Diversity of Archaea Domain in Cuatro Cienegas Basin: Archaeal Domes

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Abstract

Herein we describe the Archaea diversity in a shallow pond in the Cuatro Ciénegas Basin (CCB), Northeast Mexico, with fluctuating hypersaline conditions containing elastic microbial mats that can form small domes where their anoxic inside reminds us of the characteristics of the Archaeal Eon, rich in methane and sulfur gases; thus, we named this site the Archaeal Domes (AD). These domes only form after heavy rains that are rare in the Chihuahuan desert. CCB is a unique oasis with hundreds of ponds, containing endemic species of animals, plants and highly diverse and unique microbial communities, despite its very biased stoichiometry, due mostly to extreme low phosphorus content (soils, water columns and sediments). This extreme oligotrophy has favored survival of ancestral microorganisms. Whole metagenome sequencing approach was performed for this unusual site in three different seasons to assess the extent of the Archaea biodiversity, with a focus on extremophiles, since members of the Archaea had been underrepresented in different study sites within the oasis. We found a highly diverse Archaea community encompassing ~5% of the metagenomes. The archaeal portion in all three metagenomes maintained its abundance and most of the strains showed to form a resilient core during three seasonal samplings (2016-2017), despite environmental fluctuations. However, relative abundances of all 230 archaeal OTUs (defined using a 97% cutoff) were low enough (<0.1%) to be considered part of the rare biosphere. AD finding and their description within CCB confirms that this particular pond is the most diverse for Archaea that we are aware of and opens new paths for understanding the forces that once drove and keep shaping microbial community assemblage.

Introduction

The Archaea domain is an essential but usually rare, component of different ecosystems, not only extreme ones as originally suggested [1-4], for instance, they are critical in hydrological systems and their abundance and composition changes according to identifiable spatial and temporal scales [5-7]. Archaea is represented in 26 phyla [8], most of which are novel and some lineages include only one species of uncultivated strains that have been assembled through metagenomics [9, 10]. Indeed, a current challenge in their study is the isolation and cultivation of novel Archaea, since they are usually restricted to extreme conditions. Traditional culture-dependent approaches have underestimated abundance and diversity of Archaea, making it hard to achieve an accurate understanding of their role in microbial communities in an ecological niche [11]. Nevertheless, a successful co-cultivation of an Asgard archaeon associated with bacteria was reported after a long-term methane-fed bioreactor culture of deep marine sediments, demonstrating that non-traditional cultures of untapped environments are reservoirs of Archaea genetic and functional diversity yet to be uncovered [12]. Another challenge is to fully comprehend their phylogenetic association, given the limited genetic information available for some Archaea phyla.

In the last decade, our knowledge on the diversity and taxonomy of Archaea has substantially improved [13] due to the rise of metagenomics and increasing available archaeal genomes. The Archaea tree is being rapidly filled up with new branches, demonstrating that the Archaea domain remains largely unexplored [14] along with a diverse metabolism [15, 16], unveiling new processes and key features involving microbes and community structure.

Cuatro Cienegas Basin (CCB) is an endangered oasis within the northern zone of the Chihuahuan Desert in Mexico, characterized by an extremely unbalanced nutrient stoichiometry of the area (N:P = 159:1), similar to the conditions of the Precambrian sea [17-21]. Strikingly, despite

this nutrient deficiency CCB is considered a biodiversity hotspot for macroorganisms [22] and one of the most diverse sites for microorganisms in the world [23-27]; this microbial biodiversity is mirrored by its extreme diversity in virus [28]. CCB biodiversity is believed to have evolved as a result of a long time environmental stability of a deep aquifer [29], as suggested by the marine affiliations in many of the studied bacterial genomes [25, 30], virus [31], and probably, Archaea. These observations led CCB scientists to raise hypothesis and propose CCB as a model of “*lost world*”, where extreme conditions favored the survival of ancestral marine lineages that in some cases persist exclusively in this area [32]. Nevertheless, Archaea in CCB has been poorly studied since they were underrepresented in previous metagenomic studies [24, 28, 33, 34].

During a late March 2016 field work, an atypical spring rain apparently dissolved the salty crust of an unnoticed shallow pond, allowing the uniquely flexible and impermeable microbial mats to arise from the ground building bubbles, or domes with a strong sulfur-like smell. Our hypothesis was that gas production of methanogenic as well as other microorganisms associated to the sulfur cycle were degassing creating a locally anaerobic atmosphere. Therefore, the anaerobic layer of the microbial mats were creating the domes by raising the flexible and impermeable mat where photosynthesis was evident given the purple, and green layers, creating an unique “alien” local landscape (Figure 1). Based on the macroscopic morphology of the microbial mats, this locality in the Pozas Azules ProNatura ranch (a private ecological reserve) was named by us the “Archean Domes” (AD thereafter) (Fig. 1a).

