

Diversity of an uncommon elastic hypersaline microbial mat along a small-scale transect

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ABSTRACT

We evaluated the microbial diversity and metabolome profile of an uncommon hypersaline elastic microbial mat from Cuatro Ciénegas Basin (CCB) in the Chihuahuan Desert of Coahuila, México. We collected ten samples on a small scale transect (1.5-m) and described its microbial diversity through NGS-based ITS and 16S rDNA gene sequencing. A very low number of taxa comprised a considerable proportion of the mat and were shared across all sampling points, whereas the rare biosphere was more phylogenetically diverse (Faith's Phylogenetic Diversity (FPD) index) and phylogenetically disperse (using a null model distribution of Phylogenetic Species Clustering (nmdPSC)) than the abundant (high read count) taxa for both analyzed libraries. We also found a distinctive metabolome profile for each sample and were able to tentatively annotate several classes of compounds with relevant biological properties.

Subjects Biodiversity, Biogeography, Ecology, Microbiology

Keywords Cuatro Ciénegas Coahuila, Microbial communities, Phylogenetic diversity, Rare biosphere, Metabolome analysis

INTRODUCTION

Microbial mats are tightly interacting communities, usually self-sustaining, that are horizontally stratified with multilayered biofilms embedded in a matrix of exopolysaccharides and inorganic substances that bind cells together (*Bolhuis, Cretoiu & Stal, 2014; Prieto-Barajas, Valencia-Cantero & Santoyo, 2018; Stolz, 2000*). These stratified structures, as well as stromatolites, are the oldest communities found in the fossil record,

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informing our understanding of ancient metabolic interactions (Gutiérrez-Preciado *et al.*, 2018; Nutman *et al.*, 2016).

Although modern microbial mats and stromatolites are geographically widespread (Gerdes, 2010), their distribution is restricted to extreme environmental conditions where multicellular algae and grazing organisms cannot grow (Bebout *et al.*, 2002). One such site, with a high diversity of microbial mats and stromatolites, is the desert oasis of the Cuatro Ciénegas Basin (CCB), found in the Chihuahuan Desert of the state of Coahuila in northern México. This small site (150,000 km²) is extremely biodiverse—in particular for microbes (Souza, Olmedo-Álvarez & Eguiarte, 2018)—and is considered of international importance by the RAMSAR convention (www.ramsar.org). CCB is characterized by an imbalanced stoichiometry (*i.e.*, very low concentration of phosphorus) (Elser *et al.*, 2005, 2006; Papineau, 2010; Planavsky *et al.*, 2010), which appears to be a strong selective pressure driving microbial lineages to adapt locally (*e.g.*, the reported *Bacillus coahuilensis* genes involved in phosphorous utilization efficiency (Alcaraz *et al.*, 2008)). The uniqueness of microbial life in CCB—deemed as an “Astrobiological Precambrian Park” (Souza *et al.*, 2012)—resides in the presence of taxa related to marine lineages (Rebollar *et al.*, 2012; Souza *et al.*, 2006; Souza, Olmedo-Álvarez & Eguiarte, 2018), rich prokaryotic communities (López-Lozano *et al.*, 2013; Pajares *et al.*, 2012, 2013; Souza, Olmedo-Álvarez & Eguiarte, 2018), and diverse fungal (Velez *et al.*, 2016) and viral communities (Taboada *et al.*, 2018), as well as a high microbial diversity within the microbial mats and stromatolites (Bonilla-Rosso *et al.*, 2012; De Anda *et al.*, 2018; Peimbert *et al.*, 2012).

In March 2016, we discovered an outermost elastic layer microbial mat in CCB, which enables the formation of dome-like structures under wet conditions (Fig. 1).

The internal microbial layer, insulated by an outer aerobic community, thrives under an anaerobic environment, rich in methane, hydrogen sulfide, and small volatile hydrocarbons that recreate the atmosphere of the Archaean Eon, hence we named these mats “Archaean Domes” (Medina-Chávez *et al.*, 2019). Similar types of elastic mats have previously been described in marine tidal zones (Gerdes *et al.*, 1993).

Microbial mats are ideal systems for the study of microbial community structure under extreme conditions (Paerl & Yannarell, 2010; Prieto-Barajas, Valencia-Cantero & Santoyo, 2018). In this sense, there are approaches that can improve our understanding of microbial mat communities, such as determining the phylogenetic diversity of communities under extreme environmental conditions where, for example, abiotic filtering and competitive exclusion have been reported as drivers of phylogenetic clustering (Goberna *et al.*, 2014); another possible approach is to describe microbial subcommunities, *e.g.*, those of rare taxa, a less-studied topic than the whole community (Liu *et al.*, 2019) but nevertheless important to explore (Jia, Dini-Andreote & Falcão Salles, 2018); other form is to analyze the metabolome of communities, which is one of the functional expressions of diversity and an underexplored aspect of microbial communities that can help complete our understanding of the observed diversity.

The investigation of microbial mats has gained special relevance in recent years with regards to the interpretation of structuring processes in microbial communities. However, hypersaline elastic mats remain poorly studied, limiting our understanding of microbial

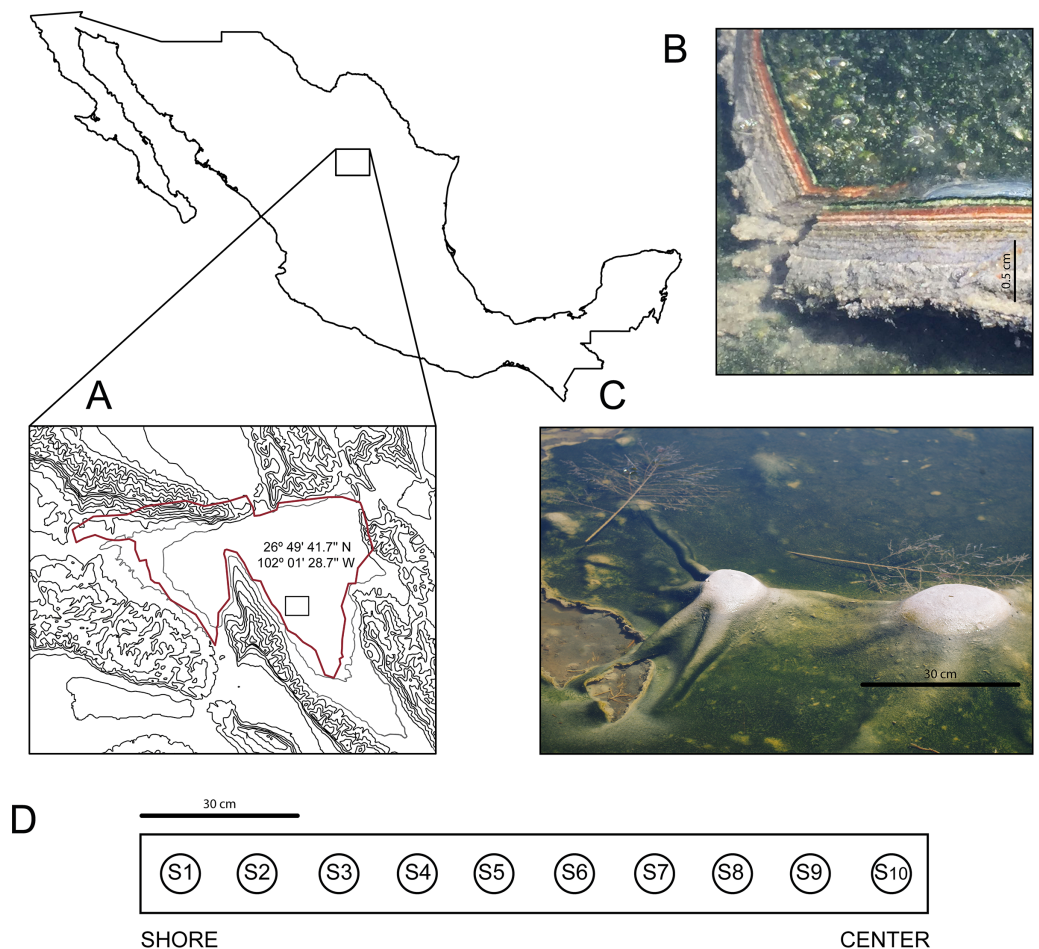


Figure 1 Site and sample. (A) The Cuatro Ciénegas Basin within México and geographic location of the Archean Domes in Pozas Azules Ranch. (B) Details of an Archean Domes slice. (C) Domes when the sampling was conducted (note the size scale). (D) Sampling design along border toward center 1.5-m transect (sample 1 (S1) to sample 10 (S10)). Photo credit: David Jaramillo.

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diversity and functioning under extreme environmental conditions (*e.g.*, high salinity concentrations, elevated evaporation and frequent desiccation cycles). In this study, we evaluated the microbial diversity and metabolome profile within this elastic hypersaline microbial mat from CCB, across ten samples collected on a 1.5-m transect. Specifically, we aimed: (1) to explore microbial diversity by analyzing amplicons of the 16S rDNA and ITS regions, (2) to determine whether rare (low read count) and abundant (high read count) taxa are phylogenetically structured; and (3) to evaluate the microbial metabolome.

MATERIALS AND METHODS

Sampling site and sample collection

The Archean Domes (26°49'41.7"N, 102°01'28.7"W) is a seasonal, water-fluctuating oval pond (50 m × 25 m (*Madrigal-Trejo, 2022*)). During the dry season (*i.e.*, most of the year at CCB; *Montiel-González et al., 2018*), the microbial mats of the Archean Domes remain

humid under a salty crust, which is then dissolved during the rainy season, when the pond fills with water up to ≈ 20 cm, allowing for the development of the dome-like structures.

The microbial mats of the Archaean Domes were sampled during the rainy season of 2018 (October), with ten evenly spaced samples (S1–S10) along a 1.5-m transect, from the border toward the center, in the ‘Pozas Azules’ ranch of Pronatura Noroeste within CCB (Fig. 1). Salinity and pH were constant along the 1.5-m transect (salinity 5.25%, pH 9.8), and the average nutrient content showed only a slight stoichiometric imbalance (C:N:P 122:42:1), as *Medina-Chávez et al. (2019)* reported.

Microbial mats were collected and transferred to sterile conical tubes (50 mL), stored at 4 °C, and subsequently frozen in liquid nitrogen until processing in the laboratory. Sampling was under the collection permit SGPA/DGVS/03188/20 issued by Subsecretaría de Gestión para la protección Ambiental, Dirección General de Vida Silvestre.

Total DNA extraction and amplicons sequencing

Prior to DNA extraction, approximately 0.5 g of each mat sample was rinsed with sterile water, removing associated sediments. Total DNA extraction was performed following the protocol reported in *De Anda et al. (2018)*.

DNA was sequenced at the Laboratorio de Servicios Genómicos, LANGEBIO (<http://langebio.cinvestav.mx/labsergen/>) for the V3 region of the 16S rDNA gene (357F universal primer 5'-CTCCTACGGGAGGCAGCAG-3'/519R universal primer 5'-GWATTACCGCGGCKGCTG-3'; (*Lane, 1991*)), and the ITS DNA region (ITS1F 5'-TCCGTAGGTGAACCTGCGG-3'/ITS4R 5'-TCCTCCGCTTATTGATATGC-3' for ITS; (*White et al., 1990*)). For each sample, 3 μ g of genomic DNA (DO 260/280 1.8) was used; 16S amplicons were sequenced within Illumina Next-Seq 500 platform (2 \times 150 PE, using forward reads; reverse reads were discarded from the analysis due to low quality), and the ITS amplicons were sequenced with a Mi-Seq platform (2 \times 300 PE).

Bioinformatics analyses

The 16S rDNA amplicon reads were quality filtered and dereplicated using the QIIME 2 bioinformatics platform version 2019.7 (*Bolyen et al., 2018*). Divisive Amplicon Denoising Algorithm 2 (dada2) denoise-single was used; sequences were demultiplexed and truncated at the 20th bp from the left, and the 120th bp from the right. ASVs were then taxonomically classified using the silva_132_99_v3v4_q2_2019-7 database (<https://www.arb-silva.de/>). For all diversity analyses the final ASV table was rarefied to 760,000 reads per sample.

The ITS amplicon reads were processed using the ITS-specific workflow of the *dada2* v1.13.1 package (*Callahan et al., 2016*) in R (*R Core Team, 2020*). Briefly, *cutadapt* was used to remove primer and adapter contamination from the raw reads (*Martin, 2011*). Prior to determining ASVs, reads with inconsistent bases were excluded, and reads were required to have less than two expected errors based on their quality scores. ASV classification was inferred from forward and reverse reads using the run-specific error rates.

