Title

Ecological relevance of abundant and rare taxa in a highly-diverse elastic hypersaline microbial mat, using a small-scale sampling

Authors

Laura Espinosa-Asuar^{1,*}, Camila Monroy¹, David Madrigal-Trejo¹, Marisol Navarro-Miranda¹, Jazmín Sánchez-Pérez¹, Jhoseline Muñoz¹, Juan Diego Villar², Julián Cifuentes², Maria Kalambokidis¹, Diego A. Esquivel-Hernández¹, Mariette Viladomat¹, Ana Elena Escalante-Hernández³, Patricia Velez⁴, Mario Figueroa⁵, Santiago Ramírez Barahona⁴, Jaime Gasca-Pineda¹, Luis E. Eguiarte¹ and Valeria Souza^{1,*}.

Affiliations

¹Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, AP 70-275, Coyoacán 04510, Ciudad de México, México

²Pontificia Universidad Javeriana, Bogotá D.C., Colombia

³Laboratorio Nacional de Ciencias de la Sostenibilidad, Instituto de Ecología, Universidad Nacional Autónoma de México, AP 70-275, Coyoacán 04510, Ciudad de México, México, City, México

⁴Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, Ciudad de México, México

⁵Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, Ciudad de México, México

*corresponding authors:

lauasuar@ecologia.unam.mx; souza@unam.mx

Abstract

We evaluated the microbial diversity and metabolomic signatures of a hypersaline elastic microbial mat from Cuatro Ciénegas Basin (CCB) in the Chihuahuan Desert of Coahuila, México. We collected ten samples within a small scale (1.5-meters transect) and found a high microbial diversity through NGS-based ITS and 16S rRNA gene sequencing. A very low number of taxa were abundant and shared between all sites, whereas the rare biosphere was more phylogenetically diverse (FPD index) and phylogenetically disperse (PSC index) than the abundant taxa for both analyzed libraries. In regard to potential biotic interactions (Pearson analysis), there were more positive correlations than negative. We also found a distinctive metabolomic signature for each sample and were able to tentatively annotate several classes of compounds with relevant biological properties. Together, these results reveal cohesive, diverse and fluctuating microbial communities, where abundant and rare taxa appear to have different ecological roles.

Keywords: biotic interactions, Cuatro Ciénegas Coahuila, metabolomic analyses, microbial communities, phylogenetic diversity, rare biosphere.

Introduction

Microbial mats are tightly interacting communities, usually self-sustaining, that are horizontally stratified with multilayered biofilms embedded in a matrix of exopolysaccharides binding cells and inorganic substances together (Bolhuis et al., 2014; Prieto-Barajas et al., 2018; Stolz, 2000). These stratified structures, as well as stromatolites, are the oldest communities found in the fossil record, 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, Eguiarte, et al., 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 and form extremely cohesive and co-adapted microbial communities (Souza, Eguiarte, et al., 2018). The uniqueness of microbial life in CCB -- characterized as an “Astrobiological Precambrian Park” (Souza et al., 2012) -- is reflected in the presence of ancient marine lineages (Rebollar et al., 2012; Souza et al., 2006; Souza, Eguiarte, et al., 2018), rich prokaryotic communities (López-Lozano et al., 2013; Pajares et al., 2012, 2013; Souza, Olmedo-Álvarez, et al., 2018) , and diverse fungal (Velez et al., 2016) and viral communities (Taboada et al., 2018), as well as a high microbial diversity within 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).

Figure 1

(A) The Cuatro Ciénegas Basin within México, and geographic location of Archaeal Domes in Pozas Azules Ranch. (B) Detail of an Archaeal Domes slice. (C) Domes when the sampling was conducted (note the size scale). Photos by David Jaramillo.

The internal microbial layer, insulated by an outer aerobic community, thrives under an anaerobic environment, rich in methane and hydrogen sulfide, and small volatile hydrocarbons that recreate the atmosphere of the Archaeal Eon, hence we named these mats “Archaeal domes” (Medina-Chávez et al., 2020). Similar types of elastic mats have previously been described in marine tidal zones, although their formation is relatively rare (Gerdes et al., 1993).

Given their functional richness, microbial mats are ideal systems for analyzing the role of microbial interactions in maintaining community structure under extreme conditions (Paerl & Yannarell, 2010; Prieto-Barajas et al., 2018). In this sense, there are three approaches that can improve our understanding of biotic interactions and processes at an evolutionary scale (Mayfield & Levine, 2010) for microbial mat communities. First, determine 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;). Second, investigate the ecology of microbial subcommunities, such as those of rare taxa, a less-studied topic than the whole community (Liu et al., 2019) but important to explore for their potential contribution to ecological processes (Jia et al., 2018). Third, analyze the metabolomics 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. Moreover, metabolomics can also elucidate ecological aspects (Sardans et al., 2011) that help uncover the reciprocal interactions between ecology and evolution, known as eco-evolutionary feedbacks (D. M. Post & Palkovacs, 2009).

In this study, we evaluated the microbial diversity, the rare biosphere, and its metabolomic signature within this elastic hypersaline microbial mat from CCB, across ten samples collected within a 1.5-meter transect. Specifically, we aimed: 1) to explore microbial diversity analyzing amplicons of the 16S rRNA and ITS regions,

2) to determine whether rare and abundant taxa are phylogenetically structured; 3) to evaluate microbial metabolomic signatures as an approximation to evolutionary feedbacks; and 4) to approximate biotic interactions.

Results

Microbial taxonomic composition

16S rRNA gene and ITS libraries revealed the occurrence of different microbial lineages, as genera composition showed (Fig. 2).

Figure 2

Genera relative abundance of taxa in Archaeal Domes, Cuatro Ciénegas Basin sample. A) Bacteria (only showed the most common taxa, more than 1% of diversity) B) Fungi (using Merge database, only fungal ASVs. NA is for ASVs that were not assigned to any genera, but phylum only).

Overall, 40 prokaryote phyla were detected from the 16S rRNA gene libraries (Suppl. Fig. 1A), and a total of 6,063 different ASVs were found, with a proportion of 11.8% of unclassified reads (Suppl. Table 2). Bacteroidetes was the most abundant phylum, representing around 23% of the prokaryotic diversity in the samples, followed by Proteobacteria with $\approx 19\%$ and Cyanobacteria with nearly 18%. Spirochaetes and Chloroflexi represented $\approx 8\%$ and $\approx 4\%$ of the prokaryotic diversity, respectively. Nearly 2% were Firmicutes, Patesibacteria and Halanaerobiota (Suppl. Fig. 1A). A total of 410 genera of Bacteria were detected in the 10 sampled sites (S1-S10) at a 1.5 m-scale. From those, only 9 genera were abundant (representing $\approx 70\%$ of total abundance, Fig. 2 A): *Coleofasciculus* (or *Microcoleus*), *Spirochaeta*, *Catalinimonas*, *Desulfovermiculus*, *Halanaerobium*, *Tangfeifania*, *Sungkyunkwania*, *Sediminispirochaeta* and *Imperialibacter*. The remaining 401 genera can be classified as rare biosphere, with $\approx 30\%$ of total abundance (less than 1% each, Fig. 2 A). Moreover, 28 ASVs of Archaea were obtained using our 16S primers, comprising two phyla, Woesearchaeota and Euryarchaeota (Suppl. Fig. 1a). Halobacteria class was the most abundant, and we detected 4 genera: *Halovivax*, *Halorubrum*, *Halorussus* and *Haloasta* (Suppl. Table 2). 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 database, we detected 923 different ASVs. The majority of the classified reads belong to Alveolata protists (77%, Suppl. Fig. 1B), 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 (Suppl. Table 2). The majority of ASVs within Forward ITS database 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 abundance in ITS reads for all sites. Using Merge ITS ASVs database for a better taxonomic assignment, Fungal ASVs comprising 18 genera were detected, with phyla Ascomycota, Basidiomycota and Rozellomycota present in all samples (Fig. 2B).

Alpha and beta diversity

Rarefaction curves (Suppl. Fig. 2A and 2B) showed similar results for both the Forward-Only ITS region libraries (S1, S2, S3, S4, S6, S7), and 16S rRNA gene libraries, as all sites reached an asymptote, suggesting an adequate sampling effort.

Only 183 ASVs (3% of the diversity) were shared between all sites for 16S rRNA gene libraries (Fig. 3A), forming a very small core, but comprised 72.4% 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 94.7% of all reads (Fig. 3B).

Figure 3

Graphic representation of shared ASVs within Archaeal Domes mats 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. Pays show taxonomy of shared ASVs within all sites (a few but abundant ASVs core). (A) 16S Archaea and Bacteria, rRNA gene database, (B) Fungi and other Eucarya, Forward-Only ITS database.

16S rRNA ASVs alpha diversity (Table 1) ranged from 2470 expected ASVs according to Chao1 in S2 (Shannon = 7.4, highest Shannon index value), to 544 expected ASVs in S9 (Shannon = 6.1). In the case of the ITS region libraries (Forward-Only ITS database), the most ASV Chao1 diversity for Eukarya was S6 with 393 expected ASVs and a Shannon diversity of 2.5; S3 had the lowest Chao1 diversity, with 199 expected ASVs, but this site had the highest Shannon value of 3 (Table 1).

