosistemas (LAMG) and Laboratorio de Física de Rocas (LAFIR) both from UNAM. Sánchez-Sánchez (CVU: 707035) received a PhD scholarship and Dra. Bárbara B. Moguel (CVU: 365865) received a Postdoctoral fellowship from Consejo Nacional de Ciencia y Tecnología (CONACyT).

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NCBI database at http://www.ncbi.nlm.nih.gov/biosample, reference number BioProject PRJNA510628.

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REFERENCES

Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M., & Neuhaus, K. (2021). Primer, pipelines, parameters: Issues in 16S rRNA gene sequencing. *mSphere*, *6*(1), e01202-20. https://doi.org/10.1128/mSphere.01202-20

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

Aguirre-Garrido, J. F., Ramírez-Saad, H. C., Toro, N., & Martínez-Abarca, F. (2016). Bacterial diversity in the soda saline crater lake from Isabel Island, Mexico. *Microbial Ecology*, *71*(1), 68–77. https://doi.org/10.1007/s00248-015-0676-6

Albarracín, V. H., Gärtner, W., & Farias, M. E. (2016). Forged under the sun: Life and art of extremophiles from Andean lakes. *Photochemistry and Photobiology*, *92*(1), 14–28. https://doi.org/10.1111/php.12555

Alcántara-Hernández, R. J., Valdespino-Castillo, P. M., Centeno, C. M., Alcocer, J., Merino-Ibarra, M., & Falcón, L. I. (2017). Genetic diversity associated with N-cycle pathways in microbialites from Lake Alchichica, Mexico. *Aquatic Microbial Ecology*, *78*(2), 121–133. https://doi.org/10.3354/ame01806

Alcocer, J. (2007). El agua epicontinental de México. *Revista Ciencia*, *66*, 26–35.

Alcocer, J. (2017). Mexican meromictic lakes: What we know so far. In R. Gulati, E. Zadereev, & A. Degermendzhi (Eds.), *Ecology of meromictic lakes* (pp. 353–375). Springer. https://doi.org/10.1007/978-3-319-49143-1_12

Alcocer, J., Escobar, E., & Lugo, A. (2000). Water use (and abuse) and its effects on the crater-lakes of Valle de Santiago, Mexico. *Lakes & Reservoirs: Research & Management*, *5*(3), 145–149. https://doi.org/10.1046/j.1440-1770.2000.00113.x

Andreote, A. P., Dini-Andreote, F., Rigonato, J., Machineski, G. S., Souza, B. C., Barbiero, L., Rezende-Filho, A. T., & Fiore, M. F. (2018). Contrasting the genetic patterns of microbial communities in soda lakes with and without cyanobacterial bloom. *Frontiers in Microbiology*, *9*, 244. https://doi.org/10.3389/fmicb.2018.0024

Aranda-Gómez, J. J., & Carrasco-Núñez, G. (2014). *The Valle de Santiago maars, México: The record of magma-water fluctuations during the formation of a basaltic maar (La Alberca) and active post-desiccation subsidence at the bottom of a maar lake (Rincón de Parangueo)*. 5th International Maar Conference. Intra-conference Field trip 5th. https://theghub.org/resources/3706

Aranda-Gómez, J. J., Cerca, M., Rocha-Treviño, L., Carrera-Hernández, J. J., Levresse, G., Pacheco, J., Yutsis, V., Arzate-Flores, J. A., Chacón, E., & Beraldi-Campesi, H. (2017). Structural evidence of enhanced active subsidence at the bottom of a maar: Rincón de Parangueo, México. *Geological Society, London, Special Publications*, *446*(1), 225–254. https://doi.org/10.1144/SP446.1

Aranda-Gómez, J. J., Levresse, G., Martínez, J. P., Ramos-Leal, J. A., Carrasco-Núñez, G., Chacón-Baca, E., Naranjo, G. G., Cabello, G. C., González, M. V., Origel, G., & Medrano, C. N. (2013). Active sinking at the bottom of the Rincón de Parangueo Maar (Guanajuato, México) and its probable relation with subsidence faults at Salamanca and Celaya. *Boletín de la Sociedad Geológica Mexicana*, *65*(1), 169–188.

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*(2), 249–258. https://doi.org/10.1016/j.jvolgeores.2008.06.019

Banciu, H. L., Gridan, I. M., Zety, A. V., & Baricz, A. (2022). Asgard archaea in saline environments. *Extremophiles*, *26*(2), 1–12. https://doi.org/10.1007/s00792-022-01266-z

Beltrán, Y., Centeno, C. M., García-Oliva, F., Legendre, P., & Falcón, L. I. (2012). N_2 fixation rates and associated diversity (nif H) of microbialite and mat-forming consortia from different aquatic environments in Mexico. *Aquatic Microbial Ecology*, *67*(1), 15–24. https://doi.org/10.3354/ame01572

Bishop, S. L., Tymm, F. J., Perry, K., Kerkovius, J. K., Menard, F., Brady, A., Slater, G., Lim, D. S. S., Metcalf, J. S., Banack, S. A., Cox, P. A., & Murch, S. J. (2020). Early-earth nonprotein amino acid metabolites in modern cyanobacterial microbialites. *Environmental Chemistry Letters*, *18*(2), 467–473. https://doi.org/10.1007/s10311-019-00943-4

Boidi, F. J., Mlewski, E. C., Fernández, G. C., Flores, M. R., Gérard, E., Farías, M. E., & Gomez, F. J. (2022). Community vertical composition of the Laguna Negra hypersaline microbial mat, Puna Region (Argentinean Andes). *Biology*, *11*(6), 831. https://doi.org/10.3390/biology11060831

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bontognali, T. R., Vasconcelos, C., Warthmann, R. J., Dupraz, C., Bernasconi, S. M., & McKenzie, J. A. (2008). Microbes produce nanobacteria-like structures, avoiding cell entombment. *Geology*, *36*(8), 663–666. https://doi.org/10.1130/G24755A.1

Boros, E., & Kolpakova, M. (2018). A review of the defining chemical properties of soda lakes and pans: An assessment on a large geographic scale of Eurasian inland saline surface waters. *PLoS One*, *13*(8), e0202205. https://doi.org/10.1371/journal.pone.0202205

Bosak, T., Liang, B., Wu, T. D., Templer, S., Evans, A., Vali, H., Guerquin-Kern, J.-. L., Klepac-Ceraj, V., Sim, M. S., & Mui, J. (2012). Cyanobacterial diversity and activity in modern conical microbialites. *Geobiology*, *10*(5), 384–401. https://doi.org/10.1111/j.1472-4669.2012.00334.x

Bundeleva, I. A., Shirokova, L. S., Bénézeth, P., Pokrovsky, O. S., Kompantseva, E. I., & Balor, S. (2012). Calcium carbonate precipitation by anoxygenic phototrophic bacteria. *Chemical Geology*, *291*, 116–131. https://doi.org/10.1016/j.chemgeo.2011.10.003

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. https://doi.org/10.1038/nmeth.3869

Carrera-Hernández, J. J., Levresse, G., Lacan, P., & Aranda-Gómez, J. J. (2016). A low cost technique for development of ultra-high resolution topography: Application to a dry maar's bottom. *Revista Mexicana de Ciencias Geológicas*, *33*(1), 122–133.

Casanueva, A., Galada, N., Baker, G. C., Grant, W. D., Heaphy, S., Jones, B., Yanhe, M., Ventosa, A., Blamey, J., & Cowan, D. A. (2008). Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. *Extremophiles*, *12*(5), 651–656. https://doi.org/10.1007/s00792-008-0170-x

Cellamare, M., Duval, C., Drelin, Y., Djediat, C., Touibi, N., Agogué, H., Leboulanger, C., Ader, M., & Bernard, C. (2018). Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean). *FEMS Microbiology Ecology*, *94*(8), fiy108. https://doi.org/10.1093/femsec/fiy108

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*(3), 724–735. https://doi.org/10.1111/j.1574-6941.2012.01447.x

Cerca, M., Rocha, L., Carreón-Freyre, D., & Aranda, J. (2015). Physical experiments of land subsidence within a maar crater: Insights for porosity variations and fracture localization. *Proceedings of the International Association of Hydrological Sciences*, *372*, 285–290. https://doi.org/10.5194/piahs-372-285-2015

Chacón, E., Aranda-Gómez, J. J., Charles-Polo, M., Sánchez-Ramos, M. A., Rivera-Muñoz, E. M., Levresse, G., & Millán-Malo, B. (2018). Biohermal thrombolites of the crater Lake Rincón de Parangueo in Central México. *Journal of South American Earth Sciences*, *85*, 236–249. https://doi.org/10.1016/j.jsames.2018.04.013

Coman, C., Chiriac, C. M., Robeson, M. S., Ionescu, C., Dragos, N., Barbu-Tudoran, L., Andrei, A.-Å., Banciu, H. L., Sicora, C., & Podar,

M. (2015). Structure, mineralogy, and microbial diversity of geothermal spring microbialites associated with a deep oil drilling in Romania. *Frontiers in Microbiology*, *6*, 253. https://doi.org/10.3389/fmicb.2015.00253

Cortés López, E. (2016). *Cianoprocariontes de microbialitas de lagos de cráter de Guanajuato, México* [Master's thesis, Universidad Nacional Autónoma de México].

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. https://doi.org/10.1371/journal.pone.0028767

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, Complex Systems*, *1695*(5), 1–9.

Domínguez-Vázquez, G., Osuna-Vallejo, V., Castro-López, V., Israde-Alcántara, I., & Bischoff, J. A. (2019). Changes in vegetation structure during the Pleistocene–Holocene transition in Guanajuato, Central Mexico. *Vegetation History and Archaeobotany*, *28*(1), 81–91. https://doi.org/10.1007/s00334-018-0685-8

Escolero, O., & Alcocer, J. (2004). Desecación de los lagos cráter del Valle de Santiago, Guanajuato. In B. Jiménez & L. Marín (Eds.), *El agua en México vista desde la Academia* (pp. 99–115). Academia Mexicana de Ciencias.

Falcón, L. I., Escobar-Briones, E., & Romero, D. (2002). Nitrogen fixation patterns displayed by cyanobacterial consortia in Alchichica crater-lake, Mexico. *Hydrobiologia*, *467*(1), 71–78. https://doi.org/10.1023/A:1014984629985

Farias, M. E., Contreras, M., Rasuk, M. C., Kurth, D., Flores, M. R., Poire, D. G., Novoa, F., & Visscher, P. T. (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. https://doi.org/10.1007/s00792-013-0617-6

Ferrari, L., Orozco-Esquivel, T., Manea, V., & Manea, M. (2012). The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico subduction zone. *Tectonophysics*, *522*, 122–149. https://doi.org/10.1016/j.tecto.2011.09.018

Gérard, E., De Goeyse, S., Hugoni, M., Agogué, H., Richard, L., Milesi, V., Guyot, F., Lecourt, L., Borensztajn, S., Joseph, M.-B., Leclerc, T., Sarazin, G., Jézéquel, D., Leboulanger, C., Ader, M., & 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. https://doi.org/10.3389/fmicb.2018.00796

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). *The ISME Journal*, *7*(10), 1997–2009. https://doi.org/10.1038/ismej.2013.81

Gómez-Acata, E. S., Ayala-Gámez, L. M., García-Covarrubias, R., Torres-Núñez, M. Y., Vargas-López, L., Hernández-Camargo, S., Becerril-Rojas, I. G., León-Domínguez, M., Zurita-García, L., Navarro-Noya, Y. E., Conde-Barajas, E., & Dendooven, L. (2021). Bioprospecting of haloalkaliphilic microorganisms isolated from a dried-out maar in the volcano "Hoya Rincón de Parangueo". *Nova Scientia*, *13*(26), 1–21. https://doi.org/10.21640/ns.v13i26.2553

Grant, W. D., & Jones, B. E. (2016). Bacteria, archaea and viruses of Soda lakes. In M. Schagerl (Ed.), *Soda lakes of East Africa* (pp. 97–147). Springer. https://doi.org/10.1007/978-3-319-28622-8_5

Green, J. (1986). Associations of zooplankton in six crater lakes in Arizona, Mexico and New Mexico. *Journal of Zoology*, *208*(1), 135–159. https://doi.org/10.1111/j.1469-7998.1986.tb04715.x

Hernández Zavala, M. E. (2016). *Caracterización hidrogeoquímica y diversidad microbiana en ambientes extremos, casos: Villa Juárez, San Luis Potosí y Rincón de Parangueo, Guanajuato* [Master's thesis, Instituto Potosino de Investigación Científica y Tecnológica]. http://hdl.handle.net/11627/4919

Himmler, T., Smrzka, D., Zwicker, J., Kasten, S., Shapiro, R. S., Bohrmann, G., & Peckmann, J. (2018). Stromatolites below the photic zone in the northern Arabian Sea formed by calcifying chemotrophic microbial mats. *Geology*, *46*(4), 339–342. https://doi.org/10.1130/G39890.1

Hollander, D., Breitbart, M., Hoare, A., Seifert, J., Edwards, R., & Rohwer, F. (2007). *The role of microbes in the precipitation of microbialites in Cuatro Cienegas, Mexico: A genomic and stable isotopic perspective*. Paper presented at the AGU Fall Meeting Abstracts.