Due to the wide range of sizes of the ITS amplicons reported for fungal species in CCB (485–782 bp; (Velez *et al.*, 2016)), two different strategies were implemented to define taxonomic diversity. First, the post-denoised ASVs were merged after excluding reads with an overlap of less than 12 bp and reads with mismatches in the overlapping region. Chimeras were removed using the consensus method of “removeBimeraDenovo” implemented in *dada2*. The taxonomic assignment was performed with the UNITE database (Nilsson *et al.*, 2019), using the RDP naive Bayesian classifier (Wang *et al.*, 2007) available in the *dada2* package with a minimum bootstrap value of 80. ASVs with a minimum of 85% identity and with E-values lower than e^{-45} were retained (Merge ITS data: Table S1). ASVs without taxonomic assignment (mostly non-fungi organisms) were classified using *blastn* (non-redundant nucleotide collection (nr/nt) database) (Altschul *et al.*, 1990), where only those ASVs within identities greater than 90% were enlisted (Table S2). The remaining ASVs were considered non-classified.

As a great proportion of the reads failed to merge (~56%), a parallel classification was implemented. The RDP naive Bayesian classifier was used on the two sets of reads (forward and reverse) to check for congruence in the classification between the ASVs. ASVs that had an assignment bootstrap value lower than 50, or showed inconsistent classifications among forward and reverse reads, were aligned to the NCBI database (non-redundant nucleotide collection (nr/nt) database), to corroborate their taxonomic assignment. A more robust classification of ASVs was reached when using forward ASVs, thus all subsequent analyses were performed using the Forward-Only ASVs; even though some ASVs reached a genus level assignment, the taxonomic classification of forward reads was limited to the phylum and kingdom level (ITS table on Tables S3 and S4); the Eukaryotic classification followed the UNITE database. The ITS community composition table (Forward-Only data) was rarefied to 164,820 reads using the *vegan* package (Oksanen *et al.*, 2013) in R (R Core Team, 2020).

In sum, the filtered 16S rDNA amplicon sequences included 760,326–1,973,982 high quality reads per sample, with a total of 6,063 ASVs (Table S5). The filtered ITS amplicon sequences included 164,822–339,394 high quality reads per sample, with a total of 923 ASVs (Table S4). ITS amplicons were obtained for only six samples (S1, S2, S3, S4, S6 and S7) due to low DNA amount that remained in four samples (S5, S8, S9 and S10), after 16S rDNA amplicon sequencing.

Alpha and beta diversity estimates

To evaluate all samples from 16S (S1–S10) and six samples (S1–S4, S6, S7) from the Forward-Only ITS data, we generated rarefied diversity plots using the *vegan* package (Oksanen *et al.*, 2013) in R (R Core Team, 2020).

In order to visualize and count the number of overlapping ASVs within the ten samples from the 16S rDNA gene and the six samples from the ITS region, we constructed an UpSet plot using the *UpSetR* package (Conway, Lex & Gehlenborg, 2017) in R (R Core Team, 2020).

Alpha diversity (Shannon index) and beta diversity (Bray-Curtis, Jaccard distances) were estimated using the 16S rDNA ASV community composition table (Table S5), as

implemented in QIIME 2. For the Forward-Only ITS ASV community composition table (Table S4), alpha and beta diversity were estimated with the *phyloseq* package (McMurdie & Holmes, 2013) in R (R Core Team, 2020). The beta diversity distance matrix was used to construct an UPGMA dendrogram of samples (Carteron et al., 2012). Mantel tests (999 permutations) (Legendre & Legendre, 2012) were conducted with the *vegan* package (Oksanen et al., 2013) to unveil the relationships between beta diversity and geographic distance, as well as between beta diversity and the metabolomic profile distance matrix (see below).

Phylogenetic diversity

We estimated phylogenetic diversity within samples using Faith's Phylogenetic Diversity index (Faith, 1992) and the Phylogenetic Species Clustering index (Helmus et al., 2007) using the *picante* package (Kembel et al., 2010) in R (R Core Team, 2020). Given a phylogenetic tree, phylogenetic diversity (PD) estimates the total length of branches encompassing a subset of taxa (e.g., sampled communities), where PD increases as the subset of taxa is more phylogenetically diverse (Faith, 1992). Phylogenetic Species Clustering (PSC) is a measure of the degree of clustering of taxa across the phylogeny (Helmus et al., 2007), where a PSC value approaching zero indicates that the taxa analyzed were phylogenetically clustered.

To estimate the two metrics, we used the rarified diversity matrices and the corresponding phylogenetic trees for bacterial and fungal ASVs, separately. We tested the observed patterns of PSC across samples against 1,000 replicates under a null model and estimated standardized effect sizes (SES) for this index (null model Phylogenetic Species Clustering, nmPSC). This allowed us to identify samples that had more or less phylogenetic clustering than expected by chance alone. To construct the null model, we randomized the entries of the rarified diversity matrices while keeping total sample diversity constant. In addition, we estimated PD and PSC for the most abundant and rare ASVs within each site. To define rare (low proportionated or low read count) and abundant (high proportionated or high read count) ASVs, we estimated the first and third quartiles (1Q, 3Q) of the distribution of ASV (organized by low to high read count), for each site separately. We used the sites' 1Q and 3Q as the thresholds to define rare and abundant ASVs; the sites' 1Qs ranged from 3 to 16 reads per ASV, whereas the 3Qs ranged from 37 to 306 reads per ASV; rare and abundant ASV numbers using this method were equivalent per site. We estimated SES for PD and PSC as defined above, using matrices for rare and abundant ASV.

The phylogenetic tree was generated by QIIME 2 (Bolyen et al., 2018) for 16S data. For the ITS data (Forward-Only data), a phylogeny was obtained by applying an equal phylogenetic QIIME 2 methodology, using Maftt (Katoh & Toh, 2010) and FastTree (Price, Dehal & Arkin, 2009) within Cipres Science Gateway (Miller, Pfeiffer & Schwartz, 2010) with default XSEDE parameters.

Metabolomics

Soluble compounds extraction and LC-MS/MS analysis

The samples (10 g each) were extracted with 60 mL of CHCl_3 -MeOH (1:1) in an orbital shaker at 150 rpm for 24 h. The mixture was filtered, and the solvent was evaporated under reduced pressure. The dried extracts were reconstituted in 60 mL of CH_3CN -MeOH (1:1) and defatted with hexanes. Then the CH_3CN -MeOH layer was dried under a vacuum and resuspended in MeOH (LC-MS grade) to yield a concentration of 1 mg mL^{-1} .

All samples were filtered with a $0.22 \mu\text{m}$ membrane and analyzed using a Waters Acquity Ultraperformance Liquid Chromatography (UPLC) system (Waters Corp., Milford, MA, USA) coupled to a Thermo Q Exactive Plus (Thermo Fisher, Waltham, MA, USA) mass spectrometer. The UPLC separations were performed using an Acquity BEH C_{18} column ($50 \text{ mm} \times 2.1 \text{ mm I.D.}$, $1.7 \mu\text{m}$; Waters) equilibrated at $40 \text{ }^\circ\text{C}$ and a flow rate set at 0.3 mL min^{-1} . The mobile phase consisted of a linear CH_3CN - H_2O (acidified with 0.1% formic acid) gradient starting at 15% to 100% of CH_3CN over 8 min. The mobile phase was held for another 1.5 min at 100% CH_3CN before returning to the starting conditions. High-resolution mass spectrometry (HRMS) data and MS/MS spectra were collected in the positive/negative switching electrospray ionization (ESI) mode at a full scan range of m/z 150–2,000, with the following settings: capillary voltage, 5 V; capillary temperature, $300 \text{ }^\circ\text{C}$; tube lens offset, 35 V; spray voltage 3.80 kV; sheath gas flow and auxiliary gas flow, 35 and 20 arbitrary units, respectively.

Untargeted metabolomics

The HRMS-MS/MS raw data was individually aligned and filtered with MZmine 2.17 software (<http://mzmine.sourceforge.net/>) (Pluskal *et al.*, 2010). Peak detection was achieved as follows: m/z values were detected within each spectrum above a baseline, a chromatogram was constructed for each of the m/z values that spanned longer than 0.1 min, and finally, deconvolution algorithms were applied to each chromatogram to recognize the individual chromatographic peaks. The parameters were set as follows for peak detection: noise level (absolute value) at 1×10^6 , minimum peak duration 0.5 s, tolerance for m/z variation 0.05, and tolerance for m/z intensity variation 20%.

Deisotoping, peak list filtering, and retention time alignment algorithm packages were employed to refine peak detection. The join align algorithm compiled a peak table according to the following parameters: the balance between m/z and retention time was set at 10.0 each, m/z tolerance at 0.05, and retention time tolerance size was defined as 2 min. The spectral data matrix (comprised of m/z , retention time, and peak area for each peak) was imported to Excel (Microsoft) for heatmap analysis using R (R Core Team, 2020).

Molecular networking was performed from the mzML files using the standard Global Natural Products Social (GNPS) molecular networking platform workflow with the spectral clustering algorithm (<http://gnps.ucsd.edu>; Aron *et al.*, 2020). For spectral networks, parent mass of 0.01 Da and fragment ion tolerance of 0.02 Da were considered. For edges construction, a cosine score over 0.70 was fitted with a minimum of four matched peaks, and two nodes at least in the top 10 cosine scores (K) and maximum of 100 connected the components. After that, the network spectra were searched against GNPS

spectral libraries, and graphic visualization of molecular networking was performed in Cytoscape 3.8.0 (Shannon *et al.*, 2003). Chemical structural information within the molecular network was obtained using the GNPS MolNetEnhancer workflow (Ernst *et al.*, 2019), which incorporated *in silico* structure annotations from the GNPS library search (metabolomic_support_info.zip in Supplemental Files). The chemical profiles were also manually dereplicated using UV-absorption maxima and HRMS-MS/MS data against the Dictionary of Natural Products v 29.1 and Dictionary of Marine Natural Products 2019 (Taylor and Francis Group, Abingdon, United Kingdom), MarinLite (University of Canterbury, Christchurch, New Zealand) and SciFinder (CAS) databases as described by El-Elimat *et al.* (2013), for fungal and bacterial small molecules. For candidate search, exact mass accuracy was set to 5 ppm (Sumner *et al.*, 2007). Heatmap visualization of metabolomic data table was generated in MetaboAnalyst (Pang *et al.*, 2020).

RESULTS

Microbial taxonomic composition

Overall, 40 prokaryote phyla were detected from the 16S rDNA gene libraries (Fig. S1A), and a total of 6,063 different ASVs were found, with a total proportion of 11.8% of unclassified reads (Table S3). Bacteroidetes was the most abundant (high read count) phylum, representing around 23% of all sequences in the samples, followed by Proteobacteria with $\approx 19\%$ and Cyanobacteria with nearly 18%. Spirochaetes and Chloroflexi represented $\approx 8\%$ and $\approx 4\%$, respectively. Nearly 2% were Firmicutes, Patesibacteria and Halanaerobiaeota (Fig. S1A).

Genera composition is shown in Fig. 2. A total of 219 genera of Bacteria were detected in the 10 sampled sites (S1–S10) at a 1.5-m scale. From those, only nine genera had high read counts (representing $\approx 80\%$ of the total proportion, Fig. 2A bottom): *Coleofasciculus* (formerly *Microcoleus*), *Spirochaeta*, *Catalinimonas*, *Desulfovermiculus*, *Halanaerobium*, *Tangfeifania*, *Sungkyunkwania*, *Sediminispirochaeta* and *Imperialibacter*. The remaining 210 genera can be classified as rare biosphere ($<1\%$ relative abundance each), with $\approx 20\%$ of the total proportion (Fig. 2A, top), having a heterogeneous distribution between sites (percentage of relative abundance and percentage of total proportion are used as synonyms).

As for 16S rDNA ASVs, 18 of the 6,063 were abundant ($>1\%$ relative abundance each), representing 49% of the relative abundance of reads (Table S5). This 18 ASVs were distributed within the previously mentioned highly abundant (high read count) genera and phyla (except Firmicutes and Patesibacteria).

Conversely, 6,045 ASVs were rare taxa ($<1\%$ relative abundance each) which represents 51% of total reads: 4,124 of them were non-classified, and the rest (1,921) were distributed within the whole 40 bacterial phyla and almost all (217) genera (Table S5). The two exceptions of genera with no rare ASVs were *Tangfeifania* with two highly proportionated ASVs, and *Coleofasciculus* with only one high-read count ASV. Moreover, 28 ASVs of Archaea were obtained using the 16S primers, comprising two phyla, Woearchaeota and Euryarchaeota (Fig. S1A). We detected four genera within Halobacteria class: *Halovivax*, *Halorubrum*, *Halorussus* and *Haloasta*.