ITS region						
α diversity				Phylogenetic diversity		
Sample	Observed	Chao	Shannon	Faith PD	Faith PD rare	Faith PD abundant
1	322	322	1.827	86.309	42.596	18.161
2	226	226	2.713	60.622	40.846	14.516
3	199	199	3.062	47.289	30.370	14.774
4	270	275	1.783	83.008	51.513	16.287
6	383	393	2.501	131.435	75.352	28.794
7	337	344	2.008	114.983	60.352	18.865

186

16S rRNA						
α diversity				Phylogenetic diversity		
Sample	Observed	Chao	Shannon	Faith PD	Faith PD rare	Faith PD abundant
1	1761	1773.203	6.863	544.704	290.491	44.159
2	2399	2470.398	7.402	789.807	368.430	84.442
3	1068	1071.318	6.256	211.745	156.204	20.064
4	1564	1568.664	6.499	500.787	259.828	40.627
5	1344	1358.842	6.882	220.536	172.299	21.557
6	2345	2353.855	6.301	851.168	302.103	131.337
7	2156	2172.218	6.838	759.536	328.238	97.486
8	1195	1217.568	6.487	205.987	139.839	22.798
9	544	544	6.126	160.368	94.201	18.577
10	828	875.634	5.115	283.017	148.655	36.514

187

188 Table1

189 Alpha diversity indices for 16S rRNA gene and Forward-Only ITS region databases
190 from Archaean Domes CCB microbial mats.

Paired beta bacterial diversity among microbial mat samples for both the 16S rRNA gene and ITS region databases ranged from 0.2 to 0.7 for the Bray-Curtis diversity values and 0.3 to 0.7 according to the Jaccard-1 values (Suppl. Fig. 3 A and B). For both indexes, S10 is different from the rest of the 16S rRNA gene libraries, and S1, S2, S4, S6, S7 were associated in a cluster. 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 ($p=0.0402$) 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.

Phylogenetic Diversity

For both libraries, Faith Phylogenetic Diversity showed that rare ASVs were more diverse than abundant ASVs (Table 1), and Phylogenetic Species Clustering (PSC) measures showed that abundant ASVs were clustered within phylogeny, while rare ASVs were disperse (Fig. 4 A and B).

Figure 4
Graph representing null model distribution of Phylogenetic Species Clustering (nmdPSC) values for all community, abundant and rare taxa from 16S rRNA database (A) and Forward-Only ITS database (B). 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 Suppl. Table 5.

Approximation to biotic interactions: Pearson analysis

In order to identify potential biotic interactions within the dominant taxa from the three domains of life within the Archaeal Domes, we calculated Pearson correlations between ASVs (Fig. 5).

Figure 5
Pearson correlation analysis within the most common (i.e., involving more than 1% of diversity) Bacteria, and all genera of Archaea and Fungi (from Merge ITS database). Colored squares are for significant correlations ($P \leq 0.05$), blue for positive ones and red for negatives.

We analyzed the most abundant ASVs (above 1%) from the 16S rRNA gene libraries and both ITS databases, along with 22 Archaea ASVs. We observed that most of the negative co-occurrence correlations are associated with Bacteria, in particular

the Bacteroidetes phylum. Within the complete correlation matrix, we observed 22 negative co-occurrence correlations, along with 9 for Archaea, 14 for Fungi, and 6 for Eukarya (see Suppl. Table 3 for interaction counts). In addition, we observed that Archaea-Archaea correlations (Fig. 5) were mostly positive (80), the majority of them enclosed within Archaea ASVs (32), whereas Eukarya-Eukarya interactions had the fewest positive correlations (4). Remarkably, in the cross-kingdom fungal interactions, most of the negative fungal correlations (8 of 14) were observed in Rozellomycota.

Metabolomic profile

We found a wide range of metabolites based on heatmap and GNPS molecular networking analysis (Fig. 6 and Suppl. Fig. 4 A&B).

Figure 6

Molecular network of soil samples grouped metabolite features into 140 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).

Untargeted metabolomics analysis of microbial communities is complicated, due to the complexity and heterogeneity of this matrix and the scarcity of the secondary metabolites extracted. The 1,341 molecular features at unique retention times (Suppl. Fig. 4 A&B) shared several common regions across all samples, 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, Suppl. Fig. 4B). We found highly heterogeneous metabolic profiles in all samples, and no significant associations were observed between metabolite features and ITS or 16SrRNA abundance matrices.

On the other hand, GNPS molecular networking analysis grouped the metabolite features in 18,547 nodes distributed into 83 chemical families (sub-networks) of ≥ 3 members, 57 families of two metabolite features, and 875 singletons (Fig. 6). Detected compounds in the GNPS and detailed metabolomic analysis of the main ions observed in LC-MS/MS data, focused in microbial metabolites, allowed us to tentatively identify several secondary metabolites that passed our quality criteria (≤ 5 ppm mass error; Suppl. Table 4 and Fig. 7).

Figure 7

Structures of microbial metabolites tentatively annotated in samples S1-S10 (≤ 5 ppm mass error).

Discussion

Microbial composition at a microscale

Our study revealed a remarkably high microbial diversity within a 1.5 m scale in an elastic microbial mat located at an extreme site. Altogether, we found 40 prokaryotic phyla, including 410 genera of Bacteria and 4 of Archaea, as well as 18 Fungal genera (Fig. 2 and Suppl. Fig. 1).

Bacteria

The bacterial phyla (Suppl. Fig. 1) detected in our study site are common components of hypersaline microbial mats (Bolhuis et al., 2014; Visscher et al., 2010): Bacteroidetes, Proteobacteria, Cyanobacteria, Chloroflexi, and Firmicutes, among other abundant 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* (*Microcoleus*) (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 et al., 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 et al., 2014; Margulis et al., 2006), an anaerobic chemo-organotrophic taxa abundant in hypersaline marine mats (Ben Hania et al., 2015). Interestingly, 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 of which are common genera in marine microbial mats (Bolhuis et al., 2014). This suggests that this shallow salty pond could be more similar to marine lineages than other sites in CCB (see Rebollar et al., 2012; Souza et al., 2006, 2018).

Previous metagenomic analysis from a mat in Churince within CCB reported 1,431 OTUs (de Anda et al., 2018) – four times less diverse than the Archaeal Domes (6063 ASVs). Other studies within CCB, also with complete metagenomes, retrieved 250 expected OTUs using 31 core genes (Bonilla-Rosso et al., 2012). Therefore, it is possible that using high coverage 16S tags may reveal a hidden high-diverse rare biosphere in these microbial mats.

Regarding the high diversity within the Archaeal Domes and comparing it with other microbial mats (since ASVs and OTUs are reported providing similar ecological results (Glassman & Martiny, 2018)), Cardoso et al. (2019) reported 977 OTUs in marine microbial mats, and Wong et al. (2015) found 4800 OTUs in Shark Bay, which are considered some of the most diverse microbial mats worldwide.

Archaea

In a recent metagenomic study of the Archaeal Domes (Medina-Chávez et al., 2020), Archaea taxa formed a constant core that is more diverse than any other site within or outside of CCB. In those metagenomes, Archaea represented nearly 5% of the total reads, accounting for 230 ASVs.

In our data, by using 16S rRNA gene amplicons, we only detected two main phyla, as expected given the nature of the amplified region (Suppl. Fig. 1 and Suppl. Table 2). These were Woesearchaeota, a mainly halophilic phylum and potentially syntrophic within 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 within saline habitats and sediments (McGonigle et al., 2019; Zaitseva et al., 2018). The metagenomic data (Medina-Chávez et al., 2020) and the 16S rRNA gene amplicons were sampled from the same pond at different times and space, and both showed 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 unique niche within the micro-scale of microbial mats, where they can degrade the abundant EPS produced in the CCB microbial mats (Breitbart et al., 2010; De Anda et al., 2018; Peimbert et al., 2012). In particular, halotolerant taxa such as members within 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 occupy a unique niche within the micro-scale of the Archaean Domes (Cantrell et al., 2011).

Other eukaryotes

The overall diversity of protists in the Archaean Domes is comparable to the diversity observed in other microbial mats (329 ASVs, Suppl. Table 2). For example, Saghaï et al. (2017), reported nearly half of the 586 18S rRNA OTUs they analyzed as Protozoa in a saline shallow pond mat in Chile, while ≈ 500 eukaryotic 18S rRNA OTUs were observed in mats from hydrothermal vents in Guaymas Basin (Pasulka et al., 2019), where half of them were likewise classified as Protozoa taxa. More than 1500 eukaryotic 18S rRNA OTUs were detected in microbialites in Highborne Cay, Bahamas, and Hamelin Pool (Shark Bay), Australia (Edgcomb et al., 2014), with also a half proportion being Protozoa.

In our sample, there are three abundant ASVs of Alveolata protists that represent nearly 70% of the ITS region (Suppl. Fig. 1B, Forward-Only ITS database) in all sequenced sites (S1-S4, S6, S7). 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 abundance (Suppl. Fig. 1B).

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 abundance ITS ASVs in our study (Merge database, Suppl. Table 2). 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 et al., 2001; Hauer & Rogerson, 2005; F. 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., 2020), strongly suggests that predator-prey interactions could be very important for the dynamics of the system (Carreira et al., 2020).

Alpha and beta diversity

The studied microbial mats –which are subject to extreme and fluctuating environmental conditions– have a similar Shannon diversity as soil bacterial communities (Dang et al., 2019; Wang et al., 2018). The Shannon Index accounts for both species evenness and abundance; hence, it could sub-represent diversity if most of the diversity is part of the rare biosphere. For example, Site 3 for the Eukarya database had the highest Shannon value, despite having the lowest ASV number (Table 1), due to its higher evenness distribution of taxa. Meanwhile, Chao1 only compares the observed diversity with the expected, given a species distribution, which more accurately reflects the total diversity of the site (Table 1; Suppl. Fig. 2A and 2B).

Given the extraordinary patchiness of the observed diversity, exemplified by genera composition differences between sample sites (Fig 2A), as well as unique metabolomic patterns in each sample (Suppl. Fig. 4A&B), beta diversity analysis showed a large turnover between sites despite the small scale (Suppl. Fig. 3). Accordingly, the different domains analyzed (i.e., the Bacteria database or the Eukarya database) had a distinctive association pattern, showing a statistically significant geographic association only for the bacterial database.