Hoshino, T., & Inagaki, F. (2019). Abundance and distribution of archaea in the subseafloor sedimentary biosphere. *The ISME Journal*, *13*(1), 227–231. https://doi.org/10.1038/s41396-018-0253-3

Ibarra-Sánchez, C. L., Prince, L., Aguirre-Noyola, J. L., Sánchez-Cerda, K. E., Navaro-Noya, Y. E., Luna-Guido, M., Conde-Barajas, E., Dendooven, L., & Gomez-Acata, E. S. (2020). The microbial community in an alkaline saline sediment of a former maar lake bed. *Journal of Soils and Sediments*, *20*(1), 542–555. https://doi.org/10.1007/s11368-019-02371-x

Iniesto, M., Moreira, D., Reboul, G., Deschamps, P., Benzerara, K., Bertolino, P., Saghaï, A., Tavera, R., & López-García, P. (2021). Core microbial communities of lacustrine microbialites sampled along an alkalinity gradient. *Environmental Microbiology*, *23*(1), 51–68. https://doi.org/10.1111/1462-2920.15252

Jeske, J. T., & Gallert, C. (2022). Microbiome analysis via OTU and ASV-based pipelines—A comparative interpretation of ecological data in WWTP systems. *Bioengineering*, *9*(4), 146. https://doi.org/10.3390/bioengineering9040146

Johnson, D., Beddows, P., Flynn, T., & Osburn, M. (2018). Microbial diversity and biomarker analysis of modern freshwater microbialites from Laguna Bacalar, Mexico. *Geobiology*, *16*(3), 319–337. https://doi.org/10.1111/gbi.12283

Jones, B. E., Grant, W. D., Duckworth, A. W., & Owenson, G. G. (1998). Microbial diversity of soda lakes. *Extremophiles*, *2*(3), 191–200. https://doi.org/10.1007/s007920050060

Joos, L., Beirinckx, S., Haegeman, A., Debode, J., Vandecasteele, B., Baeyen, S., Goormachtig, S., Clement, L., & De Tender, C. (2020). Daring to be differential: Metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units. *BMC Genomics*, *21*(1), 1–17. https://doi.org/10.1186/s12864-020-07126-4

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. https://doi.org/10.1007/s10347-010-0255-8

Kerrigan, Z., & D'Hondt, S. (2022). Patterns of relative bacterial richness and community composition in seawater and marine sediment are robust for both operational taxonomic units and amplicon sequence variants. *Frontiers in Microbiology*, *96*, 796758. https://doi.org/10.3389/fmicb.2022.796758

Kienel, U., Bowen, S. W., Byrne, R., Park, J., Böhnel, H., Dulski, P., Luhr, J. F., Siebert, L., Haug, G. H., & Negendank, J. F. (2009). First lacustrine varve chronologies from Mexico: Impact of droughts, ENSO and human activity since AD 1840 as recorded in maar sediments from Valle de Santiago. *Journal of Paleolimnology*, *42*(4), 587–609. https://doi.org/10.1007/s10933-009-9307-x

Kubo, K., Lloyd, K. G., Biddle, F., J., Amann, R., Teske, A., & Knittel, K. (2012). Archaea of the miscellaneous crenarchaeotal group are abundant, diverse and widespread in marine sediments. *The ISME Journal*, *6*(10), 1949–1965. https://doi.org/10.1038/ismej.2012.37

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., Gulin, M. B., & Pape, T. (2002). Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, *297*(5583), 1013–1015. https://doi.org/10.1126/science.1072502

Mundo Velázquez, B. (2020). *Análisis microbiano y funcional de las microbialitas y tapetes microbianos de tres lagos tipo MAAR de México* [Thesis, Benemérita Universidad Autónoma de Puebla]. https://hdl.handle.net/20.500.12371/10097

Munson-McGee, J. H., Field, E. K., Bateson, M., Rooney, C., Stepanauskas, R., & Young, M. J. (2015). Nanoarchaeota, their Sulfolobales host, and Nanoarchaeota virus distribution across Yellowstone National Park hot springs. *Applied and Environmental Microbiology*, *81*(22), 7860–7868. https://doi.org/10.1128/AEM.01539-15

Nagpal, S., Haque, M. M., Singh, R., & Mande, S. S. (2019). iVikodak—A platform and standard workflow for inferring, analyzing, comparing, and visualizing the functional potential of microbial communities. *Frontiers in Microbiology*, *9*, 3336. https://doi.org/10.3389/fmicb.2018.03336

Nguyen, S. T., Vardeh, D. P., Nelson, T. M., Pearson, L. A., Kinsela, A. S., & Neilan, B. A. (2022). Bacterial community structure and metabolic potential in microbialite-forming mats from South Australian saline lakes. *Geobiology*, *20*, 546–559. https://doi.org/10.1111/gbi.12489

Nolla-Ardèvol, V., Strous, M., Sorokin, D. Y., Merkel, A. Y., & Tegetmeyer, H. E. (2012). Activity and diversity of haloalkaliphilic methanogens in Central Asian soda lakes. *Journal of Biotechnology*, *161*(2), 167–173. https://doi.org/10.1016/j.jbiotec.2012.04.003

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Weedon, J. (2021). *vegan: Community ecology package (version 2.5-7)* [Software]. https://CRAN.R-project.org/package=vegan

Otasek, D., Morris, J. H., Bouças, J., Pico, A. R., & Demchak, B. (2019). Cytoscape automation: Empowering workflow-based network analysis. *Genome Biology*, *20*, 1–15.

Paz-Jacome, M. P., Inguaggiato, C., Levresse, G., Robidoux, P., Granados, H. D., & Tassi, F. (2020). Total CO_2 output and carbon origin discharged from Rincón de Parangueo Maar (México). *Journal of Geochemical Exploration*, *215*, 106558. https://doi.org/10.1016/j.gexplo.2020.106558

Pérez-Bernal, M. F., Brito, E. M., Bartoli, M., Aubé, J., Ollivier, B., Guyoneaud, R., & Hirschler-Réa, A. (2020). *Desulfobotulus mexicanus* sp. nov., a novel sulfate-reducing bacterium isolated from the sediment of an alkaline crater lake in Mexico. *International Journal of Systematic and Evolutionary Microbiology*, *70*(5), 3219–3225. https://doi.org/10.1099/ijsem.0.004159

Pérez-Bernal, M. F., Brito, E. M. S., Bartoli, M., Aubé, J., Fardeau, M.-L., Rodriguez, G. C., Ollivier, B., Guyoneaud, R., & Hirschler-Réa, A. (2017). *Desulfonatronum parangueonense* sp. nov., a sulfate-reducing bacterium isolated from sediment of an alkaline crater lake. *International Journal of Systematic and Evolutionary Microbiology*, *67*(12), 4999–5005. https://doi.org/10.1099/ijsem.0.002399

Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A. H., Nieuwdorp, M., & Levin, E. (2020). Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLoS One*, *15*(1), e0227434. https://doi.org/10.1371/journal.pone.0227434

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*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2013). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/

Rees, H. C., Grant, W. D., Jones, B. E., & Heaphy, S. (2004). Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremophiles*, *8*(1), 63–71. https://doi.org/10.1007/s00792-003-0361-4

Rivera Martínez, L. G. (2015). *Aislamiento de bacterias anaeróbicas del lago cráter Rincón de Parangueo* [Thesis, Universidad de Guanajuato].

Rocha-Treviño, L. (2015). *Análisis de la deformación y hundimiento activo causado por la desecación del lago del mar Rincón de Parangueo (Guanajuato, México) mediante modelos análogos y perfiles de Radar de Penetración Terrestre (RPT)* [Master's thesis, Universidad Nacional Autónoma de México]. https://repositorio.unam.mx/contenidos/65241

Ruvindy, R., White, R. A., III, Neilan, B. A., & Burns, B. P. (2016). Unravelling core microbial metabolisms in the hypersaline microbial mats of Shark Bay using high-throughput metagenomics. *The ISME Journal*, *10*(1), 183–196. https://doi.org/10.1038/ismej.2015.87

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*(12), 4990–5004. https://doi.org/10.1111/1462-2920.13456

Saghaï, A., Zivanovic, Y., Zeyen, N., Moreira, D., Benzerara, K., Deschamps, P., Bertolino, P., Ragon, M., Tavera, R., López-Archilla, A. I., & 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. https://doi.org/10.3389/fmicb.2015.00797

Sánchez-Sánchez, J., Cerca, M., Alcantara-Hernandez, R. J., Aranda-Gomez, J. J., Carreon-Freyre, D., Lozano-Flores, C., Muñoz-Velasco, I., Levresse, G., & Varela-Echavarria, A. (2021). Mobile mud layer underneath the desiccated maar lake of Rincon de Parangueo and insights into its microbial fingerprints. *Revista Mexicana de Ciencias Geológicas*, *38*(3), 178–192. https://doi.org/10.22201/cgeo.20072902e.2021.3.1636

Sánchez-Sánchez, J., Cerca, M., Alcántara-Hernández, R. J., Lozano-Flores, C., Carreón-Freyre, D., Levresse, G., Vega, M., Varela-Echavarría, A., & Aranda-Gómez, J. J. (2019). Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico. *FEMS Microbiology Ecology*, *95*(5), fiz051. https://doi.org/10.1093/femsec/fiz051

Schirmack, J., Mangelsdorf, K., Ganzert, L., Sand, W., Hillebrand-Voiculescu, A., & Wagner, D. (2014). *Methanobacterium movilense* sp. nov., a hydrogenotrophic, secondary-alcohol-utilizing methanogen from the anoxic sediment of a subsurface lake. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_2), 522–527. https://doi.org/10.1099/ijs.0.057224-0

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*(6), e66662. https://doi.org/10.1371/journal.pone.0066662

Schouten, S., van der Meer, M. T., Hopmans, E. C., Rijpstra, W. I. C., Reysenbach, A.-L., Ward, D. M., & Sinninghe Damsté, J. S. (2007). Archaeal and bacterial glycerol dialkyl glycerol tetraether lipids in hot springs of Yellowstone National Park. *Applied and Environmental Microbiology*, *73*(19), 6181–6191. https://doi.org/10.1128/AEM.00630-07

Seyler, L. M., McGuinness, L. M., & Kerkhof, L. J. (2014). Crenarchaeal heterotrophy in salt marsh sediments. *The ISME Journal*, *8*(7), 1534–1543. https://doi.org/10.1038/ismej.2014.15

Shlimon, A. G., Friedrich, M. W., Niemann, H., Ramsing, N. B., & Finster, K. (2004). *Methanobacterium aarhusense* sp. nov., a novel methanogen isolated from a marine sediment (Aarhus Bay, Denmark). *International Journal of Systematic and Evolutionary Microbiology*, *54*(3), 759–763. https://doi.org/10.1099/ijs.0.02994-0

Sorokin, D. Y., Banciu, H. L., & Muyzer, G. (2015). Functional microbiology of soda lakes. *Current Opinion in Microbiology*, *25*, 88–96. https://doi.org/10.1016/j.mib.2015.05.004

Sowers, K. R., Baron, S. F., & Ferry, J. G. (1984). *Methanosarcina acetivorans* sp. nov., an acetotrophic methane-producing bacterium isolated from marine sediments. *Applied and Environmental Microbiology*, *47*(5), 971–978. https://doi.org/10.1128/aem.47.5.971-978.1984

St. John, E., Flores, G. E., Meneghin, J., & Reysenbach, A. L. (2019). Deep-sea hydrothermal vent metagenome-assembled genomes provide insight into the phylum Nanoarchaeota. *Environmental Microbiology Reports*, *11*(2), 262–270. https://doi.org/10.1111/1758-2229.12740

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*(2), 504–519. https://doi.org/10.1111/1574-6941.12411

Valdespino-Castillo, P. M., Hu, P., Merino-Ibarra, M., López-Gómez, L. M., Cerqueda-García, D., González-De Zayas, R., Pi-Puig, T., Lestayo, J. A., Holman, H.-Y., & Falcón, L. I. (2018). Exploring biogeochemistry and microbial diversity of extant microbialites in Mexico and Cuba. *Frontiers in Microbiology*, *9*, 510. https://doi.org/10.3389/fmicb.2018.00510

Valerdi Negreros, J. C. (2015). *Estudio filogenético de bacterias aisladas de lagos de cráteres volcánicos* [Thesis, Benemérita Universidad Autónoma de Puebla]. https://hdl.handle.net/20.500.12371/6549

Vavourakis, C. D., Andrei, A.-S., Mehrshad, M., Ghai, R., Sorokin, D. Y., & Muyzer, G. (2018). A metagenomics roadmap to the uncultured genome diversity in hypersaline soda lake sediments. *Microbiome*, *6*(1), 1–18. https://doi.org/10.1186/s40168-018-0548-7

Vignale, F. A., Kurth, D., Lencina, A. I., Poiré, D. G., Chihuailaf, E., Muñoz-Herrera, N., Novoa, F., Contreras, M., Turjanski, A. G., & Farías, M. E. (2021). Geobiology of andean microbial ecosystems discovered in Salar de Atacama, Chile. *Frontiers in Microbiology*, *12*, 762076. https://doi.org/10.3389/fmicb.2021.762076

Vuillemin, A., Ariztegui, D., Horn, F., Kallmeyer, J., Orsi, W., & the PASADO Science Team. (2018). Microbial community composition along a 50 000-year lacustrine sediment sequence. *FEMS Microbiology Ecology*, *94*, fiy029. https://doi.org/10.1093/femsec/fiy029