These unusual structures in an small extremophilic and fluctuating pond, are by themselves a singularity, within CCB unique nature and amazing microbial diversity that has fascinated scientists with unexpected findings [19-21, 25, 27, 29, 30, 33, 35-43]. High salt concentration in dry conditions, and the production of biogases along dome formation under wet conditions, suggested

that this fluctuating evaporitic hypersaline microbial mat would be an ideal place to explore the Archaea diversity in CCB.

Considering the taxonomic profiling of different ponds previously studied within the Cuatro Cienegas system along 20 years [17, 28, 30, 33, 37-39, 41, 44-53], a working hypothesis was proposed for the community composition of AD microbial mat: that each sample would be unique, given the drastic environmental fluctuations. A null hypothesis would predict that the AD community would constitute a unique community with a constant core community, able to construct the domes given the right environmental conditions (heavy rain). In order to explore these hypotheses for the Archaea domain, a whole genome metagenomic approach was applied to explore the community shaping the AD microbial mat, expecting to achieve a coverage depth sensitive enough for the rare biosphere. Sampling for AD was done in three seasons (dry-wet-dry).

As mentioned above, Archaea had not been observed to be diverse or abundant in CCB, however, we found in this study that the Archaeal Domes displayed an abundant and diverse archaeal community. Even more, they represent one of the most diverse archaeal communities known up to date in the world. Unexpectedly, this diversity is not only very large given the small geographical scale of this site (at cm² level), but it is constant along all sampling seasons. This novel window to the Archaea world will be a biodiversity resource that may provide us with paths to understand the uniqueness that CCB *lost world* represents for ecology, evolution, systematics and future bioprospecting studies.

2. Material and Methods

2.1 Sampling site. Water and sediment were obtained from the Archaeal Domes (AD) in Rancho Pozas Azules, from Pronatura Noreste (26° 49' 41.7" N 102° 01' 28.7" W) in CCB, under

SEMARNAT scientific permit No. SGPA/DGVS/03121/15 (Figure 1). Sampling took place during 2016-2017 at the following times: April 2016 (dry, AD1), September 2016 (wet, AD2), February 2017 (dry, AD3); microbial mats and sediments (8 cm²) were transferred to sterile conical tubes (50 mL) and used for metagenomic analysis.

2.2 Metadata.

Following the National Meteorological System data base for the Pozas Azules ranch weather station (Supplementary material), we observed that in March 2016, there was an anomalous spring rain peak (90 mm) that apparently allowed us to discover the AD. The 10th of April sampling occurred when maximum air temperatures where ~ 30° C, and the domes were drying. In average, precipitation in early fall, reached 48 mm in September 2016, during our wet sampling date, and no rain were detected during February 2017, making it another dry sampling. The water physicochemical conditions were measured in September 2016 sampling, using a Hydrolab MS5 Water multiparameter sonde (OTT Hydromet GmbH, Germany) (Supp. Table 1). Other sampling dates were not tested due to the lack of water in the ponds (see Figure 1).

2.3 Total DNA Extraction. Extraction of total DNA from Archaeal domes sediment was performed in Experimental and Molecular Evolution Laboratory of the Ecology Institute, UNAM as reported before [24] using a modification of Purdy et al. (1996) [54] protocol.

2.4 Metagenomic Shotgun Sequencing. Total DNA from sediment of the three seasoned samples (AD1, AD2, and AD3) were sent to CINVESTAV- LANGEBIO, Irapuato, Mexico, for shotgun whole genome sequencing using Illumina Mi-Seq 2x300 technology.

2.5 Bioinformatic analysis for metagenomes. Raw data from AD1, AD2 and AD3, was quality checked using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Indexed adapter and barcodes were removed and low quality sequences were discarded with Trimmomatic v0.36 using a sliding window of 4pb and an average quality per base of 25 [55], followed by the merging

of the paired end reads using PEAR software [56]. Once reads were merged, both paired ends assembled and unassembled reads were used to cover major accuracy to obtain relative abundance for Eukarya, Bacteria and Archaeal species using MetaPhlAn2 software [57].