Our results suggest a small taxonomical and functional core in these hyper-diverse 10 samples of the Archaeal Domes, formed by bacterial and Eukarya ASVs shared among all sites, that make up a low fraction of the total taxa, but are highly abundant (183 16S rRNA ASVs, that comprises 3% of taxa (Fig. 3A) and 72.4% abundance of all reads; 56 (6%) shared ITS ASVs with 94.7% abundance of all reads) (Fig. 3B).

Interactions in the Archaeal Domes, inferred from the co-occurrence or co absence between sites.

In this study, we performed a Pearson correlation analysis (Fig. 5) to identify potential ecological roles and interactions between the most abundant ASVs within the three databases and 22 archaeal ASVs. Negative correlations (i.e. absence or lower than expected co-occurrence) can be used to identify metabolic competition, separated niches, antagonistic interactions, or any other relationship precluding co-occurrence (Saghai et al., 2017). Meanwhile, positive correlations (i.e. co-occurrence frequently or always observed) encompass a variety of relationships that include mutualism, symbiosis, parasitism, predation, common abiotic niche requirements (which are particularly relevant to complex microbial mat communities), associations with redox gradients, and metabolic syntrophy (Saghai et al., 2017). We found more positive correlations than negative (Suppl. Tab. 3) in our analysis. However, these correlations do not necessarily indicate direct interactions, since two taxa that are co-present may not be in direct physical or metabolic contact, due to the spatial heterogeneity intrinsic to microbial mats. Therefore, this result should be taken cautiously, given the Pearson correlation assumptions, as well as the consideration that only a small (but abundant) fraction of the diversity is taken into account.

On the other hand, some of the twenty-two archaeal ASVs analyzed (Fig. 5) formed a pattern of co-presence associations, which is worth noting since the same ASVs were also part of the Archaea core observed in a time series metagenomic study (Medina-Chávez et al., 2020). These positive associations reflect the presence of taxa within samples: for example *Halorubrum*, *Halobacteria* and *Wosearchaes* are positively associated because they were detected (i.e. coexist) in sample 2, and *Halovivax*, *Haolorusis* and *Halorubraceae* were detected in sample 6; a similar situation occurs for the fungi and Eukarya ASVs associated with them. Future detailed studies, including metagenomic analyses, are required to understand the mechanism of this potential cooperation, since co-cultivation techniques are difficult given the differential life strategies of Archaea, which grow very slowly compared with the faster growth of Fungi in culture.

It is interesting that, while most of the known fungal species are cosmopolitan and generalists, a few others are specialists, usually adapted to extreme conditions (Cantrell et al., 2011). For instance, Rozellomycota is a relatively large and widespread group of eukaryotes, branching at the base of the fungal tree of life (Corsaro et al., 2014). This group of fungi includes a number of amoebae parasites that have developed unique morphological and genomic adaptations to intracellular parasitism (Vávra & Lukeš, 2013). Thus, the utmost negative correlations observed in two Rozellomycota ASVs (Fig. 5) might suggest the endoparasitic lifestyle of these early-diverging fungi in the analyzed Archaeal Domes.

The majority of the negative associations found among the abundant taxa of this study were bacterial interactions (Fig. 5; Suppl. Table 3). Among them, bacteria-bacteria negative interactions were mainly between different phyla. These interactions are likely not based on metabolic competition, as they are mainly found between different phyla that could not be sharing a niche. However, antagonism has been reported previously in Cuatro Ciénegas bacterial taxa (Aguirre-von-Wobeser et al., 2014; Moreno Fontalba, 2017; Pérez-Gutiérrez et al., 2013) and within cross-kingdom interactions (Velez et al., 2018), which may account for the negative correlations observed here. Nevertheless, it is important to remember that these are

only negative correlations, and experimental data is needed to demonstrate antagonism in this system.

Phylogenetic diversity

Rare taxa

A third of the abundance of the bacterial genera detected in our samples belongs to rare bacterial taxa (Fig. 2A). The rare biosphere was more phylogenetically diverse (Faith Phylogenetic Diversity index, Table 1) and phylogenetically disperse (Phylogenetic Species Clustering, Fig. 4) than the abundant taxa within these samples for both the 16S rRNA and ITS libraries, suggesting that environmental filtering is not occurring for the rare biosphere in this site. This evidence suggests that these rare taxa could be a large reservoir of genetic diversity, as well as a matrix of rich microbial interactions and diverse metabolic functions within this microbial mat (Jia et al., 2018), perhaps presenting collective adaptations to the extreme conditions.

Abundant taxa

The pattern of phylogenetic clustering of the abundant ASVs is supported by Pearson analysis, with a higher number of positive associations in general (reflecting co-presence of taxa) and negative bacterial-bacterial associations between different phyla. This pattern 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 or 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 suggests high levels of competition among those abundant microbes.

Metabolomic profile

The dynamic and complex metabolomic profiles of the studied microbial mat was consistent with the highly diverse microbial community observed in the metagenomic analysis (Medina-Chávez et al., 2020) (Fig. 6 and Suppl. Fig. 4A&B).

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 et al., 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. 7). Many of these compounds were found to overlap between most of the samples: the antibiotics indolactam 1 (Irie et al., 1990), 8,9-dihydroindanomycin (Li et al., 2009), neoindanomycin (Rommel et al., 2011) described from *Streptomyces* species, and martensine A reported from the marine red alga *Martensia fragilis* (Kirkup & Moore, 1983); 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 colletotragarone A1, from fungi *Colletotrichum fragariae* and *C. capsiciae* (Inoue et al., 1996); benzastatin A (Kim et al., 1996) and periconiasin G (Zaghouani 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 et al., 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 et al., 2006) were identified only in S7; the antimitotic agent nostodione A from the blue-green alga *Nostoc commune* (Kobayashi et al., 1994) commune 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 (Suppl. Table 4) do not match with any data reported and could represent potentially new secondary metabolites.

Several compounds associated with 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).

Perspectives

In view of our results, the complex metabolomic data suggests local adaptive processes, following eco-evolutionary dynamics. These dynamics would create cohesive, diverse and fluctuating communities where taxa constantly and actively modify their own ecological niche and that of others (niche construction) (Laland et al., 2016; Odling-Smee et al., 2003, Van Der Hooft et al., 2020). However, this should be tested in future studies, exploring the possibility that the rare biosphere could fulfill the majority of the trophic levels and metabolic tasks, implementing metabolomics, mesocosms, and metagenomic time series approaches. Additional perspectives could evaluate the effect of environmental filtering, deepening the analysis of the biotic interactions, including the rare biosphere.

The high phylogenetic diversity and phylogenetic dispersion of the rare biosphere may suggest that there is a diverse “seed bank” in the aquifer of CCB, a deep biosphere fed by magmatic energy (Wolaver 2013) that constantly “seeds” the Archaeal Domes community, along with deep water and particular minerals. Therefore, in this scenario, eco-evolutionary feedbacks could enhance community cohesion in Red Queen-like dynamics (Haafke et al., 2016), a hypothesis stating that “biotic factors provide the basis for a self-driving perpetual motion of the effective environment and so of the evolution of species affected by it” (Van Valen, 1973). In our system, there may be a constant change in biotic interactions, as well as evolutionary landscapes, while the abiotic variables (i.e., gases, nutrients, pH, salts) are also constantly changing, as a product of community feedback within the deep aquifer. In the Archaeal Domes, active niche construction may have allowed the communities to co-evolve around a stable species core, given an influx of new neighbors of microbes and viruses (Taboada et al., 2018) from the deep biosphere,

which constantly assembles complex and new interactions. These ideas require further testing, along with the community's resilience to fluctuations within these extreme conditions.

Methods

Sampling site and sample collection

During the dry season (i. e. most of the year at CCB; Montiel-González et al., 2017), the microbial mats of the Archaeal domes remain humid and active under a salty crust, which is then dissolved during the rainy season, allowing for increased photosynthetic activity and the development of the dome-like structures.

The microbial mats of the Archaeal domes were sampled during the rainy season of 2018 (October), with ten evenly spaced samples (S1-S10) along a 1.5-meter transect (26° 49' 41.7" N 102° 01' 28.7" W) in the 'Pozas Azules' ranch of Pronatura Noroeste within CCB (Fig. 1). The salinity and pH were constant along the 1.5-meter transect (salinity 53‰, pH 9.8), and the average nutrient content showed a slight stoichiometric imbalance (C:N:P 122:42:1), similar to the one measured during the rainy season of 2016 (Medina-Chávez et al., 2020). Microbial mats and associated sediments (8 cm) 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.

Total DNA extraction and amplicons sequencing

Prior to DNA extraction, approximately 0.5 g of each sample was rinsed with sterile water. Total DNA extraction was performed following the protocol reported in de Anda et al. (2018), which is based on slight modifications from the Purdy et al. (1996) protocol.

DNA was sequenced at the Laboratorio de Servicios Genómicos, LANGE BIO for the V3-V4 region of the 16S rRNA gene (357F primer (Rudi et al., 1997), and the ITS nDNA region (ITS1F/ITS4R for ITS; (White et al., 1990). For each sample, 5 mg of genomic DNA (DO 260/280 1.8) was used; 16S amplicons were sequenced within Illumina Next-Seq 500 platform (150 SE), and the ITS amplicons were sequenced

with a Mi-Seq platform (2x300 PE). All raw sequences generated are available at GeneBank Bioproject PRJNA705156 (ITS accession SAMN18070760; 16S accession SAMN18070761).