Wickham, H. (2016). Data analysis. In H. Wickham (Ed.), *ggplot2. Use R!* (pp. 189–201). Springer. https://doi.org/10.1007/978-3-319-24277-4_9

Worakit, S., Boone, D. R., Mah, R. A., Abdel-Samie, M.-E., & El-Halwagi, M. (1986). *Methanobacterium alcaliphilum* sp. nov., an H2-utilizing methanogen that grows at high pH values. *International Journal of Systematic and Evolutionary Microbiology*, *36*(3), 380–382. https://doi.org/10.1099/00207713-36-3-380

Zeyen, N., Daval, D., Lopez-Garcia, P., Moreira, D., Gaillardet, J., & Benzerara, K. (2017). Geochemical conditions allowing the formation of modern lacustrine microbialites. *Procedia Earth and Planetary Science*, *17*, 380–383. https://doi.org/10.1016/j.proeps.2016.12.096

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sánchez-Sánchez, J., Cerca, M., Moguel, B. B., Muñoz-Velasco, I., Alcántara-Hernández, R. J., Carreón-Freyre, D., & Levresse, G. (2023). Geomicrobiology of the Rincon de Parangueo maar crater: Exploring the link between an evolving extreme environment and its potential metabolic diversity. *Freshwater Biology*, *00*, 1–21. https://doi.org/10.1111/fwb.14191

DISCUSIÓN

A continuación se discuten varios puntos clave que constituyen una lista simplificada de las aportaciones y resultados más relevantes de esta investigación:

Rincón de Parangueo: un lugar con características extremas donde la vida prospera

Principales filotipos identificados y diversidad microbiana: Rincón de Parangueo es un lugar excepcional por sus condiciones ambientales extremas. Nuestro estudio permitió identificar comunidades microbianas que se desarrollan en diferentes microambientes dentro del cráter. Las muestras analizadas incluyeron microbialitas formadas en la antigua costa del lago, tapetes microbianos, microorganismos planctónicos de las pozas hipersalinas y sedimentos lacustres en la parte central del cráter. La desecación del lago ha llevado a algunos autores a pensar que las microbialitas de Rincón de Parangueo ya no están biológicamente activas, debido a la falta de una columna de agua que provea un ambiente lacustre (Zeyen et al., 2017; Iniesto et al., 2021). Los sistemas de lagos salino-alcalinos han demostrado ser sensibles a los cambios ambientales, en especial a las variaciones hidrológicas (Wagaw et al., 2019), dichas alteraciones pueden modificar la estructura de las comunidades biológicas. En el caso de Rincón de Parangueo, las comunidades que precipitan carbonatos no han desaparecido, solo se han adaptado. Gracias a las técnicas moleculares aplicadas en nuestro estudio, pudimos identificar una gran cantidad y diversidad de microorganismos. La secuenciación del gen ADN ribosomal 16S (ADNr 16S) y ADN ribosomal 18S (ADNr 18S), y el posterior análisis de las secuencias generadas a partir de métodos de secuenciación de alto rendimiento, revelaron distintos grupos filogenéticos. En un primer análisis basado en Unidades Taxonómicas Operativas (OTU´s) fue posible identificar microorganismos pertenecientes a los dominios Bacteria, Arquea y Eucaria a diferentes niveles taxonómicos (Sánchez-Sánchez et al., 2019). La mayoría de las secuencias se clasificaron dentro del dominio Arquea, lo que no es sorpresivo debido a las condiciones fisicoquímicas extremas dentro del cráter. Filotipos bacterianos como Cyanobacteria, Proteobacteria Actinobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Chloroflexi, Spirochaetes y Verrucomicrobia fueron los más representativos en las muestras, coincidiendo con los filotipos identificados en la mayoría de microbialitas en sistemas modernos en México (Yanez-Montalvo et al., 2020; Iniesto et al., 2021) y el mundo (Quéméneur et al., 2015; Albarracín et al., 2020; Roy et al., 2020). A diferencia de la amplia diversidad de filotipos bacterianos identificados, solo un filotipo arqueano (Euryarchaeota) fue reportado en las muestras en nuestro primer análisis. Además, más de la mitad de las lecturas para el dominio Arquea no coincidieron con ninguna secuencia conocida depositada en las bases de datos hasta ese momento. Los análisis de las secuencias generadas a partir del gen ADNr 18S señalaron a Chlorophyta, Bacillariophyta y Rotifera como los filotipos más abundantes, dichos microorganismos han probado formar asociaciones microbianas con las bacterias antes mencionadas en ambientes salino-alcalinos (Sánchez-Sánchez et al., 2019).

En un segundo análisis, el conjunto de datos generados por secuenciación del gen 16S ADNr fue re-evaluada con un método bioinformático basado en Variantes de Secuencia de Amplicones (ASV´s) y una base de datos actualizada (SILVA database v. 138, última actualización agosto 2020) (Sánchez-Sánchez et al., 2023). Nuevos filotipos entre los que destacan Asgardarchaeota, Euryarchaeota, Thermoplasmatota, Hadarchaeota, Halobacterota, Crenarchaeota, Nanoarchaeota y Patescibacteria fueron identificados. Muchos de ellos microorganismos alcalófilos, halófilos y termófilos recientemente descubiertos gracias a las tecnologías de secuenciación y nuevos en las bases de datos. Sin embargo, aún después del nuevo análisis, sigue existiendo un número elevado de secuencias no clasificadas, especialmente en muestras de agua. Dichas secuencias no clasificadas podrían representar la llamada “materia oscura genética", que en teoría representa microorganismos aún desconocidos (Isa et al., 2018). Esto evidencia una diversidad inexplorada de microorganismos dentro del cráter que debe ser investigada a mayor profundidad en el futuro.

En general, los índices de diversidad de Shannon-Wiener, que tienen en cuenta la riqueza y la equidad de una muestra (Strong, 2016), mostraron una baja diversidad en las muestras (Microbialita: H´= 4.90; Tapete microbiano: H´= 4.99; Agua: H´= 4.87; Sedimento: H´= 4.58). Comparadas con otros sitios con condiciones fisicoquímicas similares (Alchichica: H´= 6.10; Highborne Cay, Bahamas: H´= 5.35) (Centeno et al., 2012), las microbialitas de Rincón de Parangueo tienen una menor diversidad (H´= 4.90). Como era de esperarse, dada la inestabilidad del ambiente, existe una pérdida de diversidad debido a procesos adaptativos de la microbiota como respuesta a la desecación del lago y el aumento de la alcalinidad y salinidad a lo largo de los últimos años. Por otro lado, la relación entre los patrones de la comunidad dentro y entre los microambientes se probó mediante un escalamiento multidimensional no métrico (NMDS) basado en la diversidad beta de Bray–Curtis (Iniesto et al., 2021). El análisis mostró claramente cuatro grupos de muestras separadas, sugiriendo una adaptación de las comunidades microbianas al microambiente específico donde se desarrollan.

El análisis del perfil metabólico para predecir la posible contribución metabólica de la comunidad procariota, basado en los taxones identificados mediante el análisis de Variantes de Secuencia de Amplicones (ASV´s), reveló a los metabolismos asociados con fijación de carbono (*e.g.*, precipitación de carbonatos, fotosíntesis y metanogénesis) como los metabolismos más representativos en las muestras (Figura 8, Sánchez-Sánchez et al., 2023). Indicando un amplio potencial para la fijación de carbono mediada por microorganismos en todas las muestras, pero principalmente en muestras de microbialitas y sedimentos lacustres. Lo anterior tiene importantes implicaciones en la geobiología única del cráter como se explica en las siguientes secciones.

Disminución del nivel de agua, geología e hidroquímica: características fundamentales en la formación de un ambiente extremo en el cráter maar Rincón de Parangueo

En Rincón de Parangueo existen una serie de factores que al combinarse han dado lugar a un ambiente muy particular donde las comunidades microbianas prosperan.

Disminución del nivel de agua en el lago por actividad antropogénica: La desecación del lago como consecuencia de la extracción de agua del acuífero regional para uso antropogénico es un problema grave. Uno de los puntos clave en la desecación del lago es la ubicación del cráter. Rincón de Parangueo se ubica en la cuenca del Río Lerma-Santiago una de las cuencas más sobreexplotadas de México (Escolero y Alcocer, 2004), la cual desde finales del siglo pasado atraviesa por una situación dramática de extracción de agua (el sector agrícola consume el 80% del agua subterránea del acuífero regional, CONAGUA 2002). El nivel del lago está determinado por un imbalance, las altas tasas de evaporación (1935 mm/año) y bajas tasas de precipitación (715-738 mm/año) que se registran en la zona (Escolero y Alcocer, 2004) contribuyen en la desecación del lago. La disminución progresiva y acelerada de agua en el lago ha dado paso a cambios fisicoquímicos dentro del cráter, aumentando la salinidad (CE= 25,000 µS/cm en 1982; CE= 165,000 µS/cm en 1999) y alcalinidad (pH= 9.8 en 1995; pH= 11.16 en 2012) del agua considerablemente en las últimas décadas (Tabla 1, Sánchez-Sánchez et al., 2023). A diferencia de los lagos de cráteres aledaños, una mayor salinidad y alcalinidad (CE= 165,000 µS/cm; pH= 10.2) fueron identificados en el lago Rincón de Parangueo (Armienta et al. 2008) a pocos años del inicio de su desecación (año 1999). Dichos valores podrían ser indicativos de factores propios del cráter (como se discute más adelante) que, sumados a la disminución del nivel del acuífero regional y las altas tasas de evaporación en la zona, estarían favoreciendo una mayor salinidad y alcalinidad del agua.

***Subsidencia*:** Rincón de Parangueo es el cráter de la región que muestra las mayores evidencias de deformación relacionadas con el proceso de subsidencia, lo que no ocurre en los cráteres aledaños (*e.g.*, La Alberca, La Cintora, San Nicolás de Parangueo) que también se han desecado en los últimos años. Aranda-Gómez et al. (2013, 2017) han demostrado que la subsidencia dentro del cráter es un proceso activo relacionado con el descenso en el nivel del acuífero regional. El descenso gradual en el nivel del terreno dentro del cráter como consecuencia de la subsidencia es evidente. En el año 1982 la profundidad máxima del lago era de 9 m (Escolero y Alcocer, 2004) y para el año 2022, una medición hecha con una estación total, arrojó una diferencia de 20 m de altura entre la antigua línea de costa (1,690 m s. n. m.) y el nivel del depocentro donde se encuentran las pozas remanentes (1,670 m s. n. m.). Dicho dato coincide con una medición realizada en 2016 (Carrera et al., 2021), comparando la profundidad original del lago en 1982 el descenso del terreno alcanzaría los 11 metros en 40 años, con una tasa promedio de alrededor de 30 cm/año. Dentro del cráter se pueden observar un gran número de fallas y fracturas asociadas con la deformación diferencial, estas se encuentran principalmente en la zona que abarca el centro y la antigua costa del lago. La principal de ellas es una falla extensional con una altura de entre 12 y 15 m (Aranda-Gómez et al. 2017; Rocha-Treviño, 2015), la cual cobra gran relevancia en la química del agua como se explica a continuación.

Química del agua, entrada de agua subterránea y desgasificación en Rincón de Parangueo: La desecación del lago combinado con el proceso de subsidencia han provocado la pérdida de estructura en los sedimentos suaves, principalmente en la zona de la falla principal, algunos de los cuales tienen una densidad aparente menor a 1000 kg m^{-3} (Sánchez-Sánchez et al., 2019). Esta condición los vuelve altamente vulnerables al intemperismo y la erosión provocando la liberación de iones de Ca^{2+} y Mg^{2+} provenientes de minerales de silicato a una tasa más elevada que en cráteres aledaños. Aunado a lo anterior, un componente de suma importancia es el enorme flujo de gases, mayormente CO_2 (10.6 ton/día) y CH_4 (6.3 ton/día) (Paz-Jacome et al., 2020), que son liberados hacia la superficie principalmente por la falla principal. Los iones de Ca^{2+} y Mg^{2+}, en conjunto con el elevado flujo de CO_2 por las fallas y fracturas, han contribuido en el aumento paulatino de la alcalinidad y salinidad del agua. El incremento en las propiedades físicoquímicas antes mencionadas pudo haber comenzado mucho antes del proceso de desecación del lago (Kempe & Kazmierczak, 2007), lo que explicaría los valores elevados en comparación con otros lagos de cráteres aledaños a pocos años del comienzo de la desecación en Rincón de Parangueo. Hoy en día, la composición química del agua remanente se caracteriza por contener Ca^{2+} y Na^{+} como principales cationes y un elevado contenido de HCO_3^{-} y CO_3^{2-} (Tabla suplementaria 2), los cuales saturan el medio llevando a la precipitación de carbonatos (Figura 2 y 3, Sánchez-Sánchez et al., 2019). La presencia de carbonatos en medios acuáticos saturados es un proceso que se puede dar de forma abiogénica y/o biogénica mediante un proceso enzimático llamado bio-calcificación (Kempe & Kazmierczak, 2007). En Rincón de Parangueo, la precipitación de carbonatos tales como calcita [$CaCO_3$], aragonita [$CaCO_3$] e hidromagnesita [$Mg_5(CO_3)_4(OH)_2(H_2O)_4$], son producidos de forma abiótica gracias al elevado flujo de CO_2 que es expulsado desde el interior del cráter, pero además por procesos biogénicos como se explica más adelante.