2.6 Diversity analysis and species accumulation curve. Using normalized relative abundances of Archaeal taxa within each metagenome, α and β diversity were calculated using EstimateS 9.1.0 and Past Software, respectively [58, 59].

2.7 Phylogenetic analysis. Reference 16S rRNA sequences were downloaded from the NCBI- *Gen Bank* and *RNA Central* Database according to identifications of MetaPhlAn2 hits. The sequences were aligned with ClustalW [60] and trimmed using MEGA V 7.0 [61]. The phylogeny was reconstructed with a Maximum Likelihood (ML) algorithm in MEGA and K-2 + G (Kimura 2-parameter + gamma distribution) evolutionary model with 10,000 bootstraps. Also reference strains were added to supply a robust interpretation.

2.8 Identification number of Metagenomic Data. The identification number for the metagenomic data used in this study are available in MG-RAST with the following ID: AD 1: mgm4856917.3, mgm4856915.3; AD 2: mgm4856913.3, mgm4856914.3; AD 3: mgm4856916.3, mgm4856918.3, and also using the following link: <https://www.mg-rast.org/linkin.cgi?project=mgp90438>.

3. Results

3.1. The “Archaean Domes” are hypersaline non-lithifying microbial mats.

After obtaining the necessary collecting permits and adequate equipment, sample collection of the AD was conducted in early April 2016. By then, the AD site was starting to dry, and the mat exhibited a morphology in which a photosynthetic upper layer became evident in the dome-like structure, while the interior of the domes maintained a wet black layer (Fig. 1b). Physicochemical and environmental conditions of AD were registered on April 2016, September 2016, and February 2017, that is, during dry and wet conditions (Table 1 and Supp. Table 1). Further observations (not shown) after four years revealed that during the dry seasons, AD microbial mats are active even if they do not produce domes. Moreover, it is only after heavy rains dissolve the salts, that domes emerge due to the elastic nature of the mats, producing its typical sulfur-like smell. We have observed the same phenomena up to last exploration September 2019.

The AD mats sampled on April 10th, 2016, looked different from the original observation (less than 3 weeks earlier). What firstly seemed like a shallow pond, was already drying up when the first sample was collected for sequencing (AD1), and the dense salty liquid started to turn into a salty-white-carbonate crust. As can be seen in Figure 1C, in, the second sampling (AD2, September 2016) represented a more wet environment, and the site looked similar to the one observed originally (i.e., March 2016), although the domes were smaller. The last sample of this study was collected in February 2017, by the end of the cold-dry season (AD3).

The microbial community of the AD is approximately 200 m away from the most described, stromatolite-rich and iconic blue pond from Rancho Pozas Azules, but physicochemical conditions found in the AD are extreme in salinity and higher in pH (Table 1). While Blue Pond pH is 7.9 [62,

63], pH of AD almost peaks 10. Moreover, in the first sampling (AD1), salinity was 53%, and it reached saturation later in that year, when the upper layer of the mat became a white salty crust, which prevented measurement with the available equipment for consistent results. Considering all the above, we can describe the AD as a hypersaline microbial mat [64].

3.2. *The Archaea domain in the Archaean Domes.*

The Illumina MiSeq run of 300-bp paired-ends delivered 28,859,454 reads for AD1, 24,772,053 reads for AD2 and 28,203,484 reads for AD3 of total DNA metagenomics, which were normalized for bioinformatic analysis. The percentage for relative abundance of the Archaea domain in AD1, AD2, and AD3, were 3.6%, 5% and 5% respectively. The binning of the metagenomic reads showed that the archaeal community in the AD metagenomes was dominated by Euryarchaeota-halophilic archaeal lineages. Sequences from Crenarchaeota, Thaumarchaeota, Korarchaeota, and Nanoarchaeota phyla were also retrieved (Fig. 2). All five phyla comprise 15 classes dominated by members of Halobacteria and Methanomicrobia classes (Fig. 2), both important groups of the Euryarchaeota phylum. Additionally, 25 orders, 36 families, 93 genera, and 230 *species* (defined as OTUs at 97% cutoff) were detected in the three metagenomes. It is noteworthy that from those 230 OTUs, a total of 24 could not be further classified (Supp. Fig.2).

To depict the extent of the biodiversity present within the AD metagenomes, we retrieved from the marker databases the 16S rRNA sequences corresponding to the species identified by MetaPhlAn2. We used these sequences to reconstruct a phylogeny (Fig. 3). Several sequences belonging to the same genera were omitted to avoid redundancy; others without a full-length marker sequence were also omitted. The phylogenetic reconstruction displays 213 sequences, 169 belonging to our mining and 44 are reference sequences. Five phyla were represented, that gave rise to 11 major clades.

