1　**Diversity of Archaea Domain in Cuatro Cienegas Basin: Archaean 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 Archaean Eon, rich in methane and sulfur gases; thus, we named this site the Archaean