Microambientes en Rincón de Parangueo: correlación de factores bióticos y abióticos que intervienen en la formación de las estructuras sedimentarias en Rincón de Parangueo

Microbialitas: Los datos recabados en campo mostraron que las estructuras organo-sedimentarias más sobresalientes son las microbialitas, las cuales presentaron una amplia gama de morfologías y tamaños, siendo las microbialitas, ubicadas en la falla principal, las más grandes y con una morfología particular (macroestructura: domica, microestructura: trombolítica y estromatolítica; Figura 3 Sánchez- Sánchez et al., 2023). Las condiciones que llevaron a la formación de microbialitas de mayor tamaño en esta zona del cráter están directamente relacionadas con las características mencionadas en las secciones anteriores. En primer lugar, la falla extensional más prominente dentro del cráter (altura:12-15m) juega un papel muy importante al ser el principal conducto para la salida de gases desde el interior del cráter. El elevado flujo de gases aporta la energía química necesaria para que las comunidades microbianas puedan crecer. Las propiedades fisicoquímicas medidas en el microambiente donde se desarrollan mostraron que la alta porosidad de los sedimentos permite el flujo de los gases, y su capacidad natural para retener agua provee un medio estable durante todo el año. Además, se presume que existió un flujo de agua hidrotermal en el pasado reciente (antes del inicio de la desecación del lago), la falla principal del cráter actuó como un importante conducto de este fluido, provocando la entrada de agua saturada en carbonatos, lo que provocó su precipitación a una tasa más elevada en comparación con otras zonas del cráter. Al igual que en Rincón de Parangueo, el flujo de agua subterránea por fallas y el crecimiento de estructuras biológicas se ha reportado en varios lugares del mundo, como es el caso de Lake Van (Cukur et al., 2015), Lake Clifton (Moore et al., 1994; Warden et al., 2019), Great Salt Lake (Lindsay et al., 2020) y el lago Alchichica (Kaźmierczak et al., 2011). Dichos estudios revelaron que las microbialitas se desarrollan mejor en áreas donde el flujo de agua subterránea a través de fallas geológicas es más prominente, lo que proporciona nutrientes y condiciones adecuadas para el crecimiento microbiano. El elevado flujo de CO_2 por la falla principal y su uso para la precipitación de carbonatos mediada por microbios en muestras de microbialitas, coincide con los resultados del perfil metabólico funcional que indicó una mayor fijación de carbono en este tipo de muestras (Figura 8, Sánchez-Sánchez et al., 2023).

Tapetes microbianos y microorganismos planctónicos en las pozas: En este microambiente, se observaron tapetes microbianos no litificantes que flotan en el agua, principalmente en los márgenes de las pozas. La presencia de Cl^-, Na^+ y carbonatos que dominan la química del agua, causan la precipitación de carbonatos de sodio (trona, termonatrita, natrita y eitelita) y evaporitas (halita y silvita), los cuales sirven como sustrato para el desarrollo de los microorganismos. Los tapetes microbianos y comunidades microbianas planctónicas, viven en un medio más salino y alcalino comparado con las microbialitas y sedimentos a profundidad (Tabla 1, Sánchez- Sánchez et al., 2023). Una mayor homogeneidad de la comunidad microbiana entre muestras de tapetes microbianos colectados de las distintas pozas fue identificada (Figura 7a, Sánchez-Sánchez et al., 2023), lo que podría ser explicado debido a su capacidad para construir su propio “nicho” y habilidad para ser autosostenibles (Souza et al., 2018). Por el contrario, las muestras de comunidades planctónicas obtenidas de las diferentes pozas resultaron ser más heterogéneas entre sí, lo que se justifica con las diferencias en tamaño y profundidad de las pozas al momento del muestreo (Tabla 1 material suplementario, Sánchez-Sánchez et al., 2023). Dichas variaciones traen como consecuencia modificaciones en las propiedades fisicoquímicas del agua, propiciando que cada poza tenga diferentes características y por lo tanto distintas comunidades microbianas. En relación con esto, el metabolismo más importante reconocido agua y tapete microbiano fue la fijación de carbono. Una mayor fijación de carbono fue predicha para muestras de tapetes microbianos (Figura 8, Sánchez- Sánchez et al., 2023), como era de esperarse debido a una mayor abundancia de Cyanobacteria (Schuler et al., 2017; Kurth et al., 2021).

Sedimentos a profundidad: El microambiente en los sedimentos a cuatro metros de profundidad es un medio con alta alcalinidad (pH 10.38 –10.65), alto contenido de agua volumétrica (> 200%), alta conductividad (23.29– 51.4 mS/cm) y rico en compuestos orgánicos (> 6 %) (Sánchez- Sánchez et al., 2021). El contenido de agua, que aumenta con la profundidad (Tabla suplementaria 2), favorece el comportamiento plástico de los sedimentos. Esto, aunado a la entrada de gases en el sistema, provoca la extrusión de domos de lodo en el depocentro del cráter. Los domos son generados, además, por deslizamiento gravitacional y sobrepresión de material sedimentario, debido al desprendimiento de material desde la zona de falla principal. La formación de domos de lodo debido a la desgasificación, sugiere un sistema geológico altamente dinámico y relacionado con la composición de las comunidades microbianas identificadas en los sedimentos lacustres. En este microambiente se identificaron microorganismos metanogénicos, entre ellos Bathyarchaeia, Methanomassiliicoccales, Methanomicrobiales y Methanobacteriales (Sánchez- Sánchez et al., 2021), los cuales toman una gran relevancia en nuestro estudio debido a su capacidad para producir metano. Su probable papel en la producción de gas en los sedimentos que provoca la sobrepresión del fluido de poro, significa un aporte biológico de gran relevancia en la geología del cráter. Los elevados porcentajes de microorganismos metanogénicos, que comprenden del 13.2 al 32.1% del ADN total de arqueas en los sedimentos, explican probablemente el abundante flujo de CH_4 (6.3 ton/d) en los sedimentos. Paz-Jacome et al. (2020) sugieren una fuerte contribución biogénica en el origen del metano, coincidiendo con nuestros resultados. La probable generación de metano en los sedimentos fue corroborada por la predicción de las funciones metabólicas, identificando el ciclo de metano y más específicamente la metanogénesis como el segundo metabolismo más abundante en las muestras de sedimentos. Los grupos metanogénicos identificados tienen la capacidad metabólica para aprovechar el CO_2 geotérmico y utilizarlo como sustrato para la producción de metano.

CONCLUSIONES

Rincón de Parangueo es un lugar que debe ser conservado por su importancia biológica y geológica. Considerado como un ambiente "extremo", representa un laboratorio natural para el estudio de comunidades microbianas extremófilas capaces de construir microbialitas, lo que lo convierte en un lugar poco común y de gran importancia a nivel mundial. Este estudio multidisciplinario abordó desde diferentes perspectivas el desarrollo de comunidades microbianas en un entorno extremo. Tiene relevancia en múltiples campos, proporcionando un conocimiento crucial para entender cómo la vida persiste y evoluciona en condiciones que inicialmente podrían considerarse inhóspitas.

Rincón de Parangueo es un lugar fascinante donde el crecimiento de comunidades microbianas únicas es impulsado por un conjunto de aspectos geobiológicos interrelacionados. A continuación se enlistan las principales conclusiones de esta investigación:

- El uso de los genes ADNr 16S y ADNr 18S como marcadores para la identificación de especies microbianas y el análisis de las secuencias por medio de metodologías bioinformáticas, permitieron la identificaron de una gran variedad de filotipos microbianos (Bacteria, Arquea y Eucaria) en diferentes microambientes en la superficie del cráter.

- La secuenciación del gen ADNr 16S resultó en la identificación de la huella microbiana en sedimentos del subsuelo del cráter, que, comparada con la comunidad microbiana en la superficie, comparten cierta similitud. También existen importantes discrepancias entre las que destacan los altos porcentajes de microorganismos metanogénicos: Bathyarchaeia, Methanomassiliicoccales, Methanomicrobiales y Methanobacteriales.

- La reevaluación del conjunto de datos derivados de la secuenciación del gen ADNr 16S mediante procedimientos bioinformáticos y bases de datos actualizadas, resultó en un perfeccionamiento de los resultados de la composición de las comunidades microbianas caracterizadas en la superficie y el subsuelo del cráter.

- La mayoría de las secuencias generadas se clasificaron dentro del dominio Arquea, los filotipos Asgardarchaeota, Thermoplasmatota, Hadarchaeota, Halobacterota y Nanoarchaeota fueron los más abundantes en las muestras, muchos de ellos alcalófilos (alta alcalinidad), halófilos (alta salinidad) y termófilos (alta temperatura) resaltando una importante adaptación de las comunidades microbianas a las condiciones extremas generalizadas del cráter.

- Los filotipos bacterianos más abundantes en las microbialitas, Cyanobacteria, Proteobacteria Actinobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Chloroflexi y Verrucomicrobia, coincidieron con los filotipos identificados en sistemas modernos de microbialitas en distintas partes del mundo con condiciones fisicoquímicas similares.

- La asignación taxonómica de las secuencias generadas no fue del todo posible, aún después del uso de una base de datos y procedimientos bioinformáticos más actualizados, evidenciando una biodiversidad importante e inexplorada que debe ser investigada a mayor profundidad en el futuro.

- La caracterización del potencial metabólico de las comunidades microbianas a partir de secuencias de ADNr 16S resultó ser una excelente vía para conocer los posibles metabolismos implicados en la formación de estructuras sedimentarias en Rincón de Parangueo. La fijación de carbono y el metabolismo del metano fueron los principales metabolismos reconocidos en las muestras.

- El proceso activo de desgasificación en el cráter está estrechamente relacionado con la precipitación de carbonatos de origen biogénico. Las fallas y fracturas distribuidas en todo el cráter proporcionan un conducto para la salida de los gases, principalmente CO_2, el cual es aprovechado por las comunidades microbianas para la producción de carbonatos.

- La cartografía de las estructuras organo-sedimentarias, las fallas y fracturas evidenció una correlación espacial entre las microbialitas más prominentes y la falla principal del cráter. La falla actúa como el conducto principal para la migración de gases desde el interior de los sedimentos, lo que proporciona una fuente de energía química ilimitada que permite un mayor crecimiento de microbialitas en esa zona.

- Las propiedades fisicoquímicas identificadas en los sedimentos carbonatados no litificados de la falla principal, mostraron un medio con alta porosidad (baja densidad) que permite el flujo de nutrientes (gases), y que tiene la capacidad para retener agua, proporcionando un medio estable para el desarrollo de comunidades endolíticas que buscan evitar las altas tasas de evaporación registradas en la zona.

- Las propiedades fisicoquímicas reconocidas en el agua revelaron ser de suma importancia para las comunidades microbianas que viven en las pozas. El medio saturado en Ca^{2+}, Na^{+}, HCO_3^{-} y CO_3^{2-} proporciona las condiciones necesarias que conducen a la formación de carbonatos de sodio y evaporitas, los cuales actúan como sustrato para el crecimiento de los microorganismos. Además, el medio saturado con carbonatos permite la producción de estructuras carbonatadas gracias a procesos de biocalcificación.

- El lodo móvil a 4 metros de profundidad está caracterizado por su elevado contenido de agua volumétrica, alto contenido de materia orgánica, alta porosidad y está compuesto principalmente por partículas de arcilla, proporcionando un medio adecuado para el crecimiento de microorganismos anaerobios (*e.g.*, microorganismos metanogénicos).

- Finalmente, a través de la cartografía de estructuras de deformación (domos de lodo) y la interpretación de perfiles de Radar de Penetración Terrestre, se identificaron una gran cantidad de domos distribuidos principalmente en la parte oeste del cráter. Dichos domos son producidos principalmente por la sobrepresión local generada en los sedimentos y la extrusión de lodo influenciada por la desgasificación. La probable producción de metano en los sedimentos por la actividad de microorganismos metanogénicos, aumenta la generación de gases en los sedimentos favoreciendo la extrusión del lodo.

REFERENCIAS

Agostini, M. D. L. Á., Monterubbianesi, M. G., Studdert, G. A., & Maurette, S. (2014). Un método simple y práctico para la determinación de densidad aparente. *Ciencia del suelo*, 32(2), 171-176.

Albarracín, V. H., Galván, F. S., & Farías, M. E. (2020). Extreme microbiology at Laguna Socompa: A high-altitude Andean lake (3570 m asl) in Salta, Argentina. Microbial Ecosystems in Central Andes Extreme Environments: Biofilms, Microbial Mats, Microbialites and Endoevaporites, 205-220. https://doi.org/10.1007/978-3-030-36192-1

Aranda-Gómez, J. J., Cerca, M., Rocha-Treviño, L., Carrera-Hernández, J. J., Levresse, G., Pacheco, J., . . . Beraldi-Campesi, H. (2017). Structural evidence of enhanced active subsidence at the bottom of a maar: Rincón de Parangueo, México. *Geological Society, London, Special Publications, 446*(1), 225-254. https://doi.org/10.1144/SP446.1

Aranda-Gómez, J. J., Levresse, G., Martínez, J. P., Ramos-Leal, J. A., Carrasco-Núñez, G., Chacón-Baca, E., . . . Origel, G. (2013). Active sinking at the bottom of the Rincón de Parangueo Maar (Guanajuato, México) and its probable relation with subsidence faults at Salamanca and Celaya. *Boletín de la Sociedad Geológica Mexicana, 65*(1), 169-188.