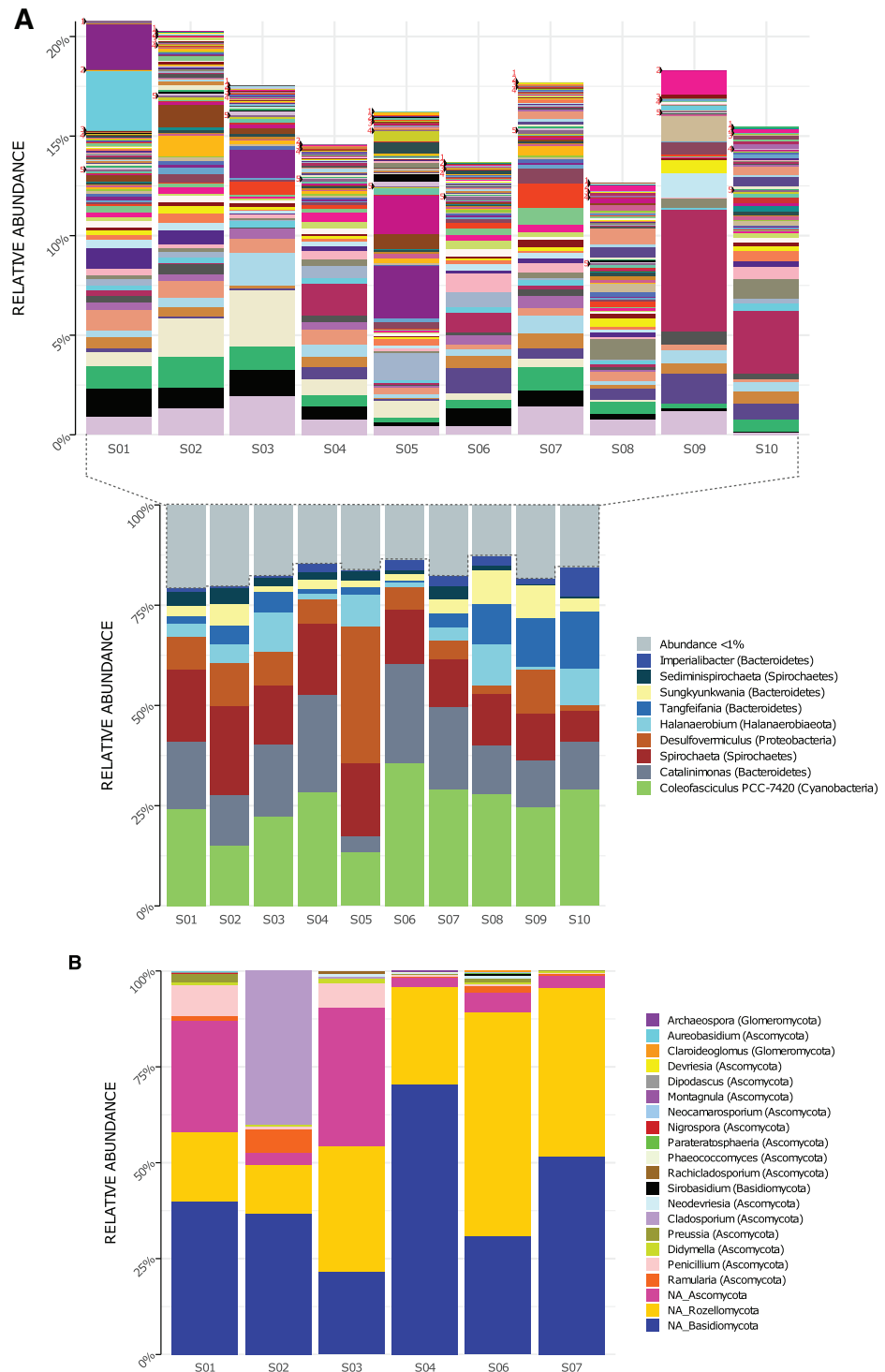


Figure 2 Genera relative abundance in Archaean Domes, Cuatro Ciénegas Basin sample. (A) Bacteria (bottom: shows the most common genera, more than 1% of total proportion each; top: shows genera less than 1% of total proportion each; for labels of 210 corresponding genera, check Table S10) (B) Fungi (using Merge data, only fungal ASVs. NA is for ASVs that were not assigned to any genera, but phylum only).

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For the ITS region libraries, we successfully obtained reads for six of the sampled sites (S1 to S4, S6 and S7). For the Forward-Only ITS data, we detected 923 different ASVs (Table S4). Many of the classified reads belong to Alveolata protists (77%, Fig. S1B), followed by Fungi (11%) and Viridiplantae (8%); Metazoa, Protista, Amoebozoa, Rhodoplantae, Chromista and Stramenophila comprised the remaining 4%. Unassigned taxa were 50% of all reads (Table S3). The majority of ASVs within Forward ITS data could not be accurately classified at lower taxonomic categories, particularly for three common ASVs (ASV0 (unclassified), ASV1 (Ciliophora) and ASV2 (Ciliophora)), which comprise nearly 70% of the proportion of ITS reads for all sites.

Using Merge ITS ASVs data (Table S1) for a better taxonomic assignment, Fungal ASVs comprising 18 genera were detected, with phyla Ascomycota, Basidiomycota and Rozellomycota present in all samples (Fig. 2B). Non-fungal Eukarya Merge ITS sequences classification (hits <90%) are shown in Table S2. *Fabrea salina* (Alveolata), and *Vannella simplex* (Amoebozoa) were two species that constituted nearly 0.5% of the total proportion of Merge ITS ASVs data, among other classified but low-read count eukaryotic ASVs.

Alpha and beta diversity

Rarefaction curves (Figs. S2A and S2B) showed similar results for both the Forward-Only ITS region libraries (S1, S2, S3, S4, S6, S7), and the 16S rDNA gene libraries, as all sites reached an asymptote, suggesting an adequate sampling effort. Only 183 ASVs (3% of the total ASV read count) were shared between all sites for 16S rDNA gene libraries (Fig. 3A), forming a very small core, but comprised 72.5% of all reads (composed of twenty-one phyla), while most of the ASVs were unique to a specific site or shared between two or three sites. For ITS libraries, the 56 shared ASVs (6%) comprised 95% of all reads (Fig. 3B).

The 16S rDNA ASVs alpha diversity (Table 1) ranged from 2,399 observed ASVs in S2 (Shannon = 7.4, highest Shannon index value), to 544 observed ASVs in S9 (Shannon = 6.1). In the case of the ITS region libraries (Forward-Only ITS data), the highest ASV number for Eukarya was S6 with 383 ASVs and a Shannon diversity of 2.5; S3 had the lowest observed ASVs with 199, but this site had the highest Shannon value of three (Table 1).

Pairwise dissimilarities between samples for both the 16S rDNA gene and ITS region data ranged from 0.2 to 0.7 (16S) and 0.2 to 0.6 (ITS) for the Bray-Curtis distance values and 0.5 to 0.8 (16S) and 0.3 to 0.7 (ITS) according to the Jaccard-1 values (Figs. S3A and S3B). For both distance values, S10 is different from the rest of the 16S rDNA gene libraries, and S1, S2, S4, S6, S7 were associated in the dendrogram clusters. In the ITS libraries, there are two main clusters (S1, S2 and S4; S3, S6 and S7). A significant geographic pattern in the Mantel test ($p = 0.0402$, $r = 0.3224$) was found for Jaccard-1 of the 16S library, however we did not find significant values based on geography for ITS libraries for either Jaccard-1 or Bray-Curtis (Table S6).

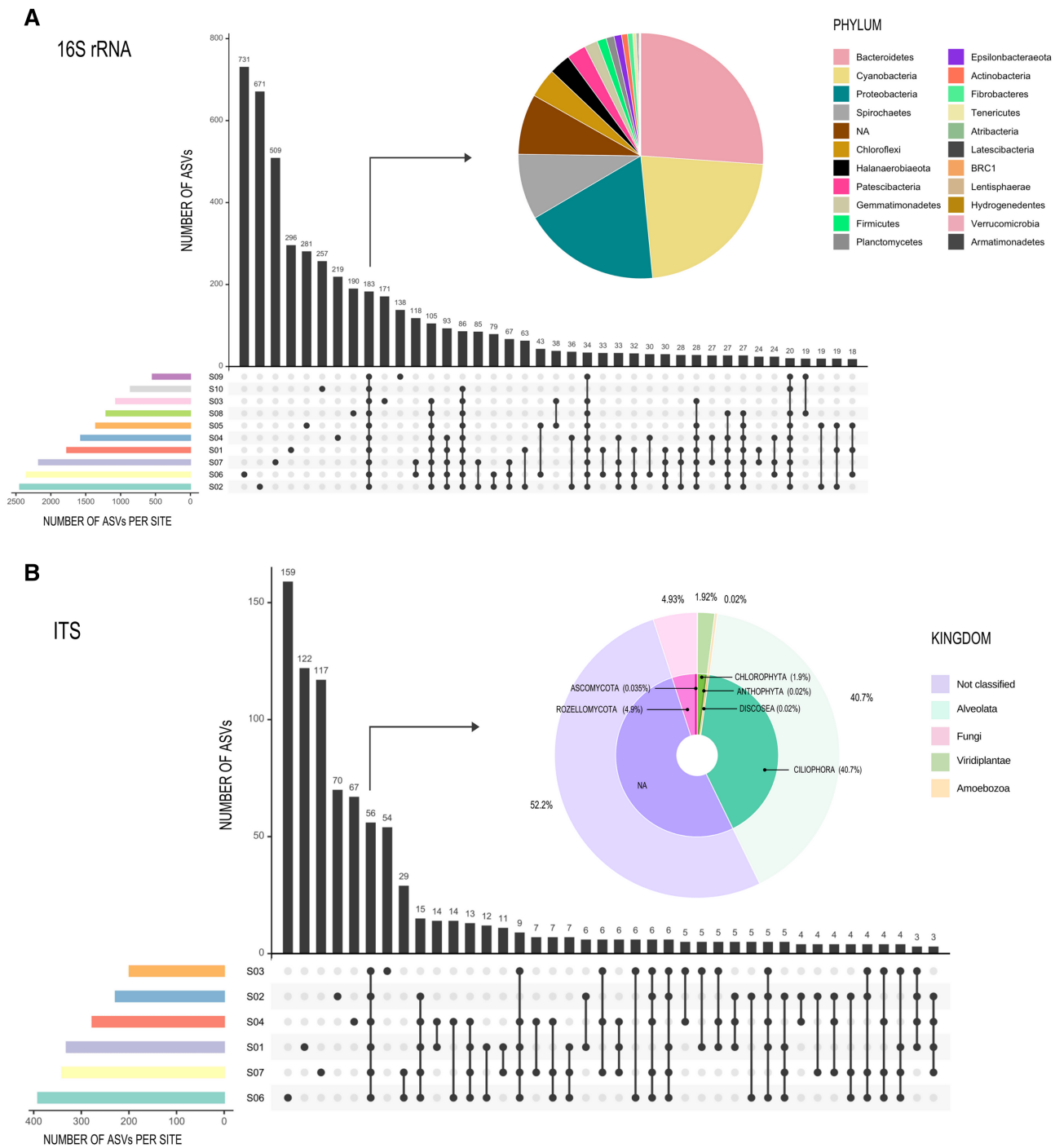


Figure 3 Graphic representation of shared ASVs within Archaeal Domes sampling sites. Number of shared ASVs is represented by black bars, showing the exact number on the top. Dots below bars denote sites where those ASVs are shared, and lines joining dots indicate the shared sites. Colored bars at bottom left of the main figure are for ASVs number within each site. Pie charts show taxonomy of the few shared ASVs (corresponding bars are pointed with arrows) within all sites. All of these shared ASVs had a high proportion within the whole ASVs (72.5% of 16S rDNA data and 95% of Forward-Only ITS data). (A) 16S Archaea and Bacteria, rDNA gene data; (B) Fungi and other Eukarya, Forward-Only ITS data.

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Table 1 Alpha diversity indices.

Sample	α diversity		Phylogenetic diversity		
	Observed	Shannon	Faith PD	Faith PD rare	Faith PD abundant
ITS data					
1	322	1.827	86.309	42.596	18.161
2	226	2.713	60.622	40.846	14.516
3	199	3.062	47.289	30.370	14.774
4	270	1.783	83.008	51.513	16.287
6	383	2.501	131.435	75.352	28.794
7	337	2.008	114.983	60.352	18.865
16S rDNA data					
1	1,761	6.863	544.704	290.491	44.159
2	2,399	7.402	789.807	368.430	84.442
3	1,068	6.256	211.745	156.204	20.064
4	1,564	6.499	500.787	259.828	40.627
5	1,344	6.882	220.536	172.299	21.557
6	2,345	6.301	851.168	302.103	131.337
7	2,156	6.838	759.536	328.238	97.486
8	1,195	6.487	205.987	139.839	22.798
9	544	6.126	160.368	94.201	18.577
10	828	5.115	283.017	148.655	36.514

Note:

Alpha diversity indices for 16S rDNA data and Forward-Only ITS region data from Archaean Domes CCB microbial mats.