Alpha diversity was calculated using Shannon, Simpson and Chao indexes (Table 2a).

Compared to the fluctuations observed in several bacterial studies in CCB [65], the archaeal diversity did not show drastic changes through sampling seasons (Table 2a). Moreover, 90% of the total archaeal richness (OTUs at 97% cutoff level) is shared among the three samples. This is confirmed by the Beta diversity estimates at species level (Table 2 b). Overall, richness evaluated at the other different taxonomic levels (i.e., using the 15 classes, the 25 orders, and/or the 36 families) of Archaea, remain similar in the three metagenomes.

In accordance with the Archaea's stable core hypothesis for the AD, the species accumulation curve shows that the inventory for every sample (AD1, AD2, AD3) was very similar. The expected diversity using the presence and absence for every identified OTUs in the metagenomic analysis increased very little with the addition of each sample. Therefore, OTU rarefaction curve with data from the 3 samples is asymptotic (Suppl. Fig. 1).

3.3 The Archaeal Domes Rare Biosphere.

Considering that 229 of the 230 identified archaeal OTUs are each present at relative abundances below 0.1%, we applied a strict threshold of 0.01% of relative abundance [66] to identify what we consider *strictly rare* archaeal taxa. Consequently, within the AD mats, 50 archaeal OTUs were defined to be strictly rare (each covering less than <0.01% of the total reads). Interestingly, such abundance profiles along the three sampling times show that 11 OTUs of the strictly rare Archaea taxa seem to be *conditionally rare* [67], that is, these taxa maintain their lower abundance in only one sample, and reach higher (while still rare <0.1%) abundances in other samples (Fig. 3A). This suggests that while the rest of the Archaea community is relatively stable, the strictly rare biosphere is dynamic and depends on the proper conditions to prosper. On the other hand, we found that 39 strictly rare Archaea OTUs were not detected in at least one metagenome (Fig. 3B). These taxa

233 exhibiting this kind of fluctuation along time series are considered *transiently rare* taxa [68]. Notice
 234 that sample season 2 (wet) shows a peak of transient rare taxa, suggesting that it is possible that
 235 dome construction during wet conditions can benefit by this change in population dynamics, while
 236 other taxa seem to prosper in the extremely salty environment.

237

238 **4. Discussion**

239 The microbial community that constitute the AD microbial mats is indeed unique among the studied
 240 CCB bacterial communities [21], displaying a relative high abundance of Archaea in AD1, AD2,
 241 and AD3, that reached 3.6%, 5% and 5% respectively. Those abundances are remarkable for such a
 242 small sampled area (cm²), but also when compared to other sites in CCB and the world (see Table
 243 1) [69-71]. After profiling the community at several taxonomic levels, it was observed that an
 244 archaean community core remained almost intact in time (April 2016-February 2017). This
 245 provided evidence for stability behind the construction of the domes (our null hypothesis).
 246 Nevertheless, within that core, the diversity and proportions of rare archaeal taxa increased or
 247 diminished across seasons, exhibiting a dynamic in the AD which might be playing a role for their
 248 functioning along different environmental conditions.

249

250 ***4.1 The Archaeal Domes at CCB are a good model of the Archaeal period, and are one of the*** 251 ***most abundant and diverse environments for Archaea.***

252 Microbial mats and stromatolites are considered analogues of early Earth, when they originated as
 253 successful ecological communities, capable of complete nutrient cycling as soon as life started to
 254 diversify in the early Archean Eon [72, 73]. Different ancestral lineages have survived at CCB [21,
 255 30], purportedly because they have adapted continuously to their neighbors, forming very tight

communities that cohesively survive despite the changing harsh abiotic factors, like those experienced along their very long evolutionary trajectory [21]. We believe, that the microbial mat of AD is not an exception in CCB, however, the observation that raised scientific interest to study these particular mats was precisely its extreme conditions of pH and salinity, besides their interesting “architectural” shape. Notwithstanding, analogue extreme environmental conditions have been reported in other archaeal rich microbial mats before, for instance in the Desert of Atacama or the human made salterns in Guerrero Negro, both stable environments (Table 1) show much lower Archaea diversity [69-71]. A possibility is that the fluctuating environment in AD are part of the community dynamics increasing possible niches, and it has been observed within CCB in other fluctuating environments that harbor large diversity due to local adaptations to each condition [37, 74]. However, the AD mat is peculiar because Archaea are more abundant and diverse than in other fluctuating microbialites within CCB [62] (Table 1). Moreover, this is the first site where there is a constant microbial core despite the environmental fluctuations among seasons. Not only Archaea diversity within AD is relatively constant, it is also considerably large for a metagenomic study using the Illumina platform [75].