Armienta, M. A., Vilaclara, G., De la Cruz-Reyna, S., Ramos, S., Ceniceros, N., Cruz, O., . . . Arcega-Cabrera, F. (2008). Water chemistry of lakes related to active and inactive Mexican volcanoes. *Journal of Volcanology and Geothermal Research, 178*(2), 249-258. https://doi.org/10.1016/j.jvolgeores.2008.06.019

Arredondo-Figueroa, J. L., Borrego-Enríquez, L. E., Castillo-Domínguez, R. M., & Valladolid-Laredo, M. A. (1983). Batimetría y morfometría de los lagos “maars” de la cuenca de Oriental, Puebla, México. *Biotica*, 8(1), 37-47.

Ballot, A., Krienitz, L., Kotut, K., Wiegand, C., & Pflugmacher, S. (2005). Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful algae*, 4(1), 139-150.

Baxter, R. M., Prosser, M. V., Talling, J. F., & Wood, R. B. (1965). Stratification in tropical African lakes at moderate altitudes (1,500 to 2,000 m). *Limnology and Oceanography*, 10(4), 510-520.

Benzerara, K., Menguy, N., López-García, P., Yoon, T. H., Kazmierczak, J., Tyliszczak, T., ... & Brown Jr, G. E. (2006). Nanoscale detection of organic signatures in carbonate microbialites. *Proceedings of the National Academy of Sciences*, 103(25), 9440-9445. https://doi.org/10.1073/pnas.0603255103

Bey, B.S., Fichot, E.B., Dayama, G., Decho, A.W., Norman, R.S., 2010, Extraction of high molecular weight DNA from microbial mats: *Biotechniques*, 49(3), 631-640. https://doi.org/10.2144/000113486

Bokulich, N. A., Rideout, J. R., Kopylova, E., Bolyen, E., Patnode, J., Ellett, Z., ... & Caporaso, J. G. (2015). A standardized, extensible framework for optimizing classification improves marker-gene taxonomic assignments. *PeerJ PrePrints*, 3, e934v2. https://dx.doi.org/10.7287/peerj.preprints.934v2

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* 30(15), 2114-2120. https://doi.org/10.1093/bioinformatics/btu170

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. https://doi.org/10.1038/nmeth.3869

Carrera-Hernández, J. J., Levresse, G., Lacan, P., & Aranda-Gómez, J. J. (2021). A low cost technique for development of ultra-high resolution topography: application to a dry maar's bottom. *Revista Mexicana de Ciencias Geológicas, 33*(1), 122-133.

Centeno, C. M., Legendre, P., Beltrán, Y., Alcántara-Hernández, R. J., Lidström, U. E., Ashby, M. N., & Falcon, L. I. (2012). Microbialite genetic diversity and composition relate to environmental variables. *FEMS microbiology ecology*, 82(3), 724-735.

Cerca, M., Carreón-Freyre, D., Aranda-Gómez, J.J., Rocha-Treviño, L. (2014). GPR profiles for characterizing subsidence deformation in lake sediments within a maar crater. *En Proceedings of the 15th International Conference on Ground Penetrating Radar* (pp. 274-278).

IEEE.https://doi.org/10.1111/j.1574-6941.2012.01447.x

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, complex systems,* 1695 (5), 1-9.

Cukur, D., Krastel, S., Çağatay, M. N., Damcı, E., Meydan, A. F., & Kim, S. P. (2015). Evidence of extensive carbonate mounds and sublacustrine channels in shallow waters of Lake Van, eastern Turkey, based on high-resolution chirp subbottom profiler and multibeam echosounder data. *Geo-Marine Letters*, 35, 329-340. https://doi.org/10.1007/s00367-015-0410-x

Dupraz, C. (2004). Microbe-carbonate mineral interactions in modern microbialite formation (open marine and hypersaline environments, Bahamas). En The 2nd Swiss Geoscience Meeting, Lausanne (Vol. 95).

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods*, 10(10), 996-998. https://doi.org/10.1038/nmeth.2604

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27(16), 2194-2200. https://doi.org/10.1093/bioinformatics/btr381

Escolero, O., & Alcocer, J. (2004). Desecación de los lagos cráter del Valle de Santiago, Guanajuato. En: El agua en México vista desde la Academia. (Eds: Jiménez, B., & Marín, L) México City, Academia Mexicana de Ciencias, 99-115.

Fassbender, H & Bornemisza, E. (1987). Química de los suelos con énfasis en suelos de América Latina. Sán José, Costa Rica: IICA, 167-168

Green, J., Moghraby, A. E., & Ali, O. M. M. (1979). Biological observations on the crater lakes of Jebel Marra, Sudan. *Journal of Zoology*, 189(4), 493-502.

Hautman, D.P., Munch, D.J. (1997). Method 300.1 determination of inorganic anions in drinking water by ion chromatography: Cincinnati, OH, US Environmental Protection Agency, 1-36

Hecky, R. E., & Kilham, P. (1973). Diatoms in alkaline, saline lakes: ecology and geochemical implications 1. *Limnology and oceanography*, 18(1), 53-71. https://doi.org/10.4319/lo.1973.18.1.0053

Iniesto, M., Moreira, D., Reboul, G., Deschamps, P., Benzerara, K., Bertolino, P., . . . López‐García, P. (2021). Core microbial communities of lacustrine microbialites sampled along an alkalinity gradient. *Environmental microbiology, 23*(1), 51-68. https://doi.org/10.1111/1462-2920.15252

Isa, P., Taboada, B., Gutiérrez, A. L., Chávez, P., del Ángel, R. M., Ludert, J. E., ... & Arias, C. F. (2018). Hyperdiverse Viral Communities in an Oligotrophic Oasis (Cuatro Ciénegas): Marine Affinities and Microgeographic Differentiation. Cuatro Ciénegas Ecology, Natural History and Microbiology, 43-55. https://doi.org/10.1007/978-3-319-93423-5_4

Kasedde, H., Kirabira, J. B., Bäbler, M. U., Tilliander, A., & Jonsson, S. (2014). Characterization of brines and evaporites of Lake Katwe, Uganda. *Journal of African Earth Sciences,* 91, 55-65.

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. https://doi.org/10.1007/s10347-010-0255-8

Kempe S, Kazmierczak J, Landmann G, Konuk T, Reimer A, Lipp A (1991) Largest known microbialites discovered in Lake Van, Turkey. *Nature*, 349:605-608

Kempe, S., & Kazmierczak, J. (2007). Hydrochemical key to the genesis of calcareous non-laminated and laminated cyanobacterial microbialites. *Algae and cyanobacteria in extreme environments*, 239-264. https://doi.org/10.1007/978-1-4020-6112-7_13

Kilham, P., & Melack, J. M. (1972). Primary northupite deposition in Lake Mahega, Uganda?. *Nature Physical Science*, 238(86), 123-123. https://doi.org/10.1038/physci238123a0

Kurth, D., Elias, D., Rasuk, M. C., Contreras, M., & Farias, M. E. (2021). Carbon fixation and rhodopsin systems in microbial mats from hypersaline lakes Brava and Tebenquiche, Salar de Atacama, Chile. *PLoS One*, 16(2), e0246656. https://doi.org/10.1371/journal.pone.0246656

Lay, C. Y., Mykytczuk, N. C., Yergeau, I., Lamarche-Gagnon, G., Greer, C. W., & Whyte, L. G. (2013). Defining the functional potential and active community members of a sediment microbial community in a high-arctic hypersaline subzero spring. *Applied and environmental microbiology*, 79(12), 3637-3648. https://doi.org/10.1128/AEM.00153-13

Lindsay, M. R.Lindsay, M.R., Dunham, E.C., Boyd, E.S. (2020). Microbialites of Great Salt Lake. En: Baxter, B., Butler, J. (eds) Great Salt Lake Biology. Springer, Cham. https://doi.org/10.1007/978-3-030-40352-2_4

Luo, W., Li, H., Kotut, K., & Krienitz, L. (2017). Molecular diversity of plankton in a tropical crater lake switching from hyposaline to subsaline conditions: Lake Oloidien, Kenya. *Hydrobiologia*, 788, 205-229. https://doi.org/10.1007/s10750-016-2998-x

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One, 8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Moore, L.S., Burne, R.V. (1994). The Modern Thrombolites of Lake Clifton, Western Australia. En: Bertrand-Sarfati, J., Monty, C. (eds) Phanerozoic Stromatolites II. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-1124-9_1

Nagpal, S., Haque, M. M., Singh, R., & Mande, S. S. (2019). iVikodak—a platform and standard workflow for inferring, analyzing, comparing, and visualizing the functional potential of microbial communities. *Frontiers in microbiology, 9*, 3336. https://doi.org/10.3389/fmicb.2018.03336

Nkambo, M., Bugenyi, F. W., Naluwayiro, J., Nayiga, S., & Leonard, W. (2015). Limnological survey of the alkaline, saline Crater Lakes of western Uganda. J*ournal of Natural Science Research*, 5(2).

Paz-Jacome, M. P., Inguaggiato, C., Levresse, G., Robidoux, P., Granados, H. D., & Tassi, F. (2020). Total CO_2 output and carbon origin discharged from Rincón de Parangueo Maar (México). *Journal of Geochemical Exploration*, 106558. https://doi.org/10.1016/j.gexplo.2020.106558

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., . . . Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research, 41*(D1), D590-D596. https://doi.org/10.1093/nar/gks1219

Quéméneur, M., Palvadeau, A., Postec, A., Monnin, C., Chavagnac, V., Ollivier, B., & Erauso, G. (2015). Endolithic microbial communities in carbonate precipitates from serpentinite-hosted hyperalkaline springs of the Voltri Massif (Ligurian Alps, Northern Italy). *Environmental Science and Pollution Research*, 22, 13613-13624.https://doi.org/10.1007/s11356-015-4113-7

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www. R-project. org/.

Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., ... & DesMarais, D. J. (2000). The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, 406(6799), 989-992. https://doi.org/10.1038/35023158

Rocha-Treviño, L. (2015). Análisis de la deformación y hundimiento activo causado por la desecación del lago del mar Rincón de Parangueo (Guanajuato, México) mediante modelos análogos y perfiles de Radar de Penetración Terrestre (RPT). Tesis de maestría, Universidad Nacional Autónoma de México. https://repositorio.unam.mx/contenidos/65241

Roy, C., Rameez, M. J., Haldar, P. K., Peketi, A., Mondal, N., Bakshi, U., ... & Ghosh, W. (2020). Microbiome and ecology of a hot spring-microbialite system on the Trans-Himalayan Plateau. *Scientific Reports*, 10(1), 5917. https://doi.org/10.1038/s41598-020-62797-z

Sánchez-Sánchez, J., Cerca, M., Alcántara-Hernández, R. J., Lozano-Flores, C., Carreón-Freyre, D., Levresse, G., . . . Aranda-Gómez, J. J. (2019). Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico. *FEMS microbiology ecology, 95*(5), fiz051. https://doi.org/10.1093/femsec/fiz051

Sánchez-Sánchez, J., Cerca, M., Alcantara-Hernandez, R. J., Aranda-Gomez, J. J., Carreon-Freyre, D., Lozano-Flores, C., Muñoz-Velasco, I., Levresse, G., & Varela-Echavarria, A. (2021). Mobile mud layer underneath the desiccated maar lake of Rincon de Parangueo and insights into its microbial fingerprints. *Revista Mexicana de Ciencias Geológicas*, 38(3), 178–192. https://doi.org/10.22201/cgeo.20072902e.2021.3.1636

Sánchez‐Sánchez, J., Cerca, M., Moguel, B. B., Muñoz‐Velasco, I., Alcántara‐Hernández, R. J., Carreón‐Freyre, D., & Levresse, G. (2023). Geomicrobiology of the Rincon de Parangueo maar crater: Exploring the link between an evolving extreme environment and its potential metabolic diversity. *Freshwater Biology*, 68, 1818–1838. https://doi.org/10.1111/fwb.14191

Schuler, C. G., Havig, J. R., & Hamilton, T. L. (2017). Hot spring microbial community composition, morphology, and carbon fixation: implications for interpreting the ancient rock record. Frontiers in Earth Science, 5, 97. https://doi.org/10.3389/feart.2017.00097

Solís-Castro, Y., Zúñiga-Zúñiga, L. A., & Mora-Alvarado, D. (2018). La conductividad como parámetro predictivo de la dureza del agua en pozos y nacientes de Costa Rica. *Revista Tecnología en Marcha*, 31(1), 35-46. http://dx.doi.org/10.18845/tm.v31i1.3495

Souza, V., Olmedo-Álvarez, G., & Eguiarte, L. E. (Eds.). (2018). Cuatro ciénegas ecology, natural history and microbiology. New York: Springer International Publishing. https://doi.org/10.1007/978-3-319-93423-5

Stoppa, F., Woolley, A. R., Lloyd, F. E., & Eby, N. (2000). Carbonatite lapilli-bearing tuff and a dolomite carbonatite bomb from Murumuli crater, Katwe volcanic field, Uganda. *Mineralogical Magazine*, 64(4), 641-650.

Strong, W. L. (2016). Biased richness and evenness relationships within Shannon–Wiener index values. Ecological indicators, 67, 703-713.