Phylogenetic diversity

For both libraries, Faith Phylogenetic Diversity indicated that rare (low read count) ASVs on each site, were more diverse than abundant (high read count) ASVs (Table 1).

In respect to the null model distribution of Phylogenetic Species Clustering (nmdPSC), the 16S and ITS communities analyzed per site had in some sites a phylogenetic clustering distribution, and in others sites a null model distribution compared with chance alone (Figs. 4A and 4B, dots between lines or below -2).

When analyzing rare and abundant subcommunities using an equivalent ASV number per site, nmdPSC indicated that abundant ASVs were phylogenetically clustered within all sites and for both libraries, while rare ASVs were subcommunities with a null model distribution in some sites, or phylogenetically dispersed in other sites, for bacterial and fungal libraries (Figs. 4A and 4B, Table S7).

Metabolomic profile

Based on the LC-MS results of S1–S10, a wide range of metabolites were visualized on a heatmap analysis chart (Figs. S4A and S4B) and a GNPS molecular networking analysis (Fig. 5 and Table S8).

Untargeted metabolome analysis of microbial communities in soil or environmental samples is difficult, due to the complexity and heterogeneity of this matrix and the scarcity

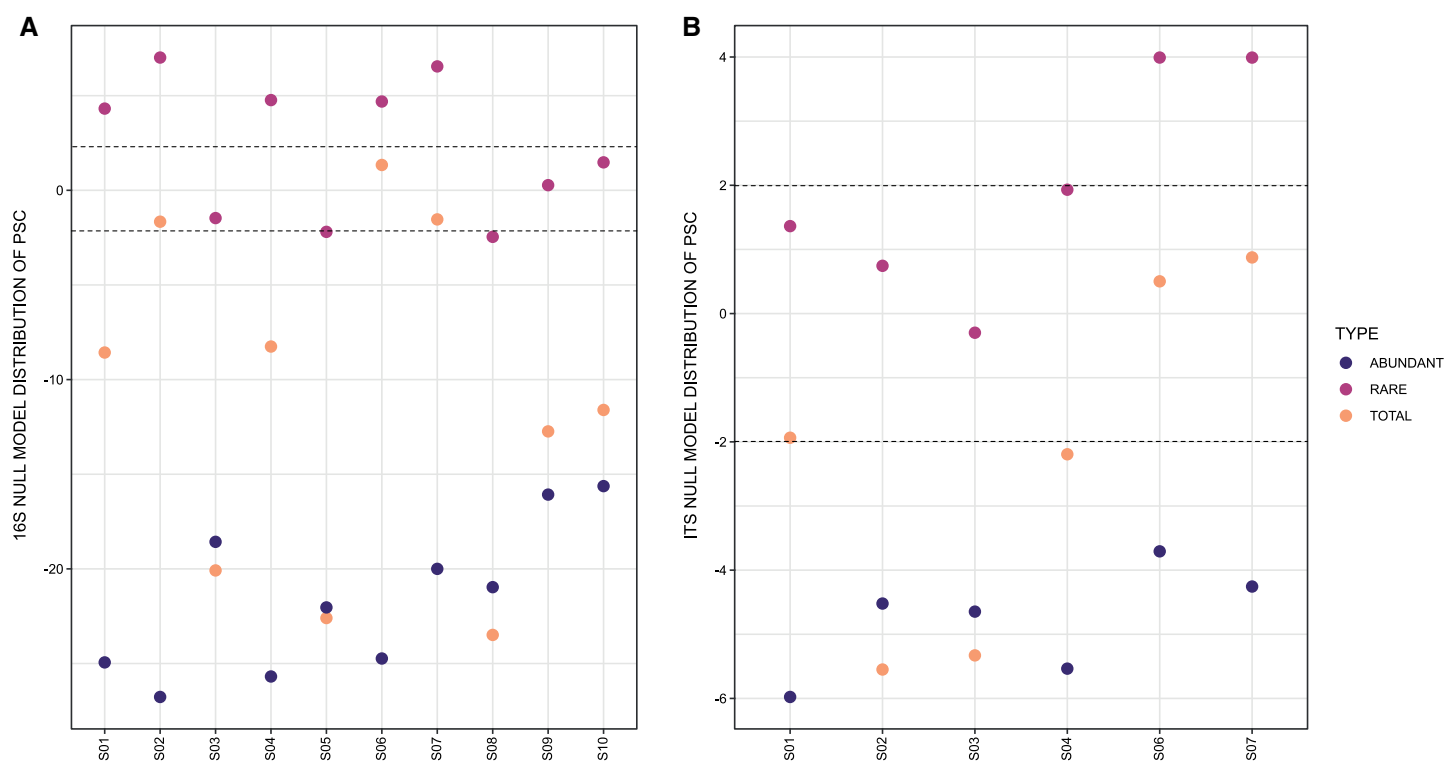


Figure 4 Graph representing null model distribution of Phylogenetic Species Clustering (nmdPSC). (A) 16S Archaea and Bacteria, rDNA gene data; (B) Forward-Only ITS data. Dotted lines are showing nmdPSC significant values: $>+2$ is consistent with a sample that had more phylogenetic overdispersion than expected by chance alone, and nmdPSC value <-2 indicates more phylogenetic clustering than expected by chance alone; values in the range $+2$ and -2 had a null model distribution. nmdPSC values are reported in Table S7. [Full-size !\[\]\(b345a1c4255362eec3746050dd71ccac_img.jpg\) DOI: 10.7717/peerj.13579/fig-4](https://doi.org/10.7717/peerj.13579/fig-4)

of the secondary metabolites extracted. In the heatmap, the 1,341 molecular features at unique retention times (Figs. S4A and S4B) shared several common regions across all samples (molecular features), allowing us to group them into three main clusters (cluster 1, samples 3–5 and 10; cluster 2, samples 1, 2 and 6; and cluster 3, samples 7–9, Fig. S4B). We found highly heterogeneous metabolic profiles in all samples, and no significant associations were observed between metabolite features and ITS or 16SrDNA community composition matrices (Table S6).

Moreover, feature-based GNPS molecular networking analysis grouped the metabolite features in 1,534 nodes distributed into 67 chemical families (sub-networks) of ≥ 3 members, 72 families of two metabolite features, and 765 singletons (Fig. 5). Chemical ontology analyses revealed the presence of superclasses of lipids and lipid-like compounds, organic acids, and organonitrogen and organoheterocyclic compounds, all commonly found in soil samples (Fig. 5). Detected compounds in the GNPS and manual metabolome analysis of the main ions observed in LC-MS/MS data focused on microbial metabolites, allowing tentative identification of several secondary metabolites that passed our quality criteria (≤ 5 ppm mass error; Table S8). Finally, some plant-derived secondary metabolites and small molecules reflecting human activity were also observed (Table S8).

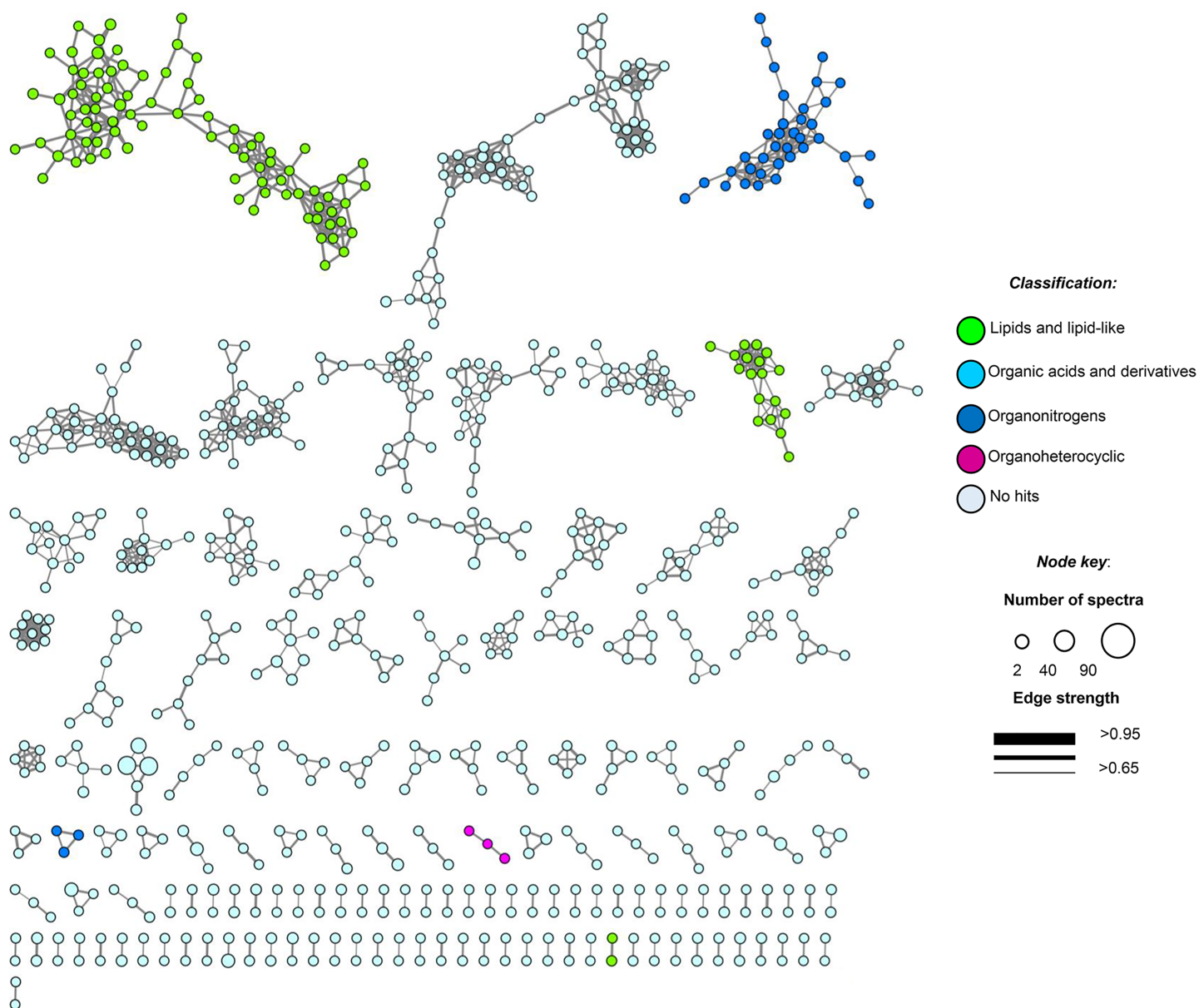


Figure 5 Molecular network of soil samples (S1-S10) grouped metabolite features into 139 chemical families. Each node in the network represents one metabolite feature. Nodes connected to each other are structurally-related (cosine MS2 similarity score ≥ 0.7). Matching compounds are not shown for clarity (included in Table S8). [Full-size !\[\]\(1679558f37f6db0dd8360a2a7e913e90_img.jpg\) DOI: 10.7717/peerj.13579/fig-5](https://doi.org/10.7717/peerj.13579/fig-5)

DISCUSSION

Microbial composition at a small scale

Our study describes microbial diversity on a 1.5-m scale in a rare elastic microbial mat located at an extreme site. Altogether, we found 40 prokaryotic phyla, including 219 genera of Bacteria and 4 of Archaea, as well as 18 Fungal genera (Fig. 2 and Fig. S1).

Bacteria

The bacterial phyla (Fig. S1A) detected in our study site are common components of hypersaline microbial mats (Bolhuis, Cretoiu & Stal, 2014; Visscher et al., 2010): Bacteroidetes, Proteobacteria, Cyanobacteria, Chloroflexi, and Firmicutes, among other abundant (high read count) phyla, which are similar at this broad taxonomic level to other sites in CCB (Bonilla-Rosso et al., 2012; Breitbart et al., 2009; De Anda et al., 2018; Nitti et al., 2012). As expected from a microbial mat, a primary producer was present and abundant in all of our samples: *Coleofasciculus* (Fig. 2), a filamentous nitrogen-fixing cyanobacteria genus well-adapted to saline conditions (De Wit et al., 2013; Ramos et al., 2017) that has been reported in Guerrero Negro microbial mats (Des Marais, 2010; Wong, Ahmed-Cox & Burns, 2016). We also found the abundant taxon *Catalinimonas*, which is a halophilic Bacteroidetes marine genus (Choi et al., 2013) that has not been previously reported in microbial mats. Another common taxon in our samples is the *Spirochaeta* genus (Bolhuis, Cretoiu & Stal, 2014; Margulis et al., 2006), an anaerobic chemo-organotrophic taxon abundant in hypersaline marine mats (Ben Hania et al., 2015). Noteworthy is that the *Spirochaetes* phylum has not been detected previously in any CCB mats (Bonilla-Rosso et al., 2012; De Anda et al., 2018; Peimbert et al., 2012). We also observed *Desulfovermiculus*, a sulfate-reducing Deltaproteobacteria, and *Halanaerobium*, a thiosulfate-reducing Firmicute that is halophilic and can form spores (Liang et al., 2016), both genera are common in marine microbial mats (Bolhuis, Cretoiu & Stal, 2014), reinforcing previous evidence from CCB studies that this site can harbor taxa that are phylogenetically similar to marine lineages (see Rebolgar et al., 2012; Souza et al., 2006, 2018).