On the other hand, targeted clone libraries for Archaeal primers have revealed a high abundance of Archaea (~10%) in stromatolites communities of the Hammelin Pool in SharkBay, Australia, which reaches one of the highest archaeal abundances. However, such archaeal community was represented by 27 archaeal clones, out of 176 total of 16S rRNA clone library from a single sample point [76]. These numbers are hard to compare to our observed AD archaeal diversity due to different experimental approaches. Other archaeal-rich hypersaline microbial mats show relative abundances of Archaea that range from 4% in a mat in Camargue, Spain. [77], 9% in Guerrero Negro hypersaline mat [78], 20% in some layers of the microbial mat in a shallow pond in

279 the Salar de Llamara [79], and above 90% of 16S rRNA of sequences amplified in a highly lithified
 280 mat in Laguna de Tebenchique, in the Salar de Atacama [69]. Nevertheless, all these Archaea-rich
 281 sites, display lower diversity [80] than our small (cm²) seasonal AD. Given the extremely small
 282 site, our 230 different species (at 97% cutoff) and 5 different phyla can be considered high.
 283 Noticeably, our 24 unclassified archaeal OTUs (Supp. Figure 2), constitute an interesting challenge
 284 for systematics and biodiscovery.

285 The CCB is well known for its outstanding microbial diversity and unique endemic lineages
 286 [25, 28, 35, 40, 74, 81] that have been isolated from the rest of the world; this uniqueness may be in
 287 part the result of its extremely biased stoichiometry [17, 19, 44]. Success of archaeal communities
 288 in the AD may rely on physiological adaptations that could for instance allow them to out-compete
 289 bacteria in specific niches with low energy or nutrients availability [82]. However, previously
 290 studied localities within CCB displaying the same low energy stress, such as Pozas Rojas microbial
 291 mat, lack similar numbers of Archaea. Pozas Rojas exhibits extreme N:P ratios (156:1), and also
 292 fluctuating environments [33]. Despite all of the above, until AD were uncovered, archaeal
 293 abundances and diversity had not been reported before as a distinctiveness in any of the numerous
 294 explored sites within CCB [24, 27, 28, 35]. The abundance of Euryarchaeota members (halophiles
 295 and methanogens) may be related to the high-carbonate, salty-crust upper layer, and the black
 296 methanogenic bottom strata, acting as barriers on both sides on the mat.

297 The estimated α and β diversity indexes for the AD samples suggest resilience of the
 298 community, which is expected to be largely influenced by species diversity, as explained by the
 299 insurance hypothesis [83]. Our data shows a slight increase in archaeal diversity and richness
 300 indexes in dry seasons compared to the sample from the wet season (Table 2), possibly because that

301 is the usual state of the AD site, since annual precipitations are not heavy enough to dissolve the
302 salts and change the ecology of the mats (Supp. Table 1)

303 Another plausible explanation for an increased abundance and diversity in the Archaea
304 domain in the AD mats is the *killing-the-winner* hypothesis [84], that proposes a negative
305 frequency-dependent selection, in which bacterial types are affected by viral pressure, promoting
306 the survival and viability of rare types, and thus maintaining high diversity. Viral reads in our AD
307 metagenomes represent a relative abundance ranging from 23.3% to 31.6%, which is remarkably
308 high compared to other metagenomes, and even higher than typical virus-rich environments, such as
309 those obtained from samples of filtered sea water in South Korea [85], several deep sea sediments
310 [86] and microbialites from SharkBay, Australia [71].

311

312 ***4.2 The rare biosphere in the AD community.***

313 Rare biosphere may represent a reservoir of genetic diversity that actively responds to
314 environmental changes [87]. Given the predominance of an equitable rare biosphere in all the
315 previously sampled sites of CCB [24], and the extreme and unpredictable conditions of the AD site,
316 we considered it was important to explore rarity within the Archaea community.