Tilahun, L. (2010). Diversity of culturable alkaliphilic denitrifying bacteria in four soda lakes of Ethiopia. Addis Ababa University.

Valenzuela-González, F., Casillas-Hernández, R., Villalpando, E., & Vargas-Albores, F. (2015). The 16S rRNA gene in the study of marine microbial communities. *Ciencias Marinas*, 41(4), 297-313.https://doi.org/10.7773/cm.v41i4.2492

Villarino, E., Watson, J. R., Jönsson, B., Gasol, J. M., Salazar, G., Acinas, S. G., ... & Chust, G. (2018). Large-scale ocean connectivity and planktonic body size. *Nature communications*, 9(1), 142. https://doi.org/10.1038/s41467-017-02535-8

Wagaw, S., Mengistou, S., & Getahun, A. (2019). Review of anthropogenic threats and biodiversity assessment of an Ethiopian soda lake, Lake Abijata. *African Journal of Aquatic Science*, 44(2), 103-111. https://doi.org/10.2989/16085914.2019.1596065

Warden, J. G., Coshell, L., Rosen, M. R., Breecker, D. O., Ruthrof, K. X., & Omelon, C. R. (2019). The importance of groundwater flow to the formation of modern thrombolitic microbialites. *Geobiology*, 17(5), 536-550. https://doi.org/10.1111/gbi.12344

Wickham, H. (2016). Data Analysis. In: ggplot2. Use R!. Springer, Cham. https://doi.org/10.1007/978-3-319-24277-4_9

Williams, W. D. (1981). 19. The limnology of saline lakes in Western Victoria: A review of some recent studies. Hydrobiologia, 81, 233-259. https://doi.org/10.1007/BF00048719

Yanez-Montalvo, A., Gomez-Acata, S., Aguila, B., Hernandez-Arana, H., & Falcon, L. I. (2020). The microbiome of modern microbialites in Bacalar Lagoon, Mexico. *PLoS One*, 15(3), e0230071. https://doi.org/10.1371/journal.pone.0230071

Zeyen, N., Daval, D., Lopez-Garcia, P., Moreira, D., Gaillardet, J., & Benzerara, K. (2017). Geochemical conditions allowing the formation of modern lacustrine microbialites. *Procedia Earth and Planetary Science*, 17, 380-383. https://doi.org/10.1016/j.proeps.2016.12.096

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, 30(5), 614-620. https://doi.org/10.1093/bioinformatics/btt593

ANEXOS

Anexo 1.
Tabla suplementaria 1: Distribución mundial de lagos salino-alcalinos con producción de microbialitas en cráteres volcánicos.

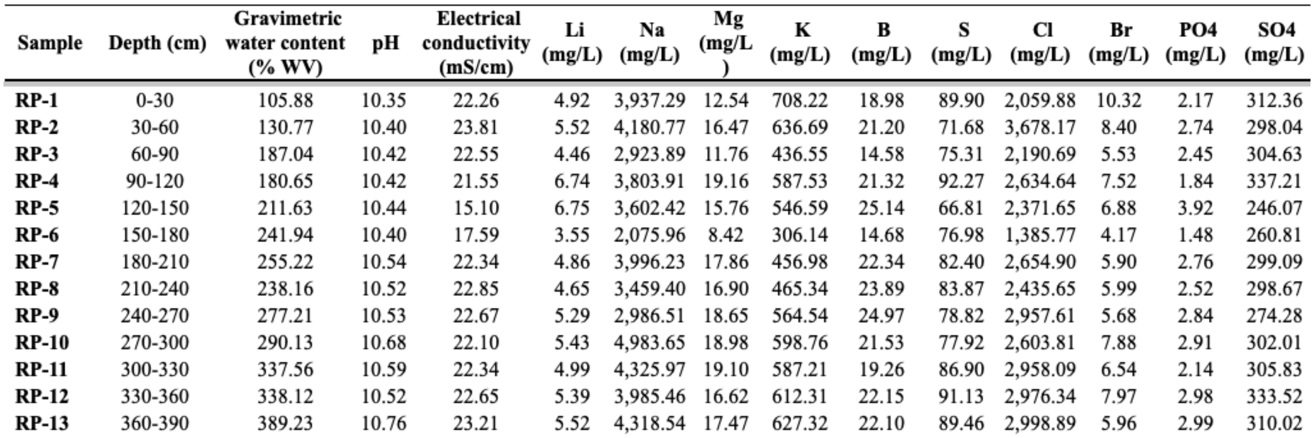
Anexo 2.
Tabla suplementaria 2: Principales características fisicoquímicas de los sedimentos en el cráter Rincón de Parangueo.

Anexo 3.
Artículo de divulgación científica: Sánchez-Sánchez, J., & Cerca, M. Explorando la vida en un ambiente extremo: extremófilos en el cráter volcánico Rincón de parangueo, Guanajuato. (2023). *Enseñanza y Comunicación de las Geociencias*, v. 2, núm. 1, p. 5-9

Tabla suplementaria 1: Distribución mundial de lagos salino-alcalinos con producción de microbialitas en cráteres volcánicos.

AMÉRICA CENTRAL		**Referencia**
México	Alchichica	Kaźmierczak *et al*., 2011
	Rincón de Parangueo	Sánchez-Sánchez *et al*., 2019
	La Alberca	Zeyen *et al*., 2017
	Quechulac	Arredondo-Figueroa *et al*., 1983
	Alberca Los Espinos	Zeyen *et al*., 2017
	Aljojuca	Zeyen *et al*., 2017
	La Preciosa	Zeyen *et al*., 2017
	Joya de Yuriria	Zeyen *et al*., 2017
	Tecuitlapa	Zeyen *et al*., 2017
	Atexcac	Zeyen *et al*., 2017
ASIA		**Reference**
Turquía	Lake Van	Kempe 1991
ÁFRICA		**Reference**
Etiopía	Lake Aranguadi	Baxter *et al*., 1965
	Lake Abiata	Baxter *et al*., 1965
	Lake Shala	Tilahun 2010
Sudán	Dariba lakes	Green *et al*., 1979
Kenia	Lake Simbi	Ballot *et al*., 2005
	Lake Sonachi	Ballot *et al*., 2005
	Lake Oloidien	Luo *et al*., 2017
Uganda	Lake Embagi	Hecky y Kilham 1973
	Lake Magad	Hecky y Kilham 1973
	Lake Katwe	Kasedde *et al*., 2014
	Lake Mahega	Kilham y Melack 1972
	Lake Kikorongo	Nkambo *et al*., 2015
	Lake Nyamunuka	Nkambo *et al*., 2015
	Lake Munyanyange	Stoppa *et al*., 2000
	Lake Murumuli	Nkambo *et al*., 2015
	Lake Nunyampaka	Nkambo *et al*., 2015
AUSTRALIA		**Reference**
Australia	Lake Corangamite	Williams *et al*., 1981
	Red Rock Lake	Williams *et al*., 1981
	Lake Werowrap	Williams *et al*., 1981

Tabla suplementaria 2: Principales características fisicoquímicas de los sedimentos en el cráter Rincón de Parangueo.



Sample	Depth (cm)	Gravimetric water content (% WV)	pH	Electrical conductivity (mS/cm)	Li (mg/L)	Na (mg/L)	Mg (mg/L)	K (mg/L)	B (mg/L)	S (mg/L)	Cl (mg/L)	Br (mg/L)	PO4 (mg/L)	SO4 (mg/L)
RP-1	0-30	105.88	10.35	22.26	4.92	3,937.29	12.54	708.22	18.98	89.90	2,059.88	10.32	2.17	312.36
RP-2	30-60	130.77	10.40	23.81	5.52	4,180.77	16.47	636.69	21.20	71.68	3,678.17	8.40	2.74	298.04
RP-3	60-90	187.04	10.42	22.55	4.46	2,923.89	11.76	436.55	14.58	75.31	2,190.69	5.53	2.45	304.63
RP-4	90-120	180.65	10.42	21.55	6.74	3,803.91	19.16	587.53	21.32	92.27	2,634.64	7.52	1.84	337.21
RP-5	120-150	211.63	10.44	15.10	6.75	3,602.42	15.76	546.59	25.14	66.81	2,371.65	6.88	3.92	246.07
RP-6	150-180	241.94	10.40	17.59	3.55	2,075.96	8.42	306.14	14.68	76.98	1,385.77	4.17	1.48	260.81
RP-7	180-210	255.22	10.54	22.34	4.86	3,996.23	17.86	456.98	22.34	82.40	2,654.90	5.90	2.76	299.09
RP-8	210-240	238.16	10.52	22.85	4.65	3,459.40	16.90	465.34	23.89	83.87	2,435.65	5.99	2.52	298.67
RP-9	240-270	277.21	10.53	22.67	5.29	2,986.51	18.65	564.54	24.97	78.82	2,957.61	5.68	2.84	274.28
RP-10	270-300	290.13	10.68	22.10	5.43	4,983.65	18.98	598.76	21.53	77.92	2,603.81	7.88	2.91	302.01
RP-11	300-330	337.56	10.59	22.34	4.99	4,325.97	19.10	587.21	19.26	86.90	2,958.09	6.54	2.14	305.83
RP-12	330-360	338.12	10.52	22.65	5.39	3,985.46	16.62	612.31	22.15	91.13	2,976.34	7.97	2.98	333.52
RP-13	360-390	389.23	10.76	23.21	5.52	4,318.54	17.47	627.32	22.10	89.46	2,998.89	5.96	2.99	310.02

EXPLORANDO LA VIDA EN UN AMBIENTE EXTREMO: EXTREMÓFILOS EN EL CRÁTER VOLCÁNICO RINCÓN DE PARANGUEO, GUANAJUATO

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RESUMEN

En la Tierra existen microorganismos que viven desafiando los límites de la vida tal y como la conocemos y son llamados "extremófilos". Hasta hace poco, creíamos que la vida sólo era capaz de desarrollarse en ciertas condiciones. Pero en los últimos años, científicos de todo el mundo han encontrado vida microscópica creciendo en condiciones ambientales extremas. En la historia de la Tierra, los microorganismos extremófilos han dejado su huella en forma de microbialitas. En la actualidad, estos pequeños seres aún participan en la formación de microbialitas en muchas partes del mundo. En México se han reportado en varios sitios, como los lagos de cráteres volcánicos en el centro del país, y Rincón de Parangueo es uno de ellos. El lago cráter Rincón de Parangueo es producto de una explosión freatomagmática que hasta hace pocas décadas contenía un lago en su interior; desafortunadamente, las actividades humanas han contribuido a la disminución del nivel del lago. La reducción paulatina del lago ha sido acompañada por el aumento significativo de la salinidad y el pH del agua, propiciando un ambiente extremo para el desarrollo de la vida microbiana extremófila. Dentro del cráter, comunidades microbianas precipitan carbonatos como parte de su actividad biológica, contribuyendo a la formación de microbialitas. Estudios geobiológicos realizados en diferentes zonas del cráter han reportado comunidades microbianas capaces de sobrevivir a las condiciones extremas del lugar. El desarrollo de comunidades microbianas extremófilas se ha documentado principalmente en tres zonas del cráter: 1) antigua costa del lago, 2) pozas remanentes y 3) sedimentos profundos del lago. El desarrollo de microorganismos extremófilos en ambientes tan peculiares como Rincón de Parangueo es de especial importancia para entender el origen, evolución y distribución de la vida en la Tierra. Además, son fuente de conocimiento científico y tecnológico, que puede ser aplicado en la búsqueda de vida en otros planetas con características extremas similares a las identificadas en la Tierra.

Palabras clave: microbialitas, cráter lago, Rincón de Parangueo, microorganismos extremófilos, ambiente extremo.

ABSTRACT

On Earth, some microorganisms that live defying the limits of life as we know it are called "extremophiles". Until recently, it was believed that life was only capable of developing under certain conditions. But in recent years, scientists around the world have found microscopic life growing in extreme environmental conditions. In Earth history, extremophile microorganisms have left their mark in the form of fossil microbialites. At present, these small beings still participate in the formation of microbialites in many parts of the world. In Mexico they have been reported in several places, such as volcanic crater lakes in the center of the country and Rincón de Parangueo is one of them. The Rincón de Parangueo crater lake is the product of a phreatomagmatic explosion, which until a few decades ago contained a perennial lake inside, unfortunately, human activities have contributed to the water level decrease. The gradual desiccation of the lake has been accompanied by a significant increase in the salinity and pH of the water, providing an extreme environment for the development of extremophile microbial life. Within the crater, microbial communities precipitate carbonates as part of their biological activity, contributing to the formation of microbialites. Geobiological studies carried out in different areas of the crater have reported microbial communities capable of surviving the extreme conditions of the place. The development of extremophile microbial communities has been documented mainly in three areas of the crater: 1) the former lake shoreline, 2) remnant pools, and 3) deep lake sediments. The development of extremophile microorganisms in such peculiar environments as Rincón de Parangueo is of special importance to understand the origin, evolution, and distribution of life on Earth. In addition, they are a source of scientific and technological knowledge that can be applied in the search for life on other planets with extreme characteristics similar to those identified on Earth.

Keywords: microbialites, crater lake, Rincón de Parangueo, extremophile microorganisms, extreme environment.