Archaea

Archaea data presented here should be taken cautiously, since the primers for 16S rDNA used are universal but were not designed specifically to amplify archaea and would likely recover only 1% or less of archaeal diversity. However, we decided to only describe the groups detected, avoiding any interpretations regarding quantity where there could be a strong bias. We detected two main phyla (Fig. S1A); these were Woesearchaeota, a phylum that potentially has a syntrophic interaction with methanogens (Liu et al., 2018; Ortiz-Alvarez & Casamayor, 2016), and Euryarchaeota, within which we found four halophilic genera: *Halovivax*, *Halorubrum*, *Halorussus* and *Haloasta*, all of them previously reported in saline habitats and sediments (McGonigle et al., 2019; Zaitseva et al., 2018).

In a recent metagenomic study of the Archaeal Domes (Medina-Chávez et al., 2019), some archaeal taxa were detected in all sampled sites, forming a “core taxa”. In those metagenomes, Archaea represented nearly 5% of the total reads. The metagenomic data (Medina-Chávez et al., 2019) and the 16S rDNA gene amplicons were sampled from the same pond at different times and locations, and both contained these lineages common to hypersaline habitats.

Fungi

Fungal members represent a diverse and common component of hypersaline water columns and mats (Cantrell *et al.*, 2013) and have been suggested to play a role in the degradation of EPS (exopolymeric substances) (Cantrell & Duval-Pérez, 2013). Our results suggest that unidentified uncultured members of the Ascomycota and Rozellomycota comprise the core mycobiota of the analyzed mats, as they occurred across all the samples (Fig. 3B). In addition, it is feasible that these abundant heterotrophs occupy a niche at the micro-scale of microbial mats, where they can degrade the abundant EPS produced in the CCB microbial mats (Breitbart *et al.*, 2009; De Anda *et al.*, 2018; Peimbert *et al.*, 2012). In particular, halotolerant taxa such as members of the genera *Aspergillus*, *Penicillium*, and *Nigrospora* have been repeatedly reported in microbial mats at other locations (Cantrell *et al.*, 2013; Cantrell & Duval-Pérez, 2013), evidencing their adaptation to extreme conditions. Thus, it appears that, similar to other microbial mats, fungal members could occupy an EPS-degrader role at the micro-scale of the Archaeal Domes (Cantrell *et al.*, 2011).

Other eukaryotes

In our sample, there are three abundant (high read count) ASVs of Alveolata protists that represent 70% of the ITS sequences (Fig. S1B, Forward-Only ITS data) in all sequenced sites (S1–S4, S6, S7). Although the ITS primer pair used in this study was originally designed for fungal amplification, it has been reported that there are potential matches with non-fungal eukaryotic sequences as well (Toju *et al.*, 2012). Nevertheless, these results should be interpreted in a descriptive sense, considering that several eukaryotic taxa are not being detected due to primer bias.

Two of the three ASVs are Ciliophora (*i.e.*, ciliates), which is the most diverse phylum of protists, with 3,500 described species, most of which are cosmopolites (Gao *et al.*, 2016). It will therefore be interesting to analyze more molecular markers in the future to distinguish the protist lineages, as well as to microscopically study the protists to reach a closer taxonomic identification. Other Protozoa ASVs were observed at low numbers and belong to the Amoebozoa phylum, and the ancestral algae from the kingdom Chromista were also observed at a low proportion (Fig. S1B).

Although research on halophilic protozoan is still young, it has made great progress in recent years (Harding & Simpson, 2018). Protozoa in general are grazing organisms, as is the case with *Fabrea salina*, one of the few classified low proportion ITS ASVs in our study (Merge data, Table S2). This ciliate is present in microbial mats and feeds on *Cyanotece sp.*, a Cyanobacteria associated with algae mats (Carrasco & Perissinotto, 2012). Indeed, in hypersaline environments, most ciliates are known to be important components of the pelagic microbial food loop (Liu *et al.*, 2016). Amoebae have also been observed as grazers (Hauer, Rogerson & Anderson, 2001; Hauer & Rogerson, 2005; Post *et al.*, 1983) on cyanobacteria in surface mud samples from a salt pond in Eilat, within the Salton sea in Southern California (Hauer & Rogerson, 2005). Even though the diversity of protists is low, their prevalence, along with the large amount of viruses found in metagenomic samples (28% of the metagenomes from Medina-Chávez *et al.*, 2019), strongly suggests that

predator-prey interactions (e.g., protist-bacteria, [Saleem et al., 2013](#)) could be very important for the dynamics of the system ([Carreira et al., 2020](#)).

Alpha and beta diversity

The Shannon Index accounts for both species evenness and abundance; hence, it might not reflect the total richness if most of the found taxa are part of the rare biosphere. For example, Site three for the Forward-Only ITS data had the highest Shannon value, despite having the lowest ASV number ([Table 1](#)), due to its higher evenness distribution of taxa. Meanwhile, the rarefaction and observed diversity reflects the richness of the site ([Figs. S2A and S2B; Table 1](#)).

Beta diversity analysis showed values that represent a large ASV turnover between sites despite the small scale ([Fig. S3](#)), illustrated by the heterogenous composition observed within bacterial genera ([Fig. 2A top](#)). Accordingly, the different domains analyzed (*i.e.*, the bacterial 16S rDNA data or the Eukarya Forward only ITS data) had a distinctive association pattern. This turnover pattern should be taken cautiously, as ASVs turnover might a methodological artifact: beta diversity analysis uses data on relative abundances, based on compositional data; this means that the presence of abundant ASVs might “push” the rare ASVs below the detection threshold. Therefore, this pattern should be corroborated in a future time-series using metagenomic data, and additionally, a transcriptome approach would explore if the rare ASVs are an active part of this community. A statistically marginal but significant geographic association was found only for the bacterial 16S rDNA data ($p = 0.0402$, $r = 0.3224$); this suggests a geographic pattern that also should be explored with a more extensive sampling.

Bacterial community comparison with other CCB layered microbial communities

We compared our data with four previously published metagenomes of layered microbial communities from the CCB: one microbialite from a large spring-fed river Rio Mesquites ([Nitti et al., 2012](#)), one stromatolite from a blue pool in Pozas Azules ([Breitbart et al., 2009](#)) and two microbial mats, one from a small and shallow pond called Lagunita in Churince ([De Anda et al., 2018](#)), and the other from a green pool in Pozas Azules ([Bonilla-Rosso et al., 2012](#)).

At the phylum level, we found seven taxa present in all these five microbial communities: Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Planctomycetes and Proteobacteria. All of them shared among all sites on Archaeal Domes mats and had a high read count ([Fig. 3A](#)).

Additionally, [Bonilla-Rosso et al. \(2012\)](#) reported 28 orders that were shared within the microbial mat from a green pool ([Bonilla-Rosso et al., 2012](#)), the stromatolite ([Breitbart et al., 2009](#)) and a coastal hypersaline non-thermophilic microbial mat from Guerrero Negro ([Kunin et al., 2008](#)). We found 18 of those 28 orders in the Archaeal Domes community ([Table S9](#)).

We also explored lower taxonomic levels but did not find a consistent pattern.

The common pattern at higher taxonomic ranks reflects, as [Bonilla-Rosso et al. \(2012\)](#) mentioned, that layered microbial communities assemble in biogeochemical gradients related to defined ecological niches (e.g., photosynthesis, sulfate reduction, heterotrophy) according to their functional traits, rather than their species ([Burke et al., 2011](#), [Escalas et al., 2019](#)).

Phylogenetic diversity

The null model distribution of Phylogenetic Species Clustering (nmdPSC, [Fig. 4](#)) of whole bacterial and eukaryotic communities showed negative or null values, indicating a clustered pattern compared with the null model. Phylogenetic clustering is predicted to be evident in environments with poor nutrient availability ([Mondav et al., 2017](#)), such as the microbial mat conditions. Since little is known about patterns and processes of rare and abundant bacterial and eukaryotic taxa in microbial mats, we also analyzed these two microbial subcommunities.

Abundant (high read count) taxa

The nmdPSC pattern of clustering for the abundant ASVs ([Fig. 4](#)) could be similar to some soil bacterial communities that typically show a coexistence of phylogenetically close relatives ([Bryant et al., 2009](#); [Horner-Devine & Bohannan, 2006](#)), which may be the product of abiotic filtering but also of competitive exclusion ([Goberna et al., 2014](#)). Competitive exclusion seems to be a likely explanation for the phylogenetic clustering observed for abundant bacterial and eukaryotic ASVs, as proposed by [Goberna et al. \(2014\)](#), since the presence of compounds with antimicrobial properties (check Metabolomic profile sub-section below) suggests high levels of competition among abundant microbes.

Rare (low read count) taxa

The rare biosphere, when analyzing equivalent numbers of ASVs within abundant taxa, was more phylogenetically diverse (Faith Phylogenetic Diversity index, [Table 1](#)), as well as more phylogenetically disperse (nmdPSC, [Fig. 4](#)) than the abundant taxa within these samples for both the 16S rDNA and ITS libraries, suggesting that environmental filtering is not occurring for the rare biosphere in this site.

The two analyzed subcommunities (abundant and rare) showed different phylogenetic dispersion patterns. The clustering pattern for abundant taxa –and also for the whole community–, suggests that the associated assembly processes are abiotic filtering or competitive exclusion ([Wang et al., 2013](#); [Mondav et al., 2017](#)). In contrast, communities that show phylogenetic dispersion, such as the rare taxa of the Archaean Domes, could be more affected by stochastic processes such as dispersal and drift ([Wang et al., 2013](#); [Mondav et al., 2017](#)). Thus, both processes, deterministic and stochastic could be occurring in the Archaean Domes community.

These rare and abundant patterns described suggest that rare taxa could be a large reservoir of genetic diversity, having diverse metabolic functions within this microbial mat ([Jia, Dini-Andreote & Falcão Salles, 2018](#)) and perhaps presenting collective adaptations to the extreme conditions. This is in correspondence with the unique metabolome profile

showed for each site, despite the homogeneous abundant taxa. These ideas should be taken cautiously, since rare taxa may not be an active part of the community, as stated previously.

Metabolomic profile

The dynamic and complex metabolomic profiles (Figs. S4A and S4B, Fig. 5 and Table S8) of the studied microbial mat was consistent with the complex microbial community observed in the metagenomic analysis (Medina-Chávez *et al.*, 2019).

Even though the expression of regulatory genes responsible for secondary metabolites production depends on several factors (*e.g.*, nitrogen or phosphate starvation, stressors like heat, pH and damage to the cell wall, among others) (Baral, Akhgari & Metsä-Ketelä, 2018), along with the lack of soil metabolomics studies and the solubility of the compounds for LCMS analysis, we were still able to tentatively annotate several classes of compounds with interesting biological properties (Fig. 5 and Table S8). Many of these compounds were found to overlap between most of the samples: the antibiotics 8,9-dihydroindanomycin (Li, Roege & Kelly, 2009), neoindanomycin (Rommel, Li & Kelly, 2011) described from *Streptomyces* species; the cytotoxic daryamide C (Asolkar *et al.*, 2006), gibbestatin B (Kinashi & Sakaguchi, 1984) and furaquinocin H (Ishibashi *et al.*, 1991), from *Streptomyces* strains; the germination inhibitor colletofragarone A1, from fungi *Colletotrichum fragariae* and *C. capsiciae* (Inoue *et al.*, 1996); periconiasin G (Zaghoulani *et al.*, 2016) from *S. nitrosporeus* and *Periconia* sp. F-31 with free radical scavenger and weak anti-HIV activities; and phomamide and tomaymycin I from *Phoma lingam* (Ferezou *et al.*, 1980) and *Nocardia* sp. (Tozuka, Takasugi & Takaya, 1983) with no biological activity described. Other metabolites of interest were found in specific samples, for example the antifungal agents griseofulvin and dechlorogriseofulvin, produced by microorganisms of the genera *Penicillium*, *Aspergillus*, *Xylaria*, *Coriolus*, *Palicourea*, *Memnoniella*, *Khauskia*, *Carpenteles*, *Arthrinium*, *Nigrospora*, *Stachybotrys*, and *Streptomyces* (Atta-ur-Rahmanro, 2005; Kimura *et al.*, 1992; Knowles *et al.*, 2019; Park, Cho & Simpson, 2006) were identified only in S7; the antimitotic agent nostodione A from the blue-green alga *Nostoc commune* (Kobayashi *et al.*, 1994) in samples S2 and S6–S8; and the mycotoxins citreohybridone B (Kosemura, 2003), setosusin (Fujimoto *et al.*, 1996), roridin E (Böhner *et al.*, 1965) and ganoboninone A (Ma *et al.*, 2015) in samples S8–S10. Interestingly, several observed ions (Fig. 5) do not match with any data reported and could represent potentially new secondary metabolites. Finally, plant- and animal-derived metabolites and compounds resulting from human activity (contaminants), were also annotated (Table S8).