Bioinformatics analyses

The 16S rRNA amplicon reads were quality filtered and dereplicated using the QIIME 2 bioinformatics platform version 2019.7 (Bolyen et al., 2018). Sequences were demultiplexed and truncated at the 20th bp from the left, and the 120th bp from the right. Divisive Amplicon Denoising Algorithm 2 (dada2) was then used to filter by sequence quality, remove chimeras, denoise, and create a table of amplicon sequence variants (ASV) per sample. ASVs were then taxonomically classified using the silva_132_99_v3v4_q2_2019-7 database. For all subsequent analyses the final ASV table was rarefied to 760,000 reads. For abundant ASVs (>1%) and Archaeobacteria ASVs, an additional taxonomic classification was conducted by selecting those taxa with coincident classification across the results of *blastN* (both type strain and 16S blast) (Altschul et al., 1990), the RDBP classifier (Q. Wang et al., 2007), and Qiime2 (Silva database) (Bolyen et al., 2018; Quast et al., 2013).

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. ASVs classification was inferred from forward and reverse reads using the run-specific error rates (Qiime2 and R code is available at <https://gitlab.com/lauasuar/archaeal-domes-repository>).

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 pre-denoised ASVs were merged after excluding reads with an overlap of less than 12bp 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 database, Suppl Table 1). ASVs without taxonomic assignment (mostly non-fungi organisms) were classified using *blastn*, where only those ASVs within identities greater than 90% were enlisted (Suppl. Table 1). The remaining ASVs were considered non-classified.

As a great proportion of the reads failed to merge (~56%), a parallel classification was implemented using the forward independently. The RDP naive Bayesian classifier was used on the two sets of reads (forward and reverse) to check for congruence in the classification between of 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 to corroborate their taxonomic assignment. Even though some ASVs reached a genus level assignment, the taxonomic classification of forward reads was limited to the phylum level (ITS table, Suppl. Table 2); a more robust classification of ASVs was reached when using forward ASVs, thus all subsequent analyses were performed using the forward-only ASVs. The Eukaryotic classification followed the UNITE database. The ITS table abundance was rarefied to 167,660 reads using the *vegan* package (Oksanen et al., 2013) in R (R Core Team, 2020).

In sum, the 16S rRNA amplicon filtered database included 760,326–1,973,982 high quality reads per sample, with a total of 6,063 ASVs. The ITS amplicon filtered database included 167,660–352,720 high quality reads per sample, with a total of 923 ASVs. ITS amplicons were obtained for only six samples (S1, S2, S3, S4, S6 and S7) due to DNA depletion in four samples (S5, S8, S9 and S10). The ASV abundance tables and the amplicons sequences are available at <https://gitlab.com/lauasuar/archaeal-domes-repository>.

Alpha and beta diversity estimates

To evaluate all samples from 16S (S1-S10) and 6 samples (S1-S4, S6, S7) from the Forward-Only ITS database, 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 rRNA gene and the six samples from the ITS region, we constructed an UpSet plot using the *UpSetR* package (Conway et al., 2017) in R (R Core Team 2020).

Alpha (i.e., Chao1, Shannon) and beta (i.e., Bray-Curtis, Jaccard) indices were estimated using the 16S rRNA ASV abundance table, as implemented in Qiime2. For the ITS ASV abundance table, alpha and beta diversity indices were estimated with the *phyloseq* package (McMurdie & Holmes, 2013) in R (R Core Team, 2020). The beta diversity indices were 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 subset of taxa are clustered within the phylogeny.

To estimate the two metrics, we used the rarefied 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. 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 and abundant ASVs, we estimated the first and third quartiles (1Q, 3Q) of the distribution of ASV abundances 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. We estimated SES for PD and PSC as defined above, using matrices for rare and abundant ASV (R code for all phylogenetic diversity analysis is available at <https://gitlab.com/lauasuar/archaeal-domes-repository>). The phylogenetic tree was generated by QIIME 2 (Bolyen et al., 2018) for 16S data. For the ITS data (Forward-Only database), a phylogeny was obtained by applying an equivalent phylogenetic QIIME 2 methodology, using Maftt (Kato & Toh, 2010) and FastTree (Price et al., 2009) within Cipres Science Gateway (Miller et al., 2010).

Pearson correlation estimate among abundant taxa

We carried out an analysis of correlated presences and absences of microbial taxa across communities to identify plausible interactions and ecological roles within microbial genera (Karpinets et al., 2018). To do this, we used a normalized abundance of six-sites matrix that included ASVs from both ITS databases and 16S rRNA gene library (Suppl Table 1): <1% abundance classified the ASV 16S database, 22 six-sites archaea ASVs, and <1% classified the ASV ITS database (calculated without the first three high abundance ASVs). Data was Hellinger transformed, with the *vegan* package (Oksanen et al., 2013) in R (R Core Team, 2020). This normalized matrix was used to calculate the correlogram with Pearson correlation (positive and negative strong correlation ($r \geq 0.9$, $P \leq 0.05$)) using the R Stats v3.6.1 library (R Core Team, 2020) and *corrplot* package for the graph (Taiyun & Viliam, 2017).

Metabolomics

Soluble compounds extraction and LC-MS/MS analysis

The samples (10 g each) were extracted with 60 mL of CHCl₃-MeOH (1:1) in an orbital shaker at 150 rpm for 24 hrs. The mixture was filtered, and the solvent was evaporated under reduced pressure. The dried extracts were reconstituted in 60 mL of CH₃CN-MeOH (1:1) and defatted with hexanes. Then the CH₃CN-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 µm membrane and analyzed using a Waters Acquity Ultraperformance Liquid Chromatography (UPLC) system (Waters Corp.) coupled to a Thermo Q Exactive Plus (ThermoFisher) mass spectrometer. The UPLC separations were performed using an Acquity BEH C₁₈ column (50 mm × 2.1 mm I.D., 1.7 µm; Waters) equilibrated at 40 °C and a flow rate set at 0.3 mL min⁻¹. The mobile phase consisted of a linear CH₃CN-H₂O (acidified with 0.1% formic acid) gradient starting at 15% to 100% of CH₃CN over 8 min. The mobile phase was held for another 1.5 min at 100% CH₃CN 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* 150–2000, with the following settings: capillary voltage, 5 V; capillary temperature, 300°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 1×10⁶, 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 4 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 (full data, feature-based molecular networking can be accessed here: <https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=547303b749f041bb88eee71145de5184>) and graphic visualization of molecular networking was performed in Cytoscape 3.8.0 (Shannon et al., 2003). The chemical profiles were also 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), MarinLite (University of Canterbury, New Zealand) and SciFinder (CAS) databases as described by El-Elmat et al. (El-Elmat et al., 2013), for fungal and bacterial small molecules. For candidate search, exact mass accuracy was set to 5 ppm. Heatmap visualization of metabolomic data table was generated in MataboAnalyst (Pang et al., 2020).

Acknowledgements

We would like to thank Dr. Erika Aguirre-Planter and Jessica Abril Hernández for technical and field assistance. We also thank PRONATURA Noreste for the access to the Pozas Azules ranch, and to Felipe García Oliva (CIEco, Biogeoquímica de suelos) for nutrient analysis of the samples. This research was supported by funding

from Sep-Ciencia Básica Conacyt grant 238245, and PAPIIT AG200319 / RG200319 and IN222220. The sampling was conducted with the collection permit SGPA/DGVS/03188/20 issued by Subsecretaría de Gestión para la protección Ambiental, Dirección General de Vida Silvestre.

Competing interests

The authors declare that no competing interests exist.

References

- Aguirre-von-Wobeser, E., Soberón-Chávez, G., Eguiarte, L. E., Ponce-Soto, G. Y., Vázquez-Rosas-Landa, M., & Souza, V. (2014). Two-role model of an interaction network of free-living γ -proteobacteria from an oligotrophic environment. *Environmental Microbiology*, 16(5), 1366–1377. <https://doi.org/10.1111/1462-2920.12305>
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Asolkar, R. N., Jensen, P. R., Kauffman, C. A., & 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. <https://doi.org/10.1021/np0603828>
- Atta-ur-Rahmanro. (2005). Foreword. In B. T.-S. in N. P. C. Atta-ur-Rahman (Ed.), *Bioactive Natural Products (Part L)* (Vol. 32). Elsevier. [https://doi.org/https://doi.org/10.1016/S1572-5995\(05\)80050-X](https://doi.org/https://doi.org/10.1016/S1572-5995(05)80050-X)
- 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. <https://doi.org/https://doi.org/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, 383–402. <https://doi.org/10.1089/153110702762470491>
- Ben Hania, W., Joseph, M., Schumann, P., Bunk, B., Fiebig, A., Spröer, C., Klenk, H. P., Fardeau, M. L., & 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(FEBRUARY2015). <https://doi.org/10.1186/1944-3277-10-7>
- Böhner, B., Fetz, E., Härri, E., Sigg, H. P., Stoll, C., & Tamm, C. (1965). Über die Isolierung von Verrucarín H, Verrucarín J, Roridin D und Roridin E aus *Myrothecium*-Arten. Verrucarine und Roridine, 8. Mitteilung. *Helvetica Chimica*