317 By defining a “strictly rare” biosphere as the taxa with relative abundances below 0.01%, we
318 found 50 archaeal OTUs. Within this group, 11 OTUs seem to be conditionally rare [67],
319 maintaining their lower abundance in only one sampling time (the wet month), and reaching higher
320 abundances in both dry samplings (Fig. 3A). Their dynamics lead us to speculate that those rare
321 OTUs from the AD, along with the more abundant OTUs, could be benefiting of a saltier
322 environment or drier conditions. In both cases, it is noticeable that although archaeal richness and
323 overall abundance does not change much among samples, it is the rare (the one driven by

environmental fluctuations) biosphere that exhibits variations. A similar response to cold versus warm conditions have been noticed in other systems within CCB [37].

Among this rare biosphere, some taxa were found to be transiently rare (i.e., absent in one or two of the samples) comprising 39 OTUs (Fig. 3B). We suggest that this type of rarity should be driven at least in part by stochastic processes, such as passive dispersal of lineages temporarily recruited from microbial seed bank, or due to immigration [88]. As an example, nine rare unclassified taxa that were not present or were in very low abundances in the initial sample AD1, became abundant in February sample (dry season) (Fig. 3; Suppl. Fig. 2). More metagenomic studies are in their way to explore such pattern in a longer time frame.

It should not be discarded that the lowest-abundant taxa of AD could be undergoing dormancy, a mechanism that maintains cells alive, but inactive and intermittently below detection thresholds [89]. Indeed, Archaea can enter a cellular dormant state [89-91], thereby providing an adaptive response to what would otherwise be a deleterious environmental perturbation. This has been experimentally studied analyzing the thermoacidophile archaeon *Metallosphaera prunae* that produces VapC toxins that drive cellular dormancy under uranium stress [92]. Another factor for dormancy in Archaea is predatory organisms, specifically, virus, which are abundant in the AD metagenomes (~28%). As an example, a research group showed that rare and even inactive viruses, induce dormancy in the model archaeon *Sulfolobus islandicus* [90]. Also oligotrophy might be playing a role in Archaea dormancy, as dormant bacteria taxa have been found to be enriched in low phosphorus environment [89] and dormant microorganisms can also escape virus predation [93]. Considering the extremely skew stoichiometry, virus may be the drivers of the community structure, not only liberating immobilized nutrients to the system by lysing cells, but by maintaining a large panoply of rare taxa coexisting and avoiding predation.

348 ***4.3 Halophiles and methanogens Archaea in the AD***

349 Halophiles and methanogens species were highly abundant in the AD, representing > 50% of the
350 total Archaeal diversity. The relationship between halophiles and methanogens is well known,
351 sharing a common ancestral habitat [78, 82, 94, 95] and both groups are found in microbial mats
352 and microbial communities associate to precipitated minerals (endoevaporites) [70]. The
353 methanogens living in the aforementioned environments need a high level of NaCl (0.5 M) for
354 optimal growth [96] and usually are halotolerant or halophilic [97]. This type of hypersaline
355 environments is very dynamic and wide spread in the world [98].

356 In our sampling site, halophilic and methanogenic Archaea are proposed to constitute the
357 stable core of the AD during hypersaline conditions under the salt crust. Accordingly, other studies
358 indicate that salinity is the major abiotic factor that allows the shaping of microbial communities,
359 especially in sediment surfaces, stromatolites, hydrothermal vents, hypersaline mats and anoxic
360 saline water. These studies have demonstrated that saline sediments contain communities with
361 higher unique biodiversity values in comparison with other environments [99]. Other studies
362 demonstrate that changes in salinity, sulfate and availability of substrates could possibly stimulate
363 the production of methane [70].

364 The most abundant OTUs in the analyzed AD metagenomes were similar to
365 *Methanohalophilus mahii*, a halophilic-methanogen Archaea described as a non-marine
366 methanogen, adapted to hypersaline environments; its metabolism requires 1.0-2.5 M NaCl for
367 optimal growth and methanogenesis, using methanol and methylamines as substrates [95]. Other
368 OTUs from AD were related to *Natrialba taiwanensis*, *Haloferax sulfurifontis*, *Methanoplanus*
369 *petrolearius* and *Haloterrigena thermotolerans*. Halophilic and methanogen species coexisted
370 within the microbial mat, along with some unexpected genera belonging to Euryarchaeota phylum.
371 Among the latter we can find, *Thermococcus*, *Natronomonas*, *Picrophylus*, *Archaeoglobus*,

372 *Aciduliprofundum* and even *Thermoplasma*, a lineage that lacks a cell wall. These microorganisms
 373 are mostly reported from hydrothermal vents, displaying a high level of tolerance to low and high
 374 temperatures and a wide range of pH [100, 101]. The presence of these taxa along with the
 375 previous observations, adds evidence to our proposal stating that CCB microbial communities have
 376 both marine and magmatic affinities [30, 32]. Therefore, the Archaea that we observed in AD are
 377 witness of both environments, the deep aquifer propelled by magmatic heat within the mountain of
 378 San Marcos y Pinos [29], and the surface life, where photosynthesis occurs, rarely, after rainy days.
 379 Hence, part of the AD diversity might have emerged from the deep sediments that contained the
 380 mineral conditions of the ancient ocean [52].