Sánchez Sánchez, Janet y Cerca, Mariano (2023). Explorando la vida en un ambiente extremo: Extremófilos en el cráter volcánico Rincón de Parangueo, Guanajuato. Enseñanza y Comunicación de las Geociencias, v. 2, núm. 1, p. 5-9.

INTRODUCCIÓN

Prepárate porque estás a punto de conocer un lugar en nuestro planeta que pareciera salir de un cuento de ciencia ficción. A pesar del importante papel que juegan los microorganismos en el ambiente, muchos de nosotros tenemos una percepción negativa sobre los microbios en nuestra vida diaria. Conocemos a los microbios por su potencial de causar enfermedades en el ser humano. Sin embargo, olvidamos que son esenciales para sostener la vida en la Tierra y que la mayoría de ellos no causan enfermedades.

Para cambiar la percepción negativa sobre los microorganismos es necesario que el conocimiento científico sea transmitido a la sociedad. Entender qué son los microorganismos, cómo funcionan y la estructura de sus comunidades es necesario para saber cuál es su papel en la naturaleza. Este conocimiento nos podría ayudar a especular sobre la pregunta de si existe vida en otros planetas. Hasta el momento es una pregunta que no es posible contestar con certeza, pero hoy sabemos que existe una gran posibilidad de que la respuesta sea sí. Hasta hace poco, creíamos que la vida sólo era capaz de desarrollarse en las condiciones que consideramos "normales" como son la presencia de oxígeno, una temperatura cercana a 25 °C, presión cercana a 1 atmósfera, pH neutro, ausencia de radiación y ausencia de gases tóxicos.

En algunos lugares de la Tierra, se han descubierto microorganismos llamados "extremófilos" (derivado del Latin "*extremus*" que significa extremo y del Griego "*philia*" que significa amor) capaces de crecer y reproducirse en condiciones muy alejadas de lo que consideramos normal y que serían letales para otras formas de vida (Wharton, 2007; Horikoshi *et al*., 2010). Organismos extremófilos han sido identificados y aislados aquí en la Tierra en lugares que parecen salir de cuentos de ciencia ficción: por ejemplo, ambientes con temperaturas superiores a los 100 °C como fumarolas submarinas, cráteres volcánicos y fuentes hidrotermales (Combie y Runnion, 1996). Otros pueden vivir en medios extremadamente fríos, como el hielo del océano Ártico y los lagos subglaciales del permafrost en la Antártica, con temperaturas de hasta -20 °C (Nicolaus *et al*., 2000). En cambio, otros se han adaptado a vivir en ambientes ácidos como los que existen en las fuentes hidrotermales o en los residuos mineros (Johnson, 2009). También se desarrollan en lugares con una alta concentración de sales y alta radiación solar, como es el caso del desierto de Atacama (Connon *et al*., 2007).

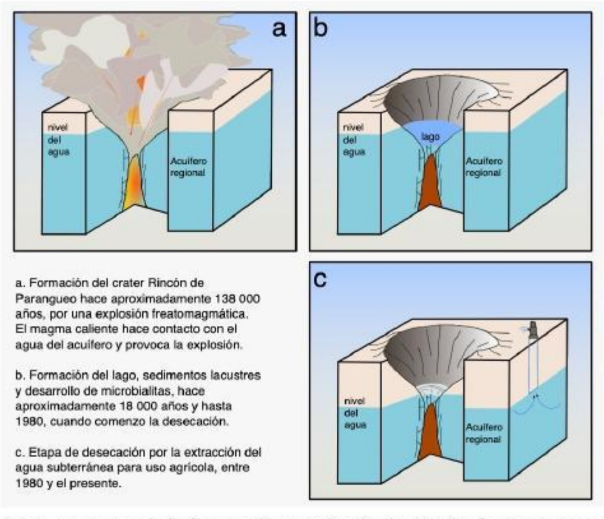
El objetivo de este trabajo es transmitir al público en general conceptos básicos sobre microorganismos extremófilos, contribuir al desarrollo de materiales que faciliten su aprendizaje e informar sobre la existencia de lugares en México y el mundo donde este tipo de microorganismos se desarrollan, en particular Rincón de Parangueo en el estado de Guanajuato, un sitio de gran valor para la comunidad científica. Aquí exploramos la presencia de microorganismos extremófilos en un cráter volcánico y buscamos que este texto y su material audiovisual sirvan como guía para el conocimiento ambiental de la zona.

Microbialitas: las primeras formas de vida en la Tierra

En la historia de la Tierra los microorganismos extremófilos han dejado su huella en forma de microbialitas. El registro fósil más antiguo de microbialitas conocido hasta el momento se encuentra en Warrawoona, Australia, con más de 3 450 millones de años de antigüedad (Allwood *et al*., 2006). En la actualidad, comunidades de microorganismos siguen formando microbialitas en condiciones extremas. Un ejemplo son las estructuras más abundantes y diversas descubiertas en 1954 en la costa de Hamelin Pool Shark Bay en Australia, las cuales se desarrollaron en un ambiente marino hipersalino donde la salinidad puede alcanzar hasta 70 ‰ (partes por mil) o 7 %, el doble del agua de mar normal (Papineau *et al.*, 2005). Otro ejemplo son las aguas termales y géiseres en el Parque Nacional Yellowstone, en los Estados Unidos, donde habitan comunidades bacterianas y algas a temperaturas de hasta 70 °C (Combie y Runnion, 1996). Se desarrollan además en lagos de cráteres intercontinentales con condiciones de alta alcalinidad: tal es el caso de Lake Van en Turquía, donde se han reportado las estructuras más grandes conocidas hasta el momento con una altura de hasta 40 metros. Otras se desarrollan en lagos con regímenes tectónicos activos, como es el caso de África del Este, donde existen condiciones como alta alcalinidad, salinidad y altas temperaturas: ejemplo de ello son el lago Bogoria y el lago Magadi en Kenia; el lago Natron, el lago Magad y el lago Embagi en Tanzania (Casanova, 1986).

¿Existen microbialitas en México?

Si, claro. No es necesario ir muy lejos para encontrarlas. En México el desarrollo de comunidades de microbios produciendo microbialitas se ha reportado en diversos sitios tales como la laguna de Bacalar en el estado de Quintana Roo, la Laguna Mormona y San Quintín en Baja California, Cuatro Ciénegas en Coahuila, Bahía Concepción en Baja California Sur y en los lagos cráter Alchichica en Puebla y Rincón de Parangueo en Valle de Santiago en Guanajuato. En particular, en las zonas volcánicas del centro de México los científicos han identificado comunidades microbianas formadoras de microbialitas en diferentes lagos dentro de cráteres volcánicos, entre los que destacan Alchichica en Puebla; Rincón de Parangueo, La Alberca y Joya de Yuriria, en Guanajuato; y Quechulac, Alberca Los Espinos, Aljojuca, La Preciosa, Tecuitlapa y Atexcac en Michoacán (Iniesto *et al.*, 2021). Estos cráteres son el producto de una explosión freático-magmática, es decir una explosión consecuencia del contacto entre magma y agua subterránea (Figura 1a). Suelen llenarse de agua años después de su formación y crean lagos de poca profundidad (Figura 1b) que en muchas ocasiones contienen agua con una alta salinidad y alcalinidad, lo que permite el desarrollo de microbialitas.



a. Formación del crater Rincón de Parangueo hace aproximadamente 138 000 años, por una explosión freatomagmática. El magma caliente hace contacto con el agua del acuífero y provoca la explosión.

b. Formación del lago, sedimentos lacustres y desarrollo de microbialitas, hace aproximadamente 18 000 años y hasta 1980, cuando comenzo la desecación.

c. Etapa de desecación por la extracción del agua subterránea para uso agrícola, entre 1980 y el presente.

Figura 1. Esquema de la formación y evolución de Rincón de Parangueo en Guanajuato.

En este trabajo presentamos un lugar accesible en el centro de México donde se puede llevar a cabo una excursión para observar de primera mano cómo los microbios pueden crecer en ambientes extremos. Como material de apoyo se elaboró un video con la finalidad de presentar las microbialitas formadas por las comunidades microbianas en este lugar y sus principales características. El video se puede consultar en la siguiente dirección electrónica:

https://www.youtube.com/watch?v=m5Txi1yAUlM

RINCÓN DE PARANGUEO

Un ambiente extremo para el desarrollo de vida extremófila

El cráter lago Rincón de Parangueo (Figura 2a) es clasificado como un ambiente extremo debido a las características fisicoquímicas del agua y a los sedimentos que se encuentran en su interior, tales como un alto contenido de sales, presencia de carbonatos, un pH alcalino ~10, altos niveles de radiación, altas tasas de evaporación y baja precipitación durante la mayor parte del año (Tabla 1, Sánchez-Sánchez *et al.*, 2021). La desecación del lago a partir del año 1980 debido a la sobreexplotación de agua subterránea para su uso en la producción agrícola de la zona (Figura 1c) ha provocado la salinización del agua remanente y ha dejado al descubierto estructuras sedimentarias (microbialitas) constituidas por comunidades microbianas (Figura 2c). Gracias a estudios geobiológicos realizados en Rincón de Parangueo se han identificado comunidades microbianas formadoras de microbialitas pertenecientes a los tres dominios de la vida (*Bacteria, Arquea y Eucaria)* y que además son capaces de sobrevivir a las condiciones extremas del cráter (Sánchez-Sánchez *et al.*, 2019).

Tabla 1. Datos generales del cráter Rincón de Parangueo y algunas propiedades fisicoquímicas del agua.

	Valor	Referencia
Coordenadas	20° 25´ N, -101° 15´ W	GPS diferencial
Altura sobre el nivel del mar	1700 m	Aranda-Gómez *et al.*, 2013
Diámetro del cráter	1300 m	Aranda-Gómez *et al.*, 2017
Edad en miles de años	137 ± 89	Aranda-Gómez *et al.*, 2013
pH	10-11	Sánchez-Sánchez *et al.*, 2021
Conductividad	165 000 µS/cm	Armienta *et al.*, 2008
Temperatura	30 °C	*In situ*
Tipo de agua	CO_2-Cl-Na	Armienta *et al.*, 2008
Nivel de radiación	Alto	www.wunderground.com
Tasa de evaporación	1935 mm/año	Escolero y Alcocer, 2004
Precipitación	715- 738 mm/año	Escolero y Alcocer, 2004

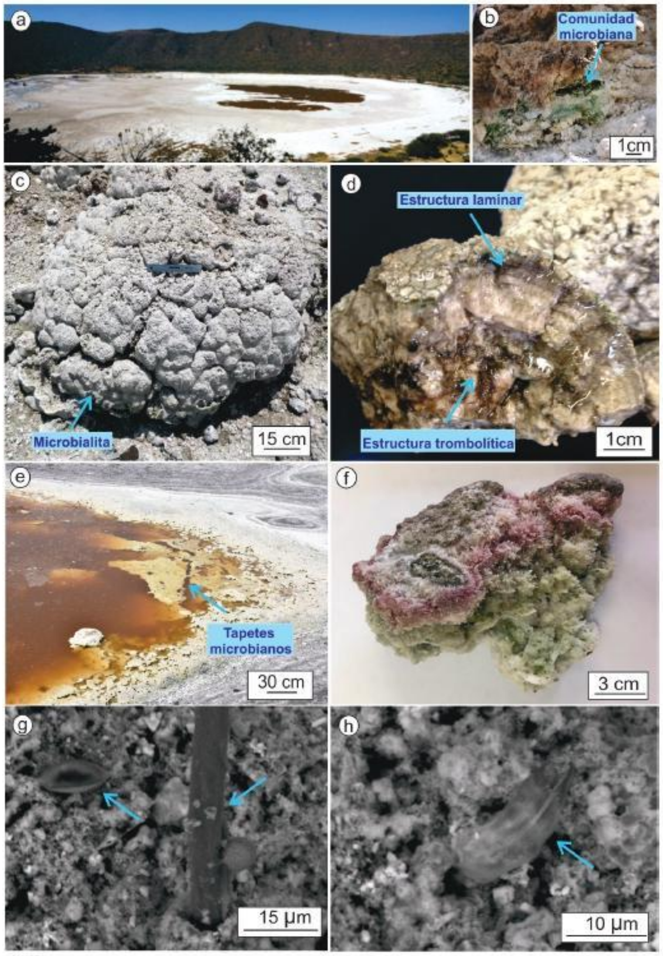


Figura 2. a) Vista panorámica del lago cráter Rincón de Parangueo. Pequeñas charcas se observan en la parte central. b) Sedimentos carbonatados ubicados en la falla geológica principal. El desarrollo de comunidades microbianas se puede apreciar por el color verde de los sedimentos. c) Microbialitas ubicadas en la falla principal del cráter. d) Estructura interna de las microbialitas, caracterizada por una estructura estromatolítica en la parte superficial y una estructura trombolítica hacia la parte central. e) Pequeñas pozas de agua en el centro del cráter. El tamaño y color varían durante el año. f) Tapetes microbianos formados en las pozas. Los colores del tapete son característicos del crecimiento de microorganismos. g), h) Imágenes de microscopía electrónica de muestras de sedimentos. En las imágenes se pueden observar microorganismos (flechas azules).