The association of compounds between fungi and osmotrophs, as well as to other Eukarya, reflect the importance of studying the potential role of microorganisms besides bacteria within microbial mats (Carreira *et al.*, 2020). It is important to mention that metabolomics studies of samples containing diverse communities are scarce; thus, this work significantly expands our understanding of soil metabolites, especially from elastic hypersaline microbial mats.

CONCLUSIONS AND PERSPECTIVES

We found a very low number of taxa that comprised a considerable proportion of the mat and were shared across all sampling points, whereas the rare biosphere was more phylogenetically diverse (FPD index) and phylogenetically disperse (using a null model distribution of Phylogenetic Species Clustering) than the abundant (high read count) taxa for both analyzed libraries. We also found a distinctive metabolome profile for each sample and were able to tentatively annotate several classes of compounds with relevant biological properties.

In view of these results, we are interested to investigate if the metabolomic data is associated with local adaptive processes that could be following an eco-evolutionary dynamic in these fluctuating communities (*i.e.*, taxa are constantly and actively modifying their own ecological niche and that of others (niche construction) (Laland, Matthews & Feldman, 2016; Odling-Smee, Laland & Feldman, 2003; Van Der Hooft *et al.*, 2020)). We also want to explore the high phylogenetic diversity and phylogenetic dispersion shown by the rare biosphere and use transcriptomic data to analyze if the rare biosphere is an active part of this community. We hypothesized that there is a diverse “seed bank” in the aquifer of CCB, a deep biosphere fed by magmatic energy (Wolaver *et al.*, 2013) that could be constantly “seeding” the Archaean Domes community, along with deep water and particular minerals.

These ideas could be tested in a deep and spatial-temporal sampling approaches.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

Luis E. Eguiarte and Valeria Souza are Academic Editors for PeerJ.

Author Contributions

- Laura Espinosa-Asuar conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Camila Monroy-Guzmán analyzed the data, prepared figures and/or tables, and approved the final draft.
- David Madrigal-Trejo analyzed the data, prepared figures and/or tables, and approved the final draft.
- Marisol Navarro-Miranda analyzed the data, prepared figures and/or tables, and approved the final draft.
- Jazmin Sánchez-Pérez analyzed the data, prepared figures and/or tables, and approved the final draft.
- Jhoselinne Buenrostro Muñoz performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Juan Villar performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Julián Felipe Cifuentes Camargo performed the experiments, prepared figures and/or tables, and approved the final draft.
- Maria Kalambokidis performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Diego A. Esquivel-Hernandez conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
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- Ana E. Escalante analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Patricia Velez conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Mario Figueroa conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Anahi Martinez-Cardenas analyzed the data, prepared figures and/or tables, and approved the final draft.
- Santiago Ramirez-Barahona conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

- Jaime Gasca-Pineda conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Luis E. Eguiarte conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Valeria Souza conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (*i.e.*, approving body and any reference numbers):

Collection permit SGPA/DGVS/03188/20 was issued by Subsecretaría de Gestión para la protección Ambiental, Dirección General de Vida Silvestre for this study.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

All raw sequences are available at GeneBank BioProject: [PRJNA705156](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA705156); BioSample ITS: [SAMN18070760](https://www.ncbi.nlm.nih.gov/biosample/SAMN18070760); BioSample 16S: [SAMN18070761](https://www.ncbi.nlm.nih.gov/biosample/SAMN18070761).

The full data of metabolome feature-based molecular networking is available at MassIVE [MSV000087545](https://massive.ucsd.edu/MSV000087545).

<https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?accession=MSV000087545>.

Data Availability

The following information was supplied regarding data availability:

The code and processed data are available at GitLab:

<https://gitlab.com/lauasuar/archaeoan-domes-repository>.

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.13579#supplemental-information>.

REFERENCES

- Alcaraz LD, Olmedo G, Bonilla G, Cerritos R, Hernández G, Cruz A, Ramírez E, Putonti C, Jiménez B, Martínez E, López V, Arvizu JL, Ayala F, Razo F, Caballero J, Siefert J, Eguiarte L, Vielle J-P, Martínez O, Souza V. 2008. The genome of *Bacillus coahuilensis* reveals adaptations essential for survival in the relic of an ancient marine environment. *Proceedings of the National Academy of Sciences of the United States of America* **105**(15):5803–5808 DOI [10.1073/pnas.0800981105](https://doi.org/10.1073/pnas.0800981105).
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**(3):403–410 DOI [10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Aron AT, Gentry EC, McPhail KL, Nothias LF, Nothias-Esposito M, Bouslimani A, Petras D, Gauglitz JM, Sikora N, Vargas F, van der Hooft JJJ, Ernst M, Kang KB, Aceves CM, Caraballo-Rodríguez AM, Koester I, Weldon KC, Bertrand S, Roullier C, Sun K, Tehan RM, Boya PCA, Christian MH, Gutiérrez M, Ulloa AM, Tejeda Mora JA, Mojica-Flores R, Lakey-Beitia J, Vásquez-Chaves V, Zhang Y, Calderón AI, Tayler N, Keyzers RA, Tugizimana F, Ndlovu N, Aksenov AA, Jarmusch AK, Schmid R, Truman AW, Bandeira N,

- Wang M, Dorrestein PC. 2020. Reproducible molecular networking of untargeted mass spectrometry data using GNPS. *Nature protocols* 15(6):1954–1991 DOI 10.1038/s41596-020-0317-5.
- Asolkar RN, Jensen PR, Kauffman CA, Fenical W. 2006. Daryamides A–C, weakly cytotoxic polyketides from a marine-derived actinomycete of the genus streptomyces strain CNQ-085. *Journal of Natural Products* 69(12):1756–1759 DOI 10.1021/np0603828.
- Atta-ur-Rahmanro. 2005. Foreword. In: *Bioactive Natural Products (Part L)*. Vol. 32. Amsterdam: Elsevier.
- Baral B, Akhgari A, Metsä-Ketelä M. 2018. Activation of microbial secondary metabolic pathways: avenues and challenges. *Synthetic and Systems Biotechnology* 3(3):163–178 DOI 10.1016/j.synbio.2018.09.001.
- Bebout B, Carpenter S, Des Marais D, Discipulo M, Embaye T, Garcia-Pichel F, Hoehler T, Hogan M, Jahnke L, Keller R, Miller S, Prufert-Bebout L, Raleigh C, Rothrock M, Turk-Kubo K. 2002. Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating earth's present and past field environments. *Astrobiology* 2(4):383–402 DOI 10.1089/153110702762470491.
- Ben Hania W, Joseph M, Schumann P, Bunk B, Fiebig A, Spröer C, Klenk HP, Fardeau ML, Spring S. 2015. Complete genome sequence and description of *Salinispira pacifica* gen. nov., sp. nov., a novel spirochaete isolated from a hypersaline microbial mat. *Standards in Genomic Sciences* 10:7 DOI 10.1186/1944-3277-10-7.
- Bolhuis H, Cretoiu MS, Stal LJ. 2014. Molecular ecology of microbial mats. *FEMS Microbiology Ecology* 90(2):335–350 DOI 10.1111/1574-6941.12408.
- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez Aés M, Chase J, Cope E, Da Silva R, Dorrestein PC, Douglas GM, Durall DM, Duvall C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley G, Janssen S, Jarmusch AK, Jiang L, Kaehler B, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolk T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton J, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson, II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2018. QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ* 6:e27295v2 DOI 10.7287/peerj.preprints.27295v2.
- Bonilla-Rosso G, Peimbert M, Alcaraz LD, Hernández I, Eguiarte LE, Olmedo-Alvarez G, Souza V. 2012. Comparative metagenomics of two microbial mats at Cuatro Ciénegas Basin II: community structure and composition in oligotrophic environments. *Astrobiology* 12(7):659–673 DOI 10.1089/ast.2011.0724.
- Breitbart Mya, Hoare A, Nitti A, Siefert J, Haynes M, Dinsdale E, Edwards R, Souza V, Rohwer F, Hollander D. 2009. Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico. *Environmental Microbiology* 11(1):16–34 DOI 10.1111/j.1462-2920.2008.01725.x.

- Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL. 2009.** Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *In the Light of Evolution* 2:127–148 DOI [10.17226/12501](https://doi.org/10.17226/12501).
- Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T. 2011.** Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of America* 108(34):14288–14293 DOI [10.1073/pnas.1101591108](https://doi.org/10.1073/pnas.1101591108).
- Böhner B, Fetz E, Härri E, Sigg HP, Stoll C, Tamm C. 1965.** Über die Isolierung von Verrucarin H, Verrucarin J, Roridin D und Roridin E aus Myrothecium -Arten. Verrucarine und Roridine, 8. Mitteilung. *Helvetica Chimica Acta* 48(5):1079–1087 DOI [10.1002/hlca.19650480515](https://doi.org/10.1002/hlca.19650480515).
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016.** DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods* 13(7):581–583 DOI [10.1038/nmeth.3869](https://doi.org/10.1038/nmeth.3869).
- Cantrell SA, Dianese J, Fell J, Gunde-Cimerman N, Zalar P. 2011.** Unusual fungal niches. *Mycologia* 103(6):1161–1174 DOI [10.3852/11-108](https://doi.org/10.3852/11-108).
- Cantrell S, Duval-Pérez L. 2013.** Microbial mats: an ecological niche for fungi. *Frontiers in Microbiology* 4:424 DOI [10.3389/fmicb.2012.00424](https://doi.org/10.3389/fmicb.2012.00424).
- Cantrell SA, Tkavc R, Gunde-Cimerman N, Zalar P, Acevedo M, Báez-Félix C. 2013.** Fungal communities of young and mature hypersaline microbial mats. *Mycologia* 105(4):827–836 DOI [10.3852/12-288](https://doi.org/10.3852/12-288).
- Carrasco NK, Perissinotto R. 2012.** Development of a halotolerant community in the St. Lucia Estuary (South Africa) during a hypersaline phase. *PLOS ONE* 7(1):e29927 DOI [10.1371/journal.pone.0029927](https://doi.org/10.1371/journal.pone.0029927).
- Carreira C, Lonborg C, Kühn M, Lillebo AI, Sandaa RA, Villanueva L, Cruz S. 2020.** Fungi and viruses as important players in microbial mats. *FEMS Microbiology Ecology* 96(11):1–13 DOI [10.1093/femsec/fiaa187](https://doi.org/10.1093/femsec/fiaa187).
- Carteron A, Jeanmougin M, Leprieur F, Spatharis S. 2012.** Assessing the efficiency of clustering algorithms and goodness-of-fit measures using phytoplankton field data. *Ecological Informatics* 9:64–68 DOI [10.1016/j.ecoinf.2012.03.008](https://doi.org/10.1016/j.ecoinf.2012.03.008).
- Choi EJ, Beatty DS, Paul LA, Fenical W, Jensen PR. 2013.** Mooreia alkaloidigena gen. nov., sp. nov. and Catalinimonas alkaloidigena gen. nov., sp. nov., alkaloid-producing marine bacteria in the proposed families Mooreiaceae fam. nov. and Catalimonadaceae fam. nov. in the phylum Bacteroidetes. *International Journal of Systematic and Evolutionary Microbiology* 63(Pt_4):1219–1228 DOI [10.1099/ij.s.0.043752-0](https://doi.org/10.1099/ij.s.0.043752-0).
- Conway JR, Lex A, Gehlenborg N. 2017.** UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics* 33(18):2938–2940 DOI [10.1093/bioinformatics/btx364](https://doi.org/10.1093/bioinformatics/btx364).
- De Anda V, Zapata-Peñasco I, Blaz J, Poot-Hernández AC, Contreras-Moreira B, González-Laffitte M, Gámez-Tamariz N, Hernández-Rosales M, Eguiarte LE, Souza V. 2018.** Understanding the mechanisms behind the response to environmental perturbation in microbial mats: a metagenomic-network based approach. *Frontiers in Microbiology* 9:11512 DOI [10.3389/fmicb.2018.02606](https://doi.org/10.3389/fmicb.2018.02606).
- De Wit R, Guerrero MC, Legaz A, Jonkers HM, Blocier L, Gumiaux C, Gautret P. 2013.** Conservation of a permanent hypersaline lake: Management options evaluated from decadal variability of coleofasciculus chthonoplastes microbial mats. *Aquatic Conservation: Marine and Freshwater Ecosystems* 23(4):532–545 DOI [10.1002/aqc.2319](https://doi.org/10.1002/aqc.2319).
- Des Marais D. 2010.** Marine hypersaline microcoleus-dominated cyanobacterial mats in the saltern at GuerRero Negro, Baja California Sur, Mexico: a system-level perspective. Vol. 14. Dordrecht: Springer, 401–420.