806 *Acta*, 48(5), 1079–1087.
807 <https://doi.org/10.1002/hlca.19650480515>
808 Bolhuis, H., Cretoiu, M. S., & Stal, L. J. (2014). Molecular ecology of microbial mats.
809 In *FEMS Microbiology Ecology* (Vol. 90, Issue 2, pp. 335–350). Oxford
810 University Press. <https://doi.org/10.1111/1574-6941.12408>
811 Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-ghalith, G.
812 A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E.,
813 Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, T., Callahan, B. J.,
814 Caraballo-rodríguez, A. M., Chase, J., ... Caporaso, J. G. (2018). QIIME 2:
815 Reproducible, interactive, scalable, and extensible microbiome data science.
816 *PeerJ*. <https://doi.org/10.7287/peerj.preprints.27295v2>
817 Bonilla-Rosso, G., Peimbert, M., Alcaraz, L. D., Hernández, I., Eguiarte, L. E.,
818 Olmedo-Alvarez, G., & Souza, V. (2012). Comparative metagenomics of two
819 microbial mats at Cuatro Ciénegas Basin II: community structure and
820 composition in oligotrophic environments. *Astrobiology*, 12(7), 659–673.
821 <https://doi.org/10.1089/ast.2011.0724>
822 Breitbart, M., Hollander, D., Nitti, A., Van Mooy, B., Siefert, J., & Souza, V. (2010).
823 Modern freshwater microbialites of Cuatro Ciénegas, México. I: metagenomic
824 and stable isotopic analyses to assess microbial community structure and
825 function. In *Astrobiology Science Conference*.
826 Breitbart, Mya, Hoare, A., Nitti, A., Siefert, J., Haynes, M., Dinsdale, E., Edwards,
827 R., Souza, V., Rohwer, F., & Hollander, D. (2009). Metagenomic and stable
828 isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas,
829 Mexico. *Environmental Microbiology*, 11(1), 16–34.
830 <https://doi.org/10.1111/j.1462-2920.2008.01725.x>
831 Bryant, J. A., Lamanna, C., Morlon, H., Kerkhoff, A. J., Enquist, B. J., & Green, J. L.
832 (2009). Microbes on mountainsides: Contrasting elevational patterns of
833 bacterial and plant diversity. In *In the Light of Evolution* (Vol. 2, pp. 127–148).
834 National Academies Press. <https://doi.org/10.17226/12501>
835 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., &
836 Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina
837 amplicon data. *Nature Methods*, 13(7), 581–583.
838 <https://doi.org/10.1038/nmeth.3869>
839 Cantrell, S. A., Dianese, J., Fell, J., Gunde-Cimerman, N., & Zalar, P. (2011).
840 Unusual fungal niches. *Mycologia*, 103(6), 1161–1174.
841 <https://doi.org/10.3852/11-108>
842 Cantrell, S. A., Tkavc, R., Gunde-Cimerman, N., Zalar, P., Acevedo, M., & Báez-
843 Félix, C. (2013). Fungal communities of young and mature hypersaline microbial
844 mats. *Mycologia*, 105(4), 827–836. <https://doi.org/10.3852/12-288>
845 Cantrell, S., & Duval-Pérez, L. (2013). Microbial mats: An ecological niche for fungi.
846 In *Frontiers in Microbiology* (Vol. 4, Issue APR). Frontiers Research Foundation.
847 Cardoso, D. C., Cretoiu, M. S., Stal, L. J., & Bolhuis, H. (2019). Seasonal
848 development of a coastal microbial mat. *Scientific Reports*, 9(1).
849 <https://doi.org/10.1038/s41598-019-45490-8>
850 Carrasco, N. K., & Perissinotto, R. (2012). Development of a halotolerant community
851 in the St. Lucia Estuary (South Africa) during a hypersaline phase. *PLoS ONE*,
852 7(1). <https://doi.org/10.1371/journal.pone.0029927>

- Carreira, C., Lonborg, C., Kühl, M., Lillebo, A. I., Sandaa, R. A., Villanueva, L., & Cruz, S. (2020). Fungi and viruses as important players in microbial mats. *FEMS Microbiology Ecology*, 96(11), 1–13. <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. <https://doi.org/10.1016/j.ecoinf.2012.03.008>
- Choi, E. J., Beatty, D. S., Paul, L. A., Fenical, W., & Jensen, P. R. (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(PART4), 1219–1228. <https://doi.org/10.1099/ijs.0.043752-0>
- Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: An R package for the visualization of intersecting sets and their properties. *Bioinformatics*, 33(18), 2938–2940. <https://doi.org/10.1093/bioinformatics/btx364>
- Corsaro, D., Walochnik, J., Venditti, D., Steinmann, J., Müller, K.-D., & Michel, R. (2014). Microsporidia-like parasites of amoebae belong to the early fungal lineage Rozellomycota. *Parasitology Research*, 113(5), 1909–1918. <https://doi.org/10.1007/s00436-014-3838-4>
- Dang, Q., Tan, W., Zhao, X., Li, D., Li, Y., Yang, T., Li, R., Zu, G., & Xi, B. (2019). Linking the response of soil microbial community structure in soils to long-term wastewater irrigation and soil depth. *Science of the Total Environment*, 688, 26–36. <https://doi.org/10.1016/j.scitotenv.2019.06.138>
- De Anda, V., Zapata-Peñasco, I., Blaz, J., Poot-Hernández, A. C., Contreras-Moreira, B., González-Laffitte, M., Gámez-Tamariz, N., Hernández-Rosales, M., Eguiarte, L. E., & Souza, V. (2018). Understanding the mechanisms behind the response to environmental perturbation in microbial mats: A metagenomic-network based approach. *Frontiers in Microbiology*, 9(NOV). <https://doi.org/10.3389/fmicb.2018.02606>
- De Wit, R., Guerrero, M. C., Legaz, A., Jonkers, H. M., 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. <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, pp. 401–420). https://doi.org/10.1007/978-90-481-3799-2_21
- Edgcomb, V. P., Bernhard, J. M., Summons, R. E., Orsi, W., Beaudoin, D., & Visscher, P. T. (2014). Active eukaryotes in microbialites from Highborne Cay, Bahamas, and Hamelin Pool (Shark Bay), Australia. *ISME Journal*, 8(2), 418–429. <https://doi.org/10.1038/ismej.2013.130>
- El-Elmat, T., Figueroa, M., Ehrmann, B. M., Cech, N. B., Pearce, C. J., & Oberlies, N. H. (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.

- 900 <https://doi.org/10.1021/np4004307>
- 901 Elser, J. J., Schampel, J. H., Garcia-Pichel, F., Wade, B. D., Souza, V., Eguiarte, L.,
- 902 Escalante, A., & Farmer, J. D. (2005). Effects of phosphorus enrichment and
- 903 grazing snails on modern stromatolitic microbial communities. *Freshwater*
- 904 *Biology*, 50(11), 1808–1825. <https://doi.org/10.1111/j.1365-2427.2005.01451.x>
- 905 Elser, J. J., Watts, J., Schampel, J. H., & Farmer, J. (2006). Early Cambrian food
- 906 webs on a trophic knife-edge? A hypothesis and preliminary data from a modern
- 907 stromatolite-based ecosystem. *Ecology Letters*, 9(3), 292–300.
- 908 <https://doi.org/10.1111/j.1461-0248.2005.00873.x>
- 909 Faith, D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological*
- 910 *Conservation*, 61(1), 1–10. [https://doi.org/https://doi.org/10.1016/0006-](https://doi.org/https://doi.org/10.1016/0006-3207(92)91201-3)
- 911 [3207\(92\)91201-3](https://doi.org/https://doi.org/10.1016/0006-3207(92)91201-3)
- 912 Ferezou, J.-P., Quesneau-Thierry, A., Barbier, M., Kollmann, A., & Bousquet, J.-F.
- 913 (1980). Structure and synthesis of phomamide, a new piperazine-2,5-dione
- 914 related to the sirodesmins, isolated from the culture medium of Phoma lingam
- 915 Tode. *Journal of the Chemical Society, Perkin Transactions 1*, 0, 113–115.
- 916 <https://doi.org/10.1039/P19800000113>
- 917 Fujimoto, H., Neghisi, E., Yamaguchi, K., Nishi, N., & Yamazaki, M. (1996). Isolation
- 918 of New Tremorgenic Metabolites from an Ascomycete, *Corynascus setosus*.
- 919 *Chemical & Pharmaceutical Bulletin*, 44(10), 1843–1848.
- 920 <https://doi.org/10.1248/cpb.44.1843>
- 921 Gerdes, G. (2010). What are microbial mats? *Microbial Mats: Modern and Ancient*
- 922 *Microorganisms in Stratified Systems*, 3–25.
- 923 Gerdes, Gisela, Claes, M., Dunajtschik-Piewak, K., Riege, H., Krumbein, W. E., &
- 924 Reineck, H.-E. (1993). Contribution of microbial mats to sedimentary surface
- 925 structures. *Facies*, 29(1), 61. <https://doi.org/10.1007/BF02536918>
- 926 Glassman, S. I., & Martiny, J. B. H. (2018). Broad-scale Ecological Patterns Are
- 927 Robust to Use of Exact. *MSphere*, 3(4), e00148-18.
- 928 Goberna, M., Navarro-Cano, J. A., Valiente-Banuet, A., García, C., & Verdú, M.
- 929 (2014). Abiotic stress tolerance and competition-related traits underlie
- 930 phylogenetic clustering in soil bacterial communities. *Ecology Letters*, 17(10),
- 931 1191–1201. <https://doi.org/10.1111/ele.12341>
- 932 Gutiérrez-Preciado, A., Saghai, A., Moreira, D., Zivanovic, Y., Deschamps, P., &
- 933 López-García, P. (2018). Functional shifts in microbial mats recapitulate early
- 934 Earth metabolic transitions. *Nature Ecology and Evolution*, 2(11), 1700–1708.
- 935 <https://doi.org/10.1038/s41559-018-0683-3>
- 936 Haafke, J., Abou Chakra, M., & Becks, L. (2016). Eco-evolutionary feedback
- 937 promotes Red Queen dynamics and selects for sex in predator populations.
- 938 *Evolution*, 70(3), 641–652. <https://doi.org/https://doi.org/10.1111/evo.12885>
- 939 Harding, T., & Simpson, A. G. B. (2018). Recent Advances in Halophilic Protozoa
- 940 Research. In *Journal of Eukaryotic Microbiology* (Vol. 65, Issue 4, pp. 556–570).
- 941 Blackwell Publishing Inc. <https://doi.org/10.1111/jeu.12495>
- 942 Hauer, G., & Rogerson, A. (2005). Heterotrophic Protozoa from Hypersaline
- 943 Environments. In Gunde-Cimerman Nina, A. and Oren, & and P. Ana (Eds.),
- 944 *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and*
- 945 *Eukarya* (pp. 519–539). Springer Netherlands.
- 946 Hauer, G., Rogerson, A., & Anderson, O. R. (2001). *Platyamoeba pseudovannellida*