382 ***4.4 Thermophilic and other related Archaea lineages in the AD***

383 Summing to the hypothesis of magmatic influence to the microbial community of this unusual site,
 384 Crenarchaeota taxa are consistently present in the AD mat. These include thermophilic genera such
 385 as *Thermoproteus*, *Caldivirga*, *Ignicoccus*, *Sulfolobus*, *Pyrolobus*, among others. In addition,
 386 Thaumarchaeota members were detected, such as “*Candidatus Nitrososphaera gargensis*”,
 387 *Nitrosoarchaeum limnia*, and Marine Group 1 Thaumarchaeota. The presence of two OTUs were
 388 unexpected: *Cenarchaeum symbiosum*, a psychrophilic archaeon, previously reported as a symbiont
 389 of a several sponge species [102], and *Nitrosopumilus maritimus*, considered ubiquitous on
 390 oligotrophic oceans [103], another AD finding consistent with the marine signatures frequently
 391 found in CCB microbes [25, 28, 30, 44, 53]. The same occurs with “*Candidatus Korarchaeota*
 392 *cryptophilum*”, a representative of Korarchaeota phylum, its presence in AD is related to the marine
 393 origin of CCB and an incoming colonization of geothermal-terrestrial environments, a feature
 394 shared among the thermophiles [104].

Both Crenarchaeota and Thaumarchaeota have in general terms similar metabolic capabilities. PCR-amplified *amoA* genes from DNA of each sample (AD1, AD2, AD3; data not shown) provided evidence of ammonia oxidation capabilities as potential energy source and nitrification [105], suggesting a role for chemolithotrophy of these taxa in CCB, using a large panoply of organic compounds as well as CO₂, iron, nitrogen and sulfur compounds as electron acceptors, as for instance methanobacteria (Euryarchaeota) and hyperthermophilic Crenarchaeota members use sulfur as an electron acceptor [106].

Other unexpected but interesting member of the phyla in the AD metagenomes were the Nanoarchaeota *Nanoarchaeum equitans*, a small-sized archaeon and an ectoparasitic relationship with the Crenarchaeota *Ignicoccus*, which was also detected in the sample [107]. Nanoarchaeota phylum has a widespread habitat distribution with diverse physicochemical features compatible with hot springs and other mesophilic hypersaline environments [108].

407

408 **5. Perspectives and conclusions.**

The Archaeal Domes microbial mats at the oasis Cuatro Ciénegas Basin, were recently discovered as a hypersaline extreme site. In these mats we uncovered through metagenomic analysis, one of the highest diversities registered so far in the Archaea domain, considering the site's geographic small scale. Most of the 230 OTUs observed in this unusual small shallow temporal pond, are part of the rare biosphere and form a stable core community. Within this core, we observed halophiles and methanogens, but also spatially unexpected archaeal taxa, that thrive under high salt concentrations. We also observed a transient rare biosphere that appears to be enriched under dry environmental conditions, suggesting seasonal dynamics shaping community assemblage. In order to explore this group of taxonomic the unclassified rare taxa more carefully, we are in the process of analyzing more metagenomes in different seasons as well as manipulating the

environment using mesocosms experiments. More sequencing effort in deep sediment cores will also help to look for the deep anaerobic biosphere, as well as eliminate blind spots in phylogeny of unclassified Archaea, and this will require differential coverage binning approach [109] using all available metagenomes from the AD to describe phylogenetic novelty within AD at CCB.

This highly diverse ecosystem within Cuatro Cienegas, Mexico, arises as an attractive novel site for evolutionary, ecological, astrobiological and bioprospecting studies. The AD is, so far, the most diverse microbial community found in CCB, despite its extreme conditions. Since this area is being subjected to intense water exploitation by agricultural practices, and desiccation has become a common occurrence in numerous ponds, it is priority for our research group to keep investigating the ecology of the adaptation of this highly diverse archaeal-rich microbial communities to fluctuating temperature and rainfall conditions, while working with shareholders on changes in policy of water usage.