El desarrollo de comunidades microbianas se ha reconocido especialmente en tres zonas:

1. Antigua costa del lago: en esta zona se ubican las microbialitas con mayor tamaño en todo el cráter, que en promedio alcanzan un tamaño de 1 m de ancho y 1.8 m de largo (Figura 2c). Su estructura interna está caracterizada por una sedimentación laminar o estromatolítica en la parte superficial y una estructura trombolítica, es decir amorfa o irregular, hacia el núcleo (Figura 2d). El desarrollo de microbialitas forma un anillo concéntrico discontinuo alrededor de todo el cráter, que coincide además con la falla geológica principal. Su cercanía a la falla habla del aporte de gases volcánicos, principalmente CO_2, que son emitidos a través de ella y son utilizados por los microorganismos (Figura 2b) para la precipitación de carbonatos, determinando así un mayor desarrollo de microbialitas en comparación con otras zonas del cráter.

2. Pozas remanentes: en la actualidad la mayor parte del lago se encuentra seco, únicamente el 10 % de la zona que cubría el lago permanece cubierto de agua, por lo cual solo existen pequeñas pozas o charcas. Las pozas pueden variar en tamaño y profundidad (siempre menor a 1 metro) dependiendo de la temporada de lluvia o sequía y contienen una gran cantidad de sales carbonatadas, mayormente producto de la evaporación del agua. Dentro de ellas (Figura 2e) habitan comunidades microbianas suspendidas en el agua creando tapetes microbianos, una especie de "balsa" que flota en el agua, y que se forman mayormente en la temporada de sequía. La presencia de microbios en los tapetes microbianos se manifiesta con colores rosa y verde que es posible observar a simple vista (Figura 2f). No solo en los tapetes microbianos existen microorganismos: una gran cantidad de ellos se encuentran también suspendidos flotando en el agua, lo que explica el cambio de color con las estaciones.

3. Sedimentos profundos del lago: si lo anterior te parece poco, también es posible que estos diminutos seres puedan sobrevivir en el subsuelo del cráter, en los sedimentos que quedaron sepultados hace miles de años. Muestras tomadas a una profundidad de 4 m fueron analizadas en un microscopio electrónico sugiriendo la existencia de microorganismos en los sedimentos (Figuras 2g y 2h). Lo anterior ha llevado a los científicos a hacerse varias preguntas: ¿Hasta dónde es posible que exista la vida en lo profundo de los sedimentos del cráter? o ¿Es posible que microorganismos como los encontrados en Rincón de Parangueo existan en lo profundo de los sedimentos en otros cráteres?

El especial interés en los microorganismos extremófilos identificados en Rincón de Parangueo es debido a las capacidades específicas de adaptación y supervivencia para desarrollarse en un ambiente tan peculiar. Hoy sabemos que dentro del cráter existen microorganismos capaces de resistir altos niveles de salinidad, radiación intensa y altos índices de evaporación, así como microorganismos capaces de sobrevivir a temperaturas relativamente altas ~ 60 °C.

Algunos de los microorganismos extremófilos identificados en Rincón de Parangueo también se han identificado en sitios del mundo con características similares, como una alta salinidad, alcalinidad y altas temperaturas (se presenta una lista con ejemplos en la Tabla 2). Sin embargo, algunos microorganismos reconocidos son exclusivos de este lugar (endémicos), por lo que es posible identificar especies con características únicas y especiales que las convierten en potenciales fuentes de conocimiento científico y tecnológico para el desarrollo de biotecnología aplicada (producción de alimentos, bebidas, antibióticos, biofertilizantes, etc.) en diferentes industrias a nivel global.

¿VIDA EN OTROS PLANETAS?

Como ya te pudiste dar cuenta, las comunidades microbianas descubiertas en Rincón de Parangueo viven en un ambiente extremo y su capacidad para desarrollar estrategias de supervivencia es un punto clave. Por ejemplo, algunos microorganismos pueden crecer en un medio hipersalino gracias a mecanismos celulares como la acumulación de grandes concentraciones de KCl en el citoplasma y tienen la capacidad de producir proteínas resistentes a las altas concentraciones de sal (DasSarma y DasSarma, 2017). Otros cuentan con mecanismos de reparación para el material genético dañado por la alta radiación solar (Crowley *et al.*, 2006) y también existen los que

Tabla 2. Principales microorganismos extremófilos identificados en Rincón de Parangueo y que también se han encontrado en otras partes del mundo.

Género	Especie	Ubicación
Rhodobaca sp.	*Rhodobaca bogoriensis* *Rhodobaca barguzinensis*	Agua y sedimentos del Lago Bogoria, Kenia Lago Barguzin valley, Siberia
Nitriliruptor sp.	*Nitriliruptor alkaliphilus*	Sedimentos del lago Estepa de Kulunda, Rusia
Nocardiopsis sp.	*Nocardiopsis valliformis*	Sedimentos del lago Xinjiang, China
Psychroflexus sp.	*Psychroflexus salis* *Psychroflexus planctonicus*	Lago salado en la provincia Qinghai, China
Defluviimonas sp.	*Defluviimonas indica*	Chimenea hidrotermal, mar del suroeste de India
Microvirga sp.	*Microvirga subterránea*	Agua del acuífero hidrotermal Great Craft Basin
Elioraea sp.	*Elioraea tepidiphila*	Fuente termal en la isla de Sao Miguel, Azores
Rubrobacter sp.	*Rubrobacter radiotolerans*	Fuente termal Sao Pedro do Sul, Portugal
Belliella sp.	*Belliella pelovolcani*	Lodo volcánico en Wandan, Taiwán
Cecembia sp.	*Cecembia lonarensis*	Agua de lago en Maharashtra, India
Thermomicrobium sp.	*Thermomicrobium carboxidum*	Tapetes microbianos del volcán Kilauea Hawái, USA
Salinarimonas sp.	*Salinarimonas ramus*	Suelo salino de un yacimiento petrolero en Shengli, China
Porphyrobacter sp.	*Porphyrobacter tepidarius*	Tapetes microbianos en aguas termales en Shidzuoka, Japón
Picocystis sp.	*Picocystis salinarum*	Lagos salinos y chimeneas hidrotermales en África del Este
Halosimplex sp.	*Halosimplex carlsbadense*	Cristales de sal de la formación Salado en el sureste de Nuevo México
Natronolimnobius sp.	*Natronolimnobius innermongolicus*	Lagos en el interior de Mongolia, China
Natrialba sp.	*Natrialba aegyptiaca*	Suelos hipersalinos en Asuán, Egipto
Methanosalsum sp.	*Methanosalsum natronophilum*	Sedimentos de lagos salados en la estepa de Kulunda, Rusia
Methanolobus sp.	*Methanolobus oregonensis*	Agua de lago salino en el cráter Lonar, India

producen vesículas de gas dentro de sus células que les permiten flotar a lugares donde la concentración de oxígeno es mayor (Pfeifer, 2015). Algunos, además, tienen la capacidad de colonizar los poros de las rocas y/o las fisuras de los sedimentos evitando cambios drásticos de temperatura y humedad, lo que les da una ventaja para sobrevivir (Sajjad *et al*., 2022). El desarrollo de comunidades extremófilas en ambientes tan peculiares es de especial importancia para entender el origen, evolución y distribución de la vida en la Tierra. Este conocimiento puede ser aplicado en la búsqueda de vida en planetas con características extremas similares a las identificadas en la Tierra. La posibilidad de que microorganismos extremófilos puedan sobrevivir en otros planetas es intrigante. Microorganismos adaptados a altas concentraciones de sal, altas temperaturas y radiación solar pueden ser modelos clave en la búsqueda de vida en otras partes del universo. Comunidades microbianas similares a las descubiertas en Rincón de Parangueo y en otros lugares de la Tierra podrían estar escondidas dentro de las rocas y/o los sedimentos en planetas como Marte, donde no existe una fuente abundante de agua, los nutrientes son escasos, el suelo contiene sales y existe una alta radiación solar.

CONCLUSIONES

Estudiar lugares con características extremas como el cráter Rincón de Parangueo en el estado de Guanajuato es muy importante para entender el desarrollo de la vida en nuestro planeta y tal vez aún más lejos. Microorganismos extremófilos podrían sobrevivir no sólo en la Tierra sino también en otros planetas. Por ello, es importante alentar a la conservación de sitios donde se desarrolla la vida extrema, pues son el hogar de una diversidad enorme de microorganismos que han sobrevivido por miles de años. Sería lamentable que lugares con características tan peculiares desaparecieran como consecuencia del impacto que las actividades humanas tienen en ellos. Desafortunadamente, Rincón de Parangueo es también un lugar donde la sobreexplotación de agua subterránea, deforestación, contaminación y uso recreativo de forma inapropiada han provocado el deterioro ambiental en los últimos años, convirtiéndose en un grave problema. La buena noticia es que aún estamos a tiempo de rescatar este hermoso lugar. La planeación e implementación de estrategias que ayuden a la conservación de las estructuras microbianas requieren de la interacción entre los tomadores de decisiones, científicos y la comunidad local.

REFERENCIAS

Altermann, W. (2008). Accretion, trapping and binding of sediment in Archean stromatolites—morphological expression of the antiquity of life. Space Science Reviews, 135(1), 55-79.

Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., y Burch, I. W. (2006). Stromatolite reef from the Early Archaean era of Australia. Nature, 441(7094), 714-718.

Aranda-Gómez, J. J., Levresse, G., Pacheco Martínez, J., Ramos-Leal, J. A., Carrasco-Núñez, G., Chacón-Baca, E. y Noyola-Medrano, C. (2013). Active sinking at the bottom of the Rincón de Parangueo Maar (Guanajuato, México) and its probable relation with subsidence faults at Salamanca and Celaya. Boletín de la Sociedad Geológica Mexicana, 65(1), 169-188.

Aranda-Gómez, J. J., Cerca, M., Rocha-Treviño, L., Carrera-Hernández, J. J., Levresse, G., Pacheco, J. y Beraldi-Campesi, H. (2017). Structural evidence of enhanced active subsidence at the bottom of a maar: Rincón de Parangueo, México. Geological Society, London, Special Publications, 446(1), 225-254.

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

Casanova, J. (1986). East African rift stromatolites. Geological Society, London, Special Publications, 25(1), 201-210.

Combie, J., y Runnion, K. (1996). Looking for diversity of Yellowstone extremophiles. Journal of industrial microbiology and biotechnology, 17(3-4), 214-218.

Connon, S. A., Lester, E. D., Shafaat, H. S., Obenhuber, D. C., y Ponce, A. (2007). Bacterial diversity in hyperarid Atacama Desert soils. Journal of Geophysical Research: Biogeosciences, 112(G4).

Crowley, D. J., Boubriak, I., Berquist, B. R., Clark, M., Richard, E., Sullivan, L., y McCready, S. (2006). The uvrA, uvrB and uvrC genes are required for repair of ultraviolet light induced DNA photoproducts in Halobacterium sp. NRC-1. Saline systems, 2, 1-13.

DasSarma, S., y DasSarma, P. (2017). Halophiles. eLS, 1-13.

Escolero, O., y Alcocer, J. (2004). Desecación de los lagos cráter del Valle de Santiago, Guanajuato. In: El agua en México vista desde la Academia. (Eds: Jiménez, B., y Marín, L) México City, Academia Mexicana de Ciencias, 99-115.

Horikoshi, K., Antranikian, G., Bull, A. T., Robb, F. T., y Stetter, K. O. (2010). Extremophiles handbook: Springer Science y Business Media.

Iniesto, M., Moreira, D., Reboul, G., Deschamps, P., Benzerara, K., Bertolino, P., y López--García, P. (2021). Core microbial communities of lacustrine microbialites sampled along an alkalinity gradient. Environmental Microbiology, 23(1), 51-68.

Johnson, D. B. (2009). Extremophiles: acidic environments. The desk encyclopedia of microbiology, 463-480.

Nicolaus, B., Lama, L., Esposito, E., Bellitti, M. R., Improta, R., Panico, A., y Gambacorta, A. (2000). Extremophiles in Antarctica. Italian Journal of Zoology, 67(S1), 169-174.

Papineau, D., Walker, J. J., Mojzsis, S. J., y Pace, N. R. (2005). Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Applied and Environmental Microbiology, 71(8), 4822-4832.

Pfeifer, F. (2015). Haloarchaea and the formation of gas vesicles. Life, 5(1), 385-402.

Sajjad, W., Ilahi, N., Kang, S., Bahadur, A., Zada, S., y Iqbal, A. (2022). Endolithic microbes of rocks, their community, function and survival strategies. International Biodeterioration y Biodegradation, 169, 105387.

Sánchez-Sánchez, J., Cerca, M., Alcántara-Hernández, R. J., Lozano-Flores, C., Carreón-Freyre, D., Levresse, G. y Aranda-Gómez, J. J. (2019). Extant microbial communities in the partially desiccated Rincon de Parangueo maar crater lake in Mexico. FEMS microbiology ecology, 95(5), fiz051.

Sánchez-Sánchez, J., Cerca, M., Alcantara-Hernandez, R. J., Aranda-Gomez, J. J., Carreon-Freyre, D., Lozano-Flores, C., y Varela-Echavarria, A. (2021). Mobile mud layer underneath the desiccated maar lake of Rincon de Parangueo and insights into its microbial fingerprints. Revista Mexicana de Ciencias Geológicas, 38(3), 178-192.

Wharton, D. A. (2007). Life at the limits: organisms in extreme environments. Cambridge University Press.

Manuscrito recibido: 7 de octubre de 2022
Manuscrito corregido recibido: 14 de marzo de 2023
Manuscrito aceptado: 18 de marzo de 2023