- n. sp., a naked amoeba with wide salt tolerance isolated from the Salton Sea, California. *Journal of Eukaryotic Microbiology*, 48(6), 663–669. <https://doi.org/10.1111/j.1550-7408.2001.tb00206.x>
- Helmus, M. R., Bland, T. J., Williams, C. K., & Ives, A. R. (2007). Phylogenetic measures of biodiversity. *The American Naturalist*, 169(3). <https://doi.org/10.1086/511334>
- Horner-Devine, M. C., & Bohannan, B. J. M. (2006). Phylogenetic clustering and overdispersion in bacterial communities. *Ecology*, 87(7 SUPPL.).
- 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. [https://doi.org/https://doi.org/10.1016/0040-4039\(96\)01212-9](https://doi.org/https://doi.org/10.1016/0040-4039(96)01212-9)
- Irie, K., Kajiyama, S., Koshimizu, K., Hayashi, H., & Arai, M. (1990). Isolation and biosynthesis of (–)-indolactam I, a new congener of indole alkaloid tumor promoter teleocidins. *Tetrahedron Letters*, 31(50), 7337–7340. [https://doi.org/https://doi.org/10.1016/S0040-4039\(00\)88559-7](https://doi.org/https://doi.org/10.1016/S0040-4039(00)88559-7)
- Ishibashi, M., Funayama, S., Anraku, Y., Komiyama, K., & Omtura, S. (1991). Novel antibiotics, furaquinocins C, D, E, F, G and H. *The Journal of Antibiotics*, 44(4), 390–395. <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. <https://doi.org/10.1016/j.tim.2018.02.011>
- Karpinets, T. V., Gopalakrishnan, V., Wargo, J., Futreal, A. P., Schadt, C. W., & Zhang, J. (2018). Linking associations of rare low-abundance species to their environments by association networks. *Frontiers in Microbiology*, 9(MAR). <https://doi.org/10.3389/fmicb.2018.00297>
- Katoh, K., & Toh, H. (2010). Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, 26(15), 1899–1900. <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, 1463–1464.
- Kim, W. G., Kim, J. P., & Yoo, I. D. (1996). Benzastatins A, B, C, and D : New free radical scavengers from *Streptomyces nitrosporeus* 30643 II. Structure determination. *Journal of Antibiotics*, 49(1), 26–30. <https://doi.org/10.7164/antibiotics.49.26>
- 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. <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. <https://doi.org/10.1080/00021369.1984.10866129>
- Kirkup, M. P., & Moore, R. E. (1983). Indole alkaloids from the marine red alga *Martensia fragilis*. *Tetrahedron Letters*, 24(20), 2087–2090. [https://doi.org/https://doi.org/10.1016/S0040-4039\(00\)81851-1](https://doi.org/https://doi.org/10.1016/S0040-4039(00)81851-1)
- Knowles, S. L., Raja, H. A., Wright, A. J., Lee, A. M. L., Caesar, L. K., Cech, N. B.,

- 994 Mead, M. E., Steenwyk, J. L., Ries, L. N. A., Goldman, G. H., Rokas, A., &
995 Oberlies, N. H. (2019). Mapping the Fungal Battlefield: Using in situ Chemistry
996 and Deletion Mutants to Monitor Interspecific Chemical Interactions Between
997 Fungi. *Frontiers in Microbiology*, 10, 285.
998 <https://doi.org/10.3389/fmicb.2019.00285>
- 999 Kobayashi, A., Kaiyama, S. ichiro, Inawaka, K., Kanzaki, H., & Kawazu, K. (1994).
1000 Nostodione A, a Novel Mitotic Spindle Poison* from a Blue-Green Alga Nostoc
1001 commune. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*,
1002 49(7–8), 464–470. <https://doi.org/10.1515/znc-1994-7-812>
- 1003 Kosemura, S. (2003). Meroterpenoids from *Penicillium citreo-viride* B. IFO 4692 and
1004 6200 hybrid. *Tetrahedron*, 59(27), 5055–5072.
1005 [https://doi.org/https://doi.org/10.1016/S0040-4020\(03\)00739-7](https://doi.org/https://doi.org/10.1016/S0040-4020(03)00739-7)
- 1006 Laland, K., Matthews, B., & Feldman, M. W. (2016). An introduction to niche
1007 construction theory. *Evolutionary Ecology*, 30(2), 191–202.
1008 <https://doi.org/10.1007/s10682-016-9821-z>
- 1009 Legendre, P., & Legendre, L. (2012). *Numerical Ecology* (3rd ed.). Elsevier,
1010 Amsterdam.
- 1011 Li, C., Roeger, K. E., & Kelly, W. L. (2009). Analysis of the Indanomycin Biosynthetic
1012 Gene Cluster from *Streptomyces antibioticus* NRRL 8167. *ChemBioChem*,
1013 10(6), 1064–1072. <https://doi.org/https://doi.org/10.1002/cbic.200800822>
- 1014 Liang, R., Davidova, I. A., Marks, C. R., Stamps, B. W., Harriman, B. H., Stevenson,
1015 B. S., Duncan, K. E., & Suflita, J. M. (2016). Metabolic capability of a
1016 predominant *Halanaerobium* sp. in hydraulically fractured gas wells and its
1017 implication in pipeline corrosion. *Frontiers in Microbiology*, 7(JUN).
1018 <https://doi.org/10.3389/fmicb.2016.00988>
- 1019 Liu, J., Meng, Z., Liu, X., & Zhang, X. (2019). Microbial assembly, interaction,
1020 functioning, activity and diversification: a review derived from community
1021 compositional data. *Marine Life Science & Technology*, 1(1), 112–128.
1022 <https://doi.org/10.1007/s42995-019-00004-3>
- 1023 Liu, W., Xu, D., Ma, H., Al-Farraj, S. A., Warren, A., & Yi, Z. (2016). Taxonomy and
1024 molecular systematics of three oligotrich (s.l.) ciliates including descriptions of
1025 two new species, *Strombidium guangdongense* sp. nov. and *Strombidinopsis*
1026 *sinicum* sp. nov. (Protozoa, Ciliophora). *Systematics and Biodiversity*, 14(5),
1027 452–465. <https://doi.org/10.1080/14772000.2016.1162872>
- 1028 Liu, X., Li, M., Castelle, C. J., Probst, A. J., Zhou, Z., Pan, J., Liu, Y., Banfield, J. F.,
1029 & Gu, J. D. (2018). Insights into the ecology, evolution, and metabolism of the
1030 widespread Woese archaeotal lineages. *Microbiome*, 6(1), 1–16.
1031 <https://doi.org/10.1186/s40168-018-0488-2>
- 1032 López-Lozano, N. E., Heidelberg, K. B., Nelson, W. C., García-Oliva, F., Eguarte,
1033 L. E., & Souza, V. (2013). Microbial secondary succession in soil microcosms
1034 of a desert oasis in the Cuatro Ciénegas Basin, Mexico. *PeerJ*, 1, e47.
1035 <https://doi.org/10.7717/peerj.47>
- 1036 Ma, K., Li, L., Bao, L., He, L., Sun, C., Zhou, B., Si, S., & Liu, H. (2015). Six new 3,4-
1037 seco-27-norlanostane triterpenes from the medicinal mushroom *Ganoderma*
1038 *boninense* and their antiplasmodial activity and agonistic activity to LXRβ.
1039 *Tetrahedron*, 71(12), 1808–1814.
1040 <https://doi.org/https://doi.org/10.1016/j.tet.2015.02.002>