Now proven as an archaeal rich extreme site, CCB is once more attracting attention as an astrobiological model [38, 41]. The Archaeal Domes not only can take us further back into the “lost world” but it is also a site that keeps providing evidences and new keys to understand how “life cycle” could have been originated on Earth, or (will be) possible on, for example, Mars.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Physicochemical parameters and Archaeal abundance in four Hypersaline Microbial Mats.

Microbial Mat	Salinity %	Temperature C°	pH	Archaea relative abundance %	Archaeal OTU's (97%)	Sequencing approach	Reference
Archaeal Domes	53%	31.45	9.94	4.96%	230	Whole Genome-Shot Gun Illumina MiSeq	This study
Guerrero Negro, Mexico	28%	30	N/A	30.45%	209	16s rRNA Tag (926F/1392R) FLX Titanium Roche	García-Maldonado <i>et al.</i> , 2018
Shark Bay, Australia	6.8%	24.8	8.13	<1%	13	Whole Genome-Shot Gun (Illumina MySeq)	Babilonia <i>et al.</i> 2018
Atacama, Chile	10.6-15%	23-27	7.65	50-66%	121	16s rRNA Tag (F515/R806) 454 pyrosequencing	Fernandez <i>et al.</i> 2016

Table 2. Diversity indexes of the Archaeal Dome Metagenomes. a) Alpha and b) beta estimator indexes for specific Archaea diversity.

AD Metagenome Samples			
a) Alpha diversity indicator	AD1	AD2	AD3
Shannon ^{mean}	5.33	5.38	5.3
Simpson Inv ^{mean}	207.3	214.17	216.38
Observed OTU's	222	200	200
Chao Taxa number	207.3	221.57	254.61
b) Beta diversity Index	AD1-AD2	AD1-AD3	AD2-AD3
Bray-Curtis	0.69	0.71	0.84

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C

AD1 (Apr.-2016)

AD2 (Sept.-2016)

AD3 (Feb.-2017)

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809 **Figure 1. Sampling site Map.** A) Cuatro Cienegas, Coahuila location within Mexico; B) Archaean
810 Domes (AD) mats with unusual and flexible bubble-containing superficial layer found in Pozas
811 Azules Ranch within Chihuahuan Desert region C) AD sampling of the three continuous seasons:
812 AD1 (April 2016), AD2 (September 2016), AD3 (February 2017) showing their obtained microbial
813 mat and the structure of microbial community in in its respective season.

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833 **Figure 2. Metagenomic data of Archaeal Domes.** Mean Community composition (left) and

834 Class-Level distribution (right) of Archaea in the three continuous season samples of Archaeal

835 Domes.

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A) Conditionally Rare Archaea Taxa through AD Temporals

B) Transiently Rare Archaea Taxa through AD Temporals

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845 **Figure 3: Archaeal rare biosphere.** A) Relative abundance of Conditionally Rare archaeal taxa
 846 through seasons in the Archaeal Domes. B) Relative abundance of the Transiently Rare archaeal
 847 taxa through seasons in the Archaeal Domes.

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871 **Figure 4.** Phylogenetic tree of the Archaea domain species found in AD1, AD2, AD3, though
872 MetaPhlAn2 hit profile, based on nearly full-length 16S rRNA gene sequences, using maximum
873 likelihood method, constructed by K-2 + G evolutionary model with 1,000 bootstraps
874 replicates.

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SUPPLEMENTARY MATERIAL

Physicochemical characteristics of AD	IBV	Temp	SPC	Salinity	TDS	pH	LDO	CH ₄	CO ₂
Water sample	7.7	31.45	77.21	53.36	49.62	9.94	78.4	12.4	Not detected
Mat Sample	8.0	34.35	77.31	52.55	48.76	9.75	1: 165.3 2: 7.3	N/A	Not detected

Supp. Table 1. Physicochemical parameters measured for Archaeal Domes on April

2016. IBV (volts); Temp: temperature °C; SPC: specific conductance (μS/cm); Salinity (psu); TDS: total dissolved solids (g/L); pH (0-14); LDO: dissolved oxygen (mg/L); CH₄ (μg/L)

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904 Supp. Fig. 1. **Rarefaction curve to estimate the richness of Archean Domain in the different**

905 **season samples.**

Supp. Fig. 2. Unclassified Rare Archaea Taxa in the AD.