- El-Elimat T, Figueroa M, Ehrmann BM, Cech NB, Pearce CJ, Oberlies NH. 2013. High-resolution MS, MS/MS, and UV database of fungal secondary metabolites as a dereplication protocol for bioactive natural products. *Journal of Natural Products* 76(9):1709–1716 DOI 10.1021/np4004307.
- Elser JJ, Schampel JH, Garcia-Pichel F, Wade BD, Souza V, Eguiarte L, Escalante A, Farmer JD. 2005. Effects of phosphorus enrichment and grazing snails on modern stromatolitic microbial communities. *Freshwater Biology* 50(11):1808–1825 DOI 10.1111/j.1365-2427.2005.01451.x.
- Elser JJ, Watts J, Schampel JH, Farmer J. 2006. Early Cambrian food webs on a trophic knife-edge? A hypothesis and preliminary data from a modern stromatolite-based ecosystem. *Ecology Letters* 9(3):292–300 DOI 10.1111/j.1461-0248.2005.00873.x.
- Ernst M, Kang KB, Caraballo-Rodríguez AM, Nothias L-F, Wandy J, Chen C, Wang M, Rogers S, Medema MH, Dorrestein PC, van der Hooft JJJ. 2019. MolNetEnhancer: enhanced molecular networks by integrating metabolome mining and annotation tools. *Metabolites* 9(7):144 DOI 10.3390/metabo9070144.
- Escalas A, Hale L, Voordeckers JW, Yang Y, Firestone MK, Alvarez-Cohen L, Zhou J. 2019. Microbial functional diversity: from concepts to applications. *Ecology and Evolution* 9(20):12000–12016 DOI 10.1002/ece3.5670.
- Faith DP. 1992. Conservation evaluation and phylogenetic diversity. *Biological Conservation* 61(1):1–10 DOI 10.1016/0006-3207(92)91201-3.
- Ferezou J-P, Quesneau-Thierry A, Barbier M, Kollmann A, Bousquet J-F. 1980. Structure and synthesis of phomamide, a new piperazine-2,5-dione related to the sirodesmins, isolated from the culture medium of *Phoma lingam* Tode. *Journal of the Chemical Society, Perkin Transactions* 1:113–115 DOI 10.1039/P19800000113.
- Fujimoto H, Neghisi E, Yamaguchi K, Nishi N, Yamazaki M. 1996. Isolation of new tremorgenic metabolites from an ascomycete, *corynascus setosus*. *Chemical and Pharmaceutical Bulletin* 44(10):1843–1848 DOI 10.1248/cpb.44.1843.
- Gao F, Warren A, Zhang Q, Gong J, Miao M, Sun P, Xu D, Huang J, Yi Z, Song W. 2016. The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). *Scientific Reports* 6:24874 DOI 10.1038/srep24874.
- Gerdes G. 2010. What are microbial mats? In: Seckbach J, Oren J, eds. *Microbial. Mats Modern and Ancient Microorganisms in Stratified Systems*. Dordrecht: Springer Netherlands, 3–25.
- Gerdes G, Claes M, Dunajtschik-Piewak K, Riege H, Krumbein WE, Reineck H-E. 1993. Contribution of microbial mats to sedimentary surface structures. *Facies* 29(1):61–74 DOI 10.1007/BF02536918.
- Goberna M, Navarro-Cano JA, Valiente-Banuet A, García C, Verdú M. 2014. Abiotic stress tolerance and competition-related traits underlie phylogenetic clustering in soil bacterial communities. *Ecology Letters* 17(10):1191–1201 DOI 10.1111/ele.12341.
- Gutiérrez-Preciado A, Saghäi A, Moreira D, Zivanovic Y, Deschamps P, López-García P. 2018. Functional shifts in microbial mats recapitulate early Earth metabolic transitions. *Nature Ecology and Evolution* 2(11):1700–1708 DOI 10.1038/s41559-018-0683-3.
- Harding T, Simpson AGB. 2018. Recent advances in halophilic protozoa research. *Journal of Eukaryotic Microbiology* 65:556–570 DOI 10.1111/jeu.12495.
- Hauer G, Rogerson A. 2005. Heterotrophic protozoa from hypersaline environments. In: Gunde-Cimerman N, Oren A, Plemenitaš A, eds. *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. Dordrecht: Springer Netherlands, 519–539.

- Hauer G, Rogerson A, Anderson OR. 2001. *Platyamoeba pseudovannellida* n. sp., a naked amoeba with wide salt tolerance isolated from the Salton Sea, California. *The Journal of Eukaryotic Microbiology* **48**(6):663–669 DOI [10.1111/j.1550-7408.2001.tb00206.x](https://doi.org/10.1111/j.1550-7408.2001.tb00206.x).
- Helmus MR, Bland TJ, Williams CK, Ives AR. 2007. Phylogenetic measures of biodiversity. *The American Naturalist* **169**(3):E68–E83 DOI [10.1086/511334](https://doi.org/10.1086/511334).
- Horner-Devine MC, Bohannan BJM. 2006. Phylogenetic clustering and overdispersion in bacterial communities. *Ecology* **87**(sp7):S100–S108 DOI [10.1890/0012-9658\(2006\)87\[100:PCAOIB\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[100:PCAOIB]2.0.CO;2).
- Inoue M, Takenaka H, Tsurushima T, Miyagawa H, Ueno T. 1996. Colletofragarones A1 and A2, novel germination self-inhibitors from the fungus *Colletotrichum fragariae*. *Tetrahedron Letters* **37**(32):5731–5734 DOI [10.1016/0040-4039\(96\)01212-9](https://doi.org/10.1016/0040-4039(96)01212-9).
- Ishibashi M, Funayama S, Anraku Y, Komiya K, Omtura S. 1991. Novel antibiotics, furaquinocins C, D, E, F, G and H. *The Journal of Antibiotics* **44**:390–395 DOI [10.7164/antibiotics.44.390](https://doi.org/10.7164/antibiotics.44.390).
- Jia X, Dini-Andreote F, Falcão Salles J. 2018. Community assembly processes of the microbial rare biosphere. *Trends in Microbiology* **26**(9):738–747 DOI [10.1016/j.tim.2018.02.011](https://doi.org/10.1016/j.tim.2018.02.011).
- Katoh K, Toh H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **26**(15):1899–1900 DOI [10.1093/bioinformatics/btq224](https://doi.org/10.1093/bioinformatics/btq224).
- Kembel S, Cowan P, Helmus M, Cornwell W, Morlon H, Ackerly D, Blomberg S, Webb C. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**(11):1463–1464 DOI [10.1093/bioinformatics/btq166](https://doi.org/10.1093/bioinformatics/btq166).
- Kimura Y, Shiojima K, Nakajima H, Hamasaki T. 1992. Structure and biological activity of plant growth regulators produced by *penicillium* sp. no. 31f. *Bioscience, Biotechnology, and Biochemistry* **56**(7):1138–1139 DOI [10.1271/bbb.56.1138](https://doi.org/10.1271/bbb.56.1138).
- Kinashi H, Sakaguchi K. 1984. Structures of antibiotics 2-11-A and B. *Agricultural and Biological Chemistry* **48**(1):245–247 DOI [10.1080/00021369.1984.10866129](https://doi.org/10.1080/00021369.1984.10866129).
- Knowles SL, Raja HA, Wright AJ, Lee AML, Caesar LK, Cech NB, Mead ME, Steenwyk JL, Ries LNA, Goldman GH, Rokas A, Oberlies NH. 2019. Mapping the fungal battlefield: using in situ chemistry and deletion mutants to monitor interspecific chemical interactions between fungi. *Frontiers in Microbiology* **10**:285 DOI [10.3389/fmicb.2019.00285](https://doi.org/10.3389/fmicb.2019.00285).
- Kobayashi A, Kajiyama S-I, Inawaka K, Kanzaki H, Kawazu K. 1994. Nostodione A, a novel mitotic spindle poison from a blue-green alga *Nostoc commune*. *Zeitschrift für Naturforschung C* **49**(7–8):464–470 DOI [10.1515/znc-1994-7-812](https://doi.org/10.1515/znc-1994-7-812).
- Kosemura S. 2003. Meroterpenoids from *Penicillium citreo-viride* B. IFO, 4692 and 6200 hybrid. *Tetrahedron* **59**(27):5055–5072 DOI [10.1016/S0040-4020\(03\)00739-7](https://doi.org/10.1016/S0040-4020(03)00739-7).
- Kunin V, Raes J, Harris JK, Spear JR, Walker JJ, Ivanova N, Von Mering C, Bebout BM, Pace NR, Bork P, Hugenholtz P. 2008. Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. *Molecular Systems Biology* **4**(1):198 DOI [10.1038/msb.2008.35](https://doi.org/10.1038/msb.2008.35).
- Laland K, Matthews B, Feldman MW. 2016. An introduction to niche construction theory. *Evolutionary Ecology* **30**(2):191–202 DOI [10.1007/s10682-016-9821-z](https://doi.org/10.1007/s10682-016-9821-z).
- Lane DJ. 1991. 16S/23S rDNA sequencing. In: Stackebrandt E, Goodfellow M, eds. *Nucleic Acid Techniques in Bacterial Systematics*. New York, NY: John Wiley and Sons, 115–175.
- Legendre P, Legendre L. 2012. *Numerical ecology*. Third Edition. Amsterdam: Elsevier.
- Li C, Roeger KE, Kelly WL. 2009. Analysis of the indanomycin biosynthetic gene cluster from *Streptomyces antibioticus* NRRL 8167. *ChemBioChem* **10**(6):1064–1072 DOI [10.1002/cbic.200800822](https://doi.org/10.1002/cbic.200800822).

- Liang R, Davidova IA, Marks CR, Stamps BW, Harriman BH, Stevenson BS, Duncan KE, Sufita JM. 2016. Metabolic capability of a predominant Halanaerobium sp. in hydraulically fractured gas wells and its implication in pipeline corrosion. *Frontiers in Microbiology* 7(e1):116 DOI 10.3389/fmicb.2016.00988.
- Liu X, Li M, Castelle CJ, Probst AJ, Zhou Z, Pan J, Liu Y, Banfield JF, Gu JD. 2018. Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeotal lineages. *Microbiome* 6(1):1–16 DOI 10.1186/s40168-018-0488-2.
- Liu J, Meng Z, Liu X, Zhang X. 2019. Microbial assembly, interaction, functioning, activity and diversification: a review derived from community compositional data. *Marine Life Science & Technology* 1(1):112–128 DOI 10.1007/s42995-019-00004-3.
- Liu W, Xu D, Ma H, Al-Farraj SA, Warren A, Yi Z. 2016. Taxonomy and molecular systematics of three oligotrich (s.l.) ciliates including descriptions of two new species, Strombidium guangdongense sp. nov. and Strombidinopsis sinicum sp. nov. (Protozoa, Ciliophora). *Systematics and Biodiversity* 14(5):452–465 DOI 10.1080/14772000.2016.1162872.
- López-Lozano NE, Heidelberg KB, Nelson WC, García-Oliva F, Eguiarte LE, Souza V. 2013. Microbial secondary succession in soil microcosms of a desert oasis in the Cuatro Ciénegas Basin, Mexico. *PeerJ* 1:e47 DOI 10.7717/peerj.47.
- Ma K, Li L, Bao L, He L, Sun C, Zhou B, Si S, Liu H. 2015. Six new 3,4-seco-27-norlanostane triterpenes from the medicinal mushroom Ganoderma boninense and their antiplasmodial activity and agonistic activity to LXR β . *Tetrahedron* 71(12):1808–1814 DOI 10.1016/j.tet.2015.02.002.
- Madrigal-Trejo D. 2022. Análisis del metametaloma en los tapetes microbianos de Domos del Arqueano, Cuatro Ciénegas, como recapitulación del uso de metales a lo largo de la historia de la Tierra. Bachelor's thesis. Mexico City, Mexico. Facultad de Ciencias, Universidad Nacional Autónoma de México. Available at <http://132.248.9.195/ptd2022/enero/0821431/Index.html>.
- Margulis L, Ashen JB, Sole M, Guerrero R. 2006. Composite, large spirochetes from microbial mats: spirochete structure review. *Proceedings of the National Academy of Sciences of the United States of America* 90(15):6966–6970 DOI 10.1073/pnas.90.15.6966.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal* 17(1):10–12 DOI 10.14806/ej.17.1.200.
- McGonigle JM, Bernau JA, Bowen BB, Brazelton WJ. 2019. Robust archaeal and bacterial communities inhabit shallow subsurface sediments of the bonnevillie salt flats. *mSphere* 4(4):1–12 DOI 10.1128/msphere.00378-19.
- McMurdie PJ, Holmes S. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* 8(4):e61217 DOI 10.1371/journal.pone.0061217.
- Medina-Chávez NO, Viladomat-Jasso M, Olmedo-Álvarez G, Eguiarte Luis E, Souza V, De la Torre-Zavala S. 2019. Diversity of Archaea domain in Cuatro Ciénegas Basin: archean domes. *bioRxiv* DOI 10.1101/766709.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *2010 Gateway Computing Environments Workshop (GCE)* 2010:1–8 DOI 10.1109/GCE.2010.5676129.
- Mondav R, McCalley CK, Hodgkins SB, Frolking S, Saleska SR, Rich VI, Chanton JP, Crill PM. 2017. Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. *Environmental Microbiology* 19(8):3201–3218 DOI 10.1111/1462-2920.13809.