- 1041 Margulis, L., Ashen, J. B., Sole, M., & Guerrero, R. (2006). Composite, large
1042 spirochetes from microbial mats: spirochete structure review. *Proceedings of*
1043 *the National Academy of Sciences*, 90(15), 6966–6970.
1044 <https://doi.org/10.1073/pnas.90.15.6966>
- 1045 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput
1046 sequencing reads. *EMBnet. Journal*, 17(1), 10–12.
- 1047 Mayfield, M. M., & Levine, J. M. (2010). Opposing effects of competitive exclusion
1048 on the phylogenetic structure of communities. *Ecology Letters*, 13, 1085–1093.
1049 <https://doi.org/https://doi.org/10.1111/j.1461-0248.2010.01509.x>
- 1050 McGonigle, J. M., Bernau, J. A., Bowen, B. B., & Brazelton, W. J. (2019). Robust
1051 Archaeal and Bacterial Communities Inhabit Shallow Subsurface Sediments of
1052 the Bonneville Salt Flats. *MSphere*, 4(4), 1–12.
1053 <https://doi.org/10.1128/msphere.00378-19>
- 1054 McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible
1055 Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, 8(4).
1056 <https://doi.org/10.1371/journal.pone.0061217>
- 1057 Medina-Chávez, N. O., Viladomat-Jasso, M., Olmedo-Álvarez, G., Eguiarte Luis, E.,
1058 Souza, V., & De la Torre-Zavala, S. (2019). Diversity of Archaea domain in
1059 Cuatro Ciénegas Basin: Archean Domes. *Submitted*.
- 1060 Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science
1061 Gateway for inference of large phylogenetic trees. *2010 Gateway Computing*
1062 *Environments Workshop*, GCE 2010.
1063 <https://doi.org/10.1109/GCE.2010.5676129>
- 1064 Moreno Fontalba, C. (2017). Ecología de las interacciones entre bacterias en Cuatro
1065 Ciénegas Coahuila México. *Instituto de Ecología UNAM Master Thesis*
1066 <https://bit.ly/2n0sdMp>.
- 1067 Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T.
1068 S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I.,
1069 Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular
1070 identification of fungi: Handling dark taxa and parallel taxonomic classifications.
1071 *Nucleic Acids Research*, 47(D1), D259–D264.
1072 <https://doi.org/10.1093/nar/gky1022>
- 1073 Nitti, A., Daniels, C. A., Siefert, J., Souza, V., Hollander, D., & Breitbart, M. (2012).
1074 Spatially Resolved Genomic, Stable Isotopic, and Lipid Analyses of a Modern
1075 Freshwater Microbialite from Cuatro Ciénegas, Mexico. *Astrobiology*, 12(7),
1076 685–698. <https://doi.org/10.1089/ast.2011.0812>
- 1077 Nutman, A. P., Bennett, V. C., Friend, C. R. L., Van Kranendonk, M. J., & Chivas, A.
1078 R. (2016). Rapid emergence of life shown by discovery of 3,700-million-year-
1079 old microbial structures. *Nature*, 537(7621), 535–538.
1080 <https://doi.org/10.1038/nature19355>
- 1081 Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *The Neglected Process*
1082 *in Evolution (MPB-37)*. Princeton University Press.
1083 <http://www.jstor.org/stable/j.ctt24hqpd>
- 1084 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B.,
1085 Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). *Vegan:*
1086 *Community Ecology Package. R package version 2.0-8*. [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)
1087 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan). Accessed 2013 Nov (R package version 2.0-8.).

- 1088 <http://cran.r-project.org/package=vegan>
- 1089 Ortiz-Alvarez, R., & Casamayor, E. O. (2016). High occurrence of Pacearchaeota
- 1090 and Woese archaeota (Archaea superphylum DPANN) in the surface waters of
- 1091 oligotrophic high-altitude lakes. *Environmental Microbiology Reports*, 8(2), 210–
- 1092 217. <https://doi.org/10.1111/1758-2229.12370>
- 1093 Paerl Hans W. and Yannarell, A. C. (2010). Environmental Dynamics, Community
- 1094 Structure and Function in a Hypersaline Microbial Mat. In A. Seckbach
- 1095 Joseph and Oren (Ed.), *Microbial Mats: Modern and Ancient Microorganisms in*
- 1096 *Stratified Systems* (pp. 421–442). Springer Netherlands.
- 1097 https://doi.org/10.1007/978-90-481-3799-2_22
- 1098 Pajares, S., Bonilla-Rosso, G., Travisano, M., Eguiarte, L. E., & Souza, V. (2012).
- 1099 Mesocosms of aquatic bacterial communities from the Cuatro Ciénegas Basin
- 1100 (Mexico): a tool to test bacterial community response to environmental stress.
- 1101 *Microbial Ecology*, 64(2), 346–358. <https://doi.org/10.1007/s00248-012-0045-7>
- 1102 Pajares, S., Eguiarte, L. E., Bonilla-Rosso, G., & Souza, V. (2013). Drastic changes
- 1103 in aquatic bacterial populations from the Cuatro Ciénegas Basin (Mexico) in
- 1104 response to long-term environmental stress. *Antonie van Leeuwenhoek*, 104(6),
- 1105 1159–1175. <https://doi.org/10.1007/s10482-013-0038-7>
- 1106 Pang, Z., Chong, J., Li, S., & Xia, J. (2020). Metaboanalyst 3.0: Toward an optimized
- 1107 workflow for global metabolomics. *Metabolites*, 10(5).
- 1108 <https://doi.org/10.3390/metabo10050186>
- 1109 Papineau, D. (2010). Global Biogeochemical Changes at Both Ends of the
- 1110 Proterozoic: Insights from Phosphorites. *Astrobiology*, 10(2), 165–181.
- 1111 <https://doi.org/10.1089/ast.2009.0360>
- 1112 Park, J. S., Cho, B. C., & Simpson, A. G. B. (2006). Halocafeteria seosinensis gen.
- 1113 et sp. nov. (Bicosoecida), a halophilic bacterivorous nanoflagellate isolated from
- 1114 a solar saltern. *Extremophiles*, 10(6), 493–504. <https://doi.org/10.1007/s00792-006-0001-x>
- 1115
- 1116 Pasulka, A., Hu, S. K., Countway, P. D., Coyne, K. J., Cary, S. C., Heidelberg, K. B.,
- 1117 & Caron, D. A. (2019). SSU-rRNA Gene Sequencing Survey of Benthic
- 1118 Microbial Eukaryotes from Guaymas Basin Hydrothermal Vent. *Journal of*
- 1119 *Eukaryotic Microbiology*. <https://doi.org/10.1111/jeu.12711>
- 1120 Peimbert, M., Alcaraz, L. D., Bonilla-Rosso, G., Olmedo-Alvarez, G., García-Oliva,
- 1121 F., Segovia, L., Eguiarte, L. E., & Souza, V. (2012). Comparative metagenomics
- 1122 of two microbial mats at Cuatro Ciénegas Basin I: ancient lessons on how to
- 1123 cope with an environment under severe nutrient stress. *Astrobiology*, 12(7),
- 1124 648–658. <https://doi.org/10.1089/ast.2011.0694>
- 1125 Pérez-Gutiérrez, R. A., López-Ramírez, V., Islas, Á., Alcaraz, L. D., Hernández-
- 1126 González, I., Olivera, B. C. L., Santillán, M., Eguiarte, L. E., Souza, V.,
- 1127 Travisano, M., & Olmedo-Alvarez, G. (2013). Antagonism influences assembly
- 1128 of a Bacillus guild in a local community and is depicted as a food-chain network.
- 1129 *ISME Journal*, 7(3), 487–497. <https://doi.org/10.1038/ismej.2012.119>
- 1130 Planavsky, N. J., Rouxel, O. J., Bekker, A., Lalonde, S. V., Konhauser, K. O.,
- 1131 Reinhard, C. T., & Lyons, T. W. (2010). The evolution of the marine phosphate
- 1132 reservoir. *Nature*, 467(7319), 1088–1090. <https://doi.org/10.1038/nature09485>
- 1133 Pluskal, T., Castillo, S., Villar-Briones, A., & Orešič, M. (2010). MZmine 2: Modular
- 1134 framework for processing, visualizing, and analyzing mass spectrometry-based

molecular profile data. *BMC Bioinformatics*, 11. <https://doi.org/10.1186/1471-2105-11-395>

Post, D. M., & Palkovacs, E. P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1523), 1629–1640. <https://doi.org/10.1098/rstb.2009.0012>

Post, F., Borowitzka, L., Borowitzka, M., Mackay, B., & Moulton, T. (1983). The protozoa of a Western Australian hypersaline lagoon. *Hydrobiologia*, 105(1), 95–113. <https://doi.org/10.1007/BF00025180>

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). Fasttree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, 26(7), 1641–1650. <https://doi.org/10.1093/molbev/msp077>

Prieto-Barajas, C. M., Valencia-Cantero, E., & Santoyo, G. (2018). Microbial mat ecosystems: Structure types, functional diversity, and biotechnological application. In *Electronic Journal of Biotechnology* (Vol. 31, pp. 48–56). Pontificia Universidad Catolica de Valparaiso. <https://doi.org/10.1016/j.ejbt.2017.11.001>

Purdy, K. J., Embley, T. M., Takii, S., & Nedwell, A. D. B. (1996). Rapid Extraction of DNA and rRNA from Sediments by a Novel Hydroxyapatite Spin-Column Method. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 62, Issue 10).

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). 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. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. R Foundation for Statistical Computing. <http://www.r-project.org/>

Ramos, V. M. C., Castelo-Branco, R., Leão, P. N., Martins, J., Carvalhal-Gomes, S., da Silva, F. S., Filho, J. G. M., & Vasconcelos, V. M. (2017). Cyanobacterial diversity in microbial mats from the hypersaline lagoon system of Araruama, Brazil: An in-depth polyphasic study. *Frontiers in Microbiology*, 8(JUN), 1–16. <https://doi.org/10.3389/fmicb.2017.01233>

Rebollar, E. A., Avitia, M., Eguiarte, L. E., 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. <https://doi.org/10.1111/j.1462-2920.2012.02784.x>

Rommel, K. R., Li, C., & Kelly, W. L. (2011). Identification of a Tetraene-Containing Product of the Indanomycin Biosynthetic Pathway. *Organic Letters*, 13(10), 2536–2539. <https://doi.org/10.1021/ol200570u>

Rudi, K., Skulberg, O. M., Larsen, F., & Jakobsen, K. S. (1997). Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the variable regions V6, V7, and V8. *Applied and Environmental Microbiology*, 63(7), 2593–2599.

Saghai, A., Gutiérrez-Preciado, A., Deschamps, P., Moreira, D., Bertolino, P., Ragon, M., & López-García, P. (2017). Unveiling microbial interactions in

- 1182 stratified mat communities from a warm saline shallow pond. *Environmental*
- 1183 *Microbiology*, 19(6), 2405–2421. <https://doi.org/10.1111/1462-2920.13754>
- 1184 Sardans, J., Peñuelas, J., & Rivas-Ubach, A. (2011). Ecological metabolomics:
- 1185 Overview of current developments and future challenges. *Chemoecology*,
- 1186 21(4), 191–225. <https://doi.org/10.1007/s00049-011-0083-5>
- 1187 Souza, V., Eguiarte, L. E., Moreno-Letelier, A., Travisano, M., Alcaraz, L. D., &
- 1188 Olmedo, G. (2018). The lost world of Cuatro Ciénegas Basin, a relictual bacterial
- 1189 niche in a desert oasis. *ELife*, 7. <https://doi.org/10.7554/eLife.38278>
- 1190 Souza, V., Espinosa-Asuar, L., Escalante, A. E., Eguiarte, L. E., Farmer, J., Forney,
- 1191 L., Lloret, L., Rodríguez-Martínez, J. M., Soberón, X., Dirzo, R., Elser, J. J.,
- 1192 Rodríguez-Martínez, J. M., Soberón, X., Dirzo, R., Elser, J. J., Forney, L.,
- 1193 Soberón, X., & Elser, J. J. (2006). An endangered oasis of aquatic microbial
- 1194 biodiversity in the Chihuahuan desert. *Proceedings of the National Academy of*
- 1195 *Sciences of the United States of America*, 103(17), 6565–6570.
- 1196 <https://doi.org/10.1073/pnas.0601434103>
- 1197 Souza, V., Olmedo-Álvarez, G., & Eguiarte, L. E. (Eds.). (2018). *Cuatro Ciénegas*
- 1198 *Ecology, Natural History and Microbiology*. Springer International Publishing.
- 1199 <https://doi.org/10.1007/978-3-319-93423-5>
- 1200 Souza, V., Siefert, J., Escalante, A. E., Elser, J. J., & Eguiarte, L. E. (2012). The
- 1201 Cuatro Ciénegas Basin in Coahuila, Mexico: an astrobiological Precambrian
- 1202 Park. *Astrobiology*, 12(7), 641–647. <https://doi.org/10.1089/ast.2011.0675>
- 1203 Stolz, J. F. (2000). Structure of Microbial Mats and Biofilms. In S. M. Riding Robert
- 1204 E. and Awramik (Ed.), *Microbial Sediments* (pp. 1–8). Springer Berlin
- 1205 Heidelberg. https://doi.org/10.1007/978-3-662-04036-2_1
- 1206 Taboada, B., Isa, P., Gutiérrez-Escolano, A. L., M. del ángel, R., Ludert, J. E.,
- 1207 Vázquez, N., Tapia-Palacios, M. A., Chávez, P., Garrido, E., Espinosa, A. C.,
- 1208 Eguiarte, L. E., López, S., Souza, V., & Arias, C. F. (2018). The geographic
- 1209 structure of viruses in the Cuatro Ciénegas Basin, a unique oasis in northern
- 1210 Mexico, reveals a highly diverse population on a small geographic scale.
- 1211 *Applied and Environmental Microbiology*, 84(11).
- 1212 <https://doi.org/10.1128/AEM.00465-18>
- 1213 Taiyun, W., & Vilam, S. (2017). *R package “corrplot”: Visualization of a Correlation*
- 1214 *Matrix (Version 0.84)*. <https://github.com/taiyun/corrplot>
- 1215 Tozuka, Z., Takasugi, H., & Takaya, T. (1983). Studies on tomaymycin. II. Total
- 1216 syntheses of the antitumor antibiotics, E- and Z-tomaymycins. *The Journal of*
- 1217 *Antibiotics*, 36(3), 276–282. <https://doi.org/10.7164/antibiotics.36.276>
- 1218 Van Der Hooft, J. J. J., Mohimani, H., Bauermeister, A., Dorrestein, P. C., Duncan,
- 1219 K. R., & Medema, M. H. (2020). Linking genomics and metabolomics to chart
- 1220 specialized metabolic diversity. *Chemical Society Reviews*, 49(11), 3297–3314.
- 1221 <https://doi.org/10.1039/d0cs00162g>
- 1222 Van Valen, L. (1973). A new evolutionary law. *Evolutionary Theory*, 1, 1–30.
- 1223 Vávra, J., & Lukeš, J. (2013). Microsporidia and ‘The Art of Living Together.’ In
- 1224 *Advances in Parasitology* (Vol. 82, pp. 253–319). Academic Press.
- 1225 <https://doi.org/10.1016/B978-0-12-407706-5.00004-6>
- 1226 Velez, P., Espinosa-Asuar, L., Figueroa, M., Gasca-Pineda, J., Aguirre-von-
- 1227 Wobeser, E., Eguiarte, L. E., Hernandez-Monroy, A., & Souza, V. (2018).
- 1228 Nutrient dependent cross-kingdom interactions: Fungi and bacteria from an

- oligotrophic desert oasis. *Frontiers in Microbiology*, 9(AUG).
<https://doi.org/10.3389/fmicb.2018.01755>
- Velez, P., Gasca-Pineda, J., Rosique-Gil, E., Eguiarte, L. E., 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, e2064. <https://doi.org/10.7717/peerj.2064>
- Visscher, P. T., Dupraz, C., Braissant, O., Gallagher, K. L., Glunk, C., Casillas, L., & Reed, R. E. S. (2010). Biogeochemistry of Carbon Cycling in Hypersaline Mats: Linking the Present to the Past through Biosignatures. In Seckbach Joseph & A. and Oren (Eds.), *Microbial Mats: Modern and Ancient Microorganisms in Stratified Systems* (pp. 443–468). Springer Netherlands.
https://doi.org/10.1007/978-90-481-3799-2_23
- Wang, B., Adachi, Y., & Sugiyama, S. (2018). Soil productivity and structure of bacterial and fungal communities in unfertilized arable soil. *PLoS ONE*, 13(9).
<https://doi.org/10.1371/journal.pone.0204085>
- Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73(16), 5261–5267.
<https://doi.org/10.1128/AEM.00062-07>
- White, Bruns, T., Lee, S., & Taylor, J. (1990). *White, T. J., T. D. Bruns, S. B. Lee, and J. W. Taylor. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics* (pp. 315–322).
- Wong, H., Ahmed-Cox, A., & Burns, B. (2016). Molecular Ecology of Hypersaline Microbial Mats: Current Insights and New Directions. *Microorganisms*, 4(1), 6.
<https://doi.org/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.
<https://doi.org/https://doi.org/10.1002/chem.201603734>
- Zaitseva, S. V., Abidueva, E. Y., Radnagurueva, A. A., Bazarov, S. M., & Buryukhaev, S. P. (2018). Structure of Microbial Communities of the Sediments of Alkaline Transbaikalia Lakes with Different Salinity. *Microbiology (Russian Federation)*, 87(4), 559–568. <https://doi.org/10.1134/S0026261718040185>

Figure legends

Figure 1

(A) The Cuatro Ciénegas Basin within México, and geographic location of Archaeal Domes in Pozas Azules Ranch. (B) Detail of an Archaeal Domes slice. (C) Domes when the sampling was conducted (note the size scale). Photos by David Jaramillo.

Figure 2

Genera relative abundance of taxa in Archaeal Domes, Cuatro Ciénegas Basin sample. A) Bacteria (only showed the most common taxa, more than 1% of diversity) B) Fungi (using Merge database, only fungal ASVs. NA is for ASVs that were not assigned to any genera, but phylum only)

Figure 3

Graphic representation of shared ASVs within Archaeal Domes mats 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. Pays show taxonomy of shared ASVs within all sites (a few but abundant ASVs core).
(A) 16S Archaea and Bacteria, rRNA gene database, (B) Fungi and other Eucarya, Forward-Only ITS database.

Figure 4

Graph representing null model distribution of Phylogenetic Species Clustering (nmdPSC) values for all community, abundant and rare taxa from 16S rRNA database (A) and Forward-Only ITS database (B). 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 Suppl. Table 5.

Figure 5

Pearson correlation analysis within the most common (i.e., involving more than 1% of diversity) Bacteria, and all genera of Archaea and Fungi (from Merge ITS database). Colored squares are for significant correlations ($P \leq 0.05$), blue for positive ones and red for negatives.

Figure 6

Molecular network of soil samples grouped metabolite features into 140 chemical families. Each node in the network represents one metabolite feature. Nodes connected to each other are structurally-related (cosine MS^2 similarity score ≥ 0.7).

Figure 7

Structures of microbial metabolites tentatively annotated in samples S1-S10 (≤ 5 ppm mass error).

Table1

Alpha diversity indices for 16S rRNA gene and Forward-Only ITS region databases from Archaeal Domes CCB microbial mats.

Suppl. Fig. 1

(A) Phylum composition within 16S rRNA gene database (B) Kingdom composition (based on UNITE database eukaryotic taxonomy) within Forward-Only ITS region database.

Suppl. Fig. 2
Rarefaction curves for 16S rRNA gene database (A) and ITS region Forward-Only ITS database (B).

Suppl. Fig. 3
UPGMA dendrogram showing composition similarities among sampling sites (Forward-Only ITS database for Eucarya UPGMA).
Branch length represent Bray-Curtis and Jaccard-1 dissimilarity coefficient, based on (A) abundance of 16S rDNA sequences ASVs, and (B) abundance of ITS region database ASVs. The numbers (height) are Bray-Curtis and Jaccard values.

Suppl. Figure 4
Heatmap analysis showing relative abundance of metabolite features (MS ions in positive mode) in the Archaeal Domes mats samples. On the scale bar, brick red color indicates increased metabolite levels, and blue color represents decreased levels. A shows non clustered samples and sites, B clustered samples and sites.

Suppl. Table 1.
List of ASVs Blast hits with <90%, of non-fungal Merge ITS database.

Suppl. Table 2.
Abundance tables (16S rRNA gene and ITS region taxa).

Suppl. Table 3.
Counts of significant negative and positive correlations from Pearson Correlation Analysis (Figure 5).

Suppl. Table 4.
Tentatively identified microbial metabolites in samples S1-S10 by GNPS and detailed metabolomic analysis.

Suppl. Table 5
nmdPSC values