- Montiel-González C, Bautista F, Delgado C, García-Oliva F. 2018.** The climate of Cuatro Ciénegas Basin: drivers and temporal patterns. In: Souza V, Olmedo-Álvarez G, Eguiarte L, eds. *Cuatro Ciénegas Ecology, Natural History and Microbiology. Cuatro Ciénegas Basin: An Endangered Hyperdiverse Oasis*. Cham: Springer.
- Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K. 2019.** The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47(D1):D259–D264 DOI 10.1093/nar/gky1022.
- Nitti A, Daniels CA, Siefert J, Souza V, Hollander D, Breitbart M. 2012.** Spatially resolved genomic, stable isotopic, and lipid analyses of a modern freshwater microbialite from Cuatro Ciénegas, Mexico. *Astrobiology* 12(7):685–698 DOI 10.1089/ast.2011.0812.
- Nutman AP, Bennett VC, Friend CRL, Van Kranendonk MJ, Chivas AR. 2016.** Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. *Nature* 537(7621):535–538 DOI 10.1038/nature19355.
- Odling-Smee FJ, Laland KN, Feldman MW. 2003.** *The neglected process in evolution (MPB-37)*. Princeton: Princeton University Press.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013.** Vegan: community ecology package. R package version 2.0-8. Available at <http://CRAN.R-project.org/package=vegan>.
- Ortiz-Alvarez R, Casamayor EO. 2016.** High occurrence of Pacearchaeota and Woese archaeota (Archaea superphylum DPANN) in the surface waters of oligotrophic high-altitude lakes. *Environmental Microbiology Reports* 8(2):210–217 DOI 10.1111/1758-2229.12370.
- Paerl HW, Yannarell AC. 2010.** Environmental dynamics, community structure and function in a hypersaline microbial mat. In: Seckbach J, Oren A, eds. *Microbial Mats: Modern and Ancient Microorganisms in Stratified Systems*. Dordrecht: Springer Netherlands, 421–442.
- Pajares S, Bonilla-Rosso G, Travisano M, Eguiarte LE, Souza V. 2012.** Mesocosms of aquatic bacterial communities from the Cuatro Ciénegas Basin (Mexico): a tool to test bacterial community response to environmental stress. *Microbial Ecology* 64(2):346–358 DOI 10.1007/s00248-012-0045-7.
- Pajares S, Eguiarte LE, Bonilla-Rosso G, Souza V. 2013.** Drastic changes in aquatic bacterial populations from the Cuatro Ciénegas Basin (Mexico) in response to long-term environmental stress. *Antonie van Leeuwenhoek* 104(6):1159–1175 DOI 10.1007/s10482-013-0038-7.
- Pang Z, Chong J, Li S, Xia J. 2020.** MetaboAnalystR 3.0: toward an optimized workflow for global metabolomics. *Metabolites* 10(5):186 DOI 10.3390/metabo10050186.
- Papineau D. 2010.** Global biogeochemical changes at both ends of the proterozoic: insights from phosphorites. *Astrobiology* 10(2):165–181 DOI 10.1089/ast.2009.0360.
- Park JS, Cho BC, Simpson AGB. 2006.** Halocafeteria seosinensis gen. et sp. nov. (Bicosoecida), a halophilic bacterivorous nanoflagellate isolated from a solar saltern. *Extremophiles* 10(6):493–504 DOI 10.1007/s00792-006-0001-x.
- Peimbert M, Alcaraz LD, Bonilla-Rosso G, Olmedo-Alvarez G, García-Oliva F, Segovia L, Eguiarte LE, Souza V. 2012.** Comparative metagenomics of two microbial mats at Cuatro Ciénegas Basin I: ancient lessons on how to cope with an environment under severe nutrient stress. *Astrobiology* 12(7):648–658 DOI 10.1089/ast.2011.0694.
- Planavsky NJ, Rouxel OJ, Bekker A, Lalonde SV, Konhauser KO, Reinhard CT, Lyons TW. 2010.** The evolution of the marine phosphate reservoir. *Nature* 467(7319):1088–1090 DOI 10.1038/nature09485.

- Pluskal T, Castillo S, Villar-Briones A, Orešič M. 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* 11(1):318 DOI 10.1186/1471-2105-11-395.
- Post F, Borowitzka L, Borowitzka M, Mackay B, Moulton T. 1983. The protozoa of a Western Australian hypersaline lagoon. *Hydrobiologia* 105(1):95–113 DOI 10.1007/BF00025180.
- Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* 26(7):1641–1650 DOI 10.1093/molbev/msp077.
- Prieto-Barajas CM, Valencia-Cantero E, Santoyo G. 2018. Microbial mat ecosystems: structure types, functional diversity, and biotechnological application. *Electronic Journal of Biotechnology* 31(4):48–56 Pontificia Universidad Católica de Valparaíso DOI 10.1016/j.ejbt.2017.11.001.
- R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <http://www.r-project.org/>.
- Ramos VMC, Castelo-Branco R, Leão PN, Martins J, Carvalhal-Gomes S, da Silva FS, Filho JGM, Vasconcelos VM. 2017. Cyanobacterial diversity in microbial mats from the hypersaline lagoon system of Araruama, Brazil: an in-depth polyphasic study. *Frontiers in Microbiology* 8:1–16 DOI 10.3389/fmicb.2017.01233.
- Rebollar EA, Avitia M, Eguiarte LE, González-González A, Mora L, Bonilla-Rosso G, Souza V. 2012. Water-sediment niche differentiation in ancient marine lineages of *Exiguobacterium* endemic to the Cuatro Ciénegas Basin. *Environmental Microbiology* 14(9):2323–2333 DOI 10.1111/j.1462-2920.2012.02784.x.
- Rommel KR, Li C, Kelly WL. 2011. Identification of a tetraene-containing product of the indanomycin biosynthetic pathway. *Organic Letters* 13(10):2536–2539 DOI 10.1021/ol200570u.
- Saleem M, Fetzer I, Harms H, Chatzinotas A. 2013. Diversity of protists and bacteria determines predation performance and stability. *The ISME Journal* 7(10):1912–1921 DOI 10.1038/ismej.2013.95.
- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research* 13(11):2498–2504 DOI 10.1101/gr.1239303.
- Souza V, Eguiarte LE, Moreno-Letelier A, Travisano M, Alcaraz LD, Olmedo G. 2018. The lost world of Cuatro Ciénegas Basin, a relictual bacterial niche in a desert oasis. *eLife* 7:598 DOI 10.7554/eLife.38278.
- Souza V, Espinosa-Asuar L, Escalante AE, Eguiarte LE, Farmer J, Forney L, Lloret L, Rodríguez-Martínez JM, Soberón X, Dirzo R, Elser JJ, Rodríguez-Martínez JM, Soberón X, Dirzo R, Elser JJ, Forney L, Soberón X, Elser JJ. 2006. An endangered oasis of aquatic microbial biodiversity in the Chihuahuan desert. *Proceedings of the National Academy of Sciences of the United States of America* 103(17):6565–6570 DOI 10.1073/pnas.0601434103.
- Souza V, Olmedo-Álvarez G, Eguiarte LE. 2018. *Cuatro ciénegas ecology, natural history and microbiology*. New York: Springer International Publishing.
- Souza V, Siefert J, Escalante AE, Elser JJ, Eguiarte LE. 2012. The Cuatro Ciénegas Basin in Coahuila, Mexico: an astrobiological Precambrian Park. *Astrobiology* 12(7):641–647 DOI 10.1089/ast.2011.0675.
- Stolz JF. 2000. Structure of microbial mats and biofilms. In: Riding RE, Awramik SM, eds. *Microbial Sediments*. Berlin, Heidelberg: Springer, 1–8.
- Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TW, Fiehn O, Goodacre R, Griffin JL, Hankemeier T, Hardy N, Harnly J, Higashi R, Kopka J, Lane AN, Lindon JC, Marriott P, Nicholls AW, Reily MD, Thaden JJ, Viant MR. 2007. Proposed

minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* 3(3):211–221 DOI 10.1007/s11306-007-0082-2.

- Taboada B, Isa P, Gutiérrez-Escolano AL, del ángel RM, Ludert JE, Vázquez N, Tapia-Palacios MA, Chávez P, Garrido E, Espinosa AC, Eguiarte LE, López S, Souza V, Arias CF, Drake HL. 2018.** The geographic structure of viruses in the Cuatro Ciénegas Basin, a unique oasis in northern Mexico, reveals a highly diverse population on a small geographic scale. *Applied and Environmental Microbiology* 84(11):3285 DOI 10.1128/AEM.00465-18.
- Toju H, Tanabe AS, Yamamoto S, Sato H. 2012.** High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLOS ONE* 7(7):e40863 DOI 10.1371/journal.pone.0040863.
- Tozuka Z, Takasugi H, Takaya T. 1983.** Studies on tomaymycin. II. Total syntheses of the antitumor antibiotics, E-and Z-tomaymycins. *The Journal of Antibiotics* 36(3):276–282 DOI 10.7164/antibiotics.36.276.
- Van Der Hooft JJJ, Mohimani H, Bauermeister A, Dorrestein PC, Duncan KR, Medema MH. 2020.** Linking genomics and metabolomics to chart specialized metabolic diversity. *Chemical Society Reviews* 49(11):3297–3314 DOI 10.1039/d0cs00162g.
- Velez P, Gasca-Pineda J, Rosique-Gil E, Eguiarte LE, Espinosa-Asuar L, Souza V. 2016.** Microfungal oasis in an oligotrophic desert: diversity patterns and community structure in three freshwater systems of Cuatro Ciénegas, Mexico. *PeerJ* 4(Special Issue):e2064 DOI 10.7717/peerj.2064.
- Visscher PT, Dupraz C, Braissant O, Gallagher KL, Glunk C, Casillas L, Reed RES. 2010.** Biogeochemistry of carbon cycling in hypersaline mats: linking the present to the past through biosignatures. In: Seckbach J, Oren A, eds. *Microbial Mats: Modern and Ancient Microorganisms in Stratified Systems*. Dordrecht: Springer Netherlands, 443–468.
- Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007.** Naive Bayesian classifier for rapid assignment of rDNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16):5261–5267 DOI 10.1128/AEM.00062-07.
- Wang J, Shen J, Wu Y, Tu C, Soininen J, Stegen JC, He J, Liu X, Zhang L, Zhang E. 2013.** Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *The ISME Journal* 7(7):1310–1321 DOI 10.1038/ismej.2013.30.
- White TJ, Bruns TD, Lee SB, Taylor JW. 1990.** Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: *PCR Protocols*. Cambridge: Academic Press, Inc, 315–322.
- Wolaver BD, Crossey LJ, Karlstrom KE, Banner JL, Cardenas MB, Ojeda CG, Sharp JM Jr. 2013.** Identifying origins of and pathways for spring waters in a semiarid basin using He, Sr, and C isotopes: Cuatrociénegas Basin. *Mexico Geosphere* 9(1):113–125 DOI 10.1130/GES00849.1.
- Wong H, Ahmed-Cox A, Burns B. 2016.** Molecular ecology of hypersaline microbial mats: current insights and new directions. *Microorganisms* 4(1):6 DOI 10.3390/microorganisms4010006.
- Zaghouani M, Kunz C, Guédon L, Blanchard F, Nay B. 2016.** First total synthesis, structure revision, and natural history of the smallest cytochalasin: (+)-Periconiasin G. *Chemistry – A European Journal* 22(43):15257–15260 DOI 10.1002/chem.201603734.
- Zaitseva SV, Abidueva EY, Radnagurueva AA, Bazarov SM, Buryukhaev SP. 2018.** Structure of microbial communities of the sediments of alkaline transbaikalia lakes with different salinity. *Microbiology* 87(4):559–568 DOI 10.1134/S0026261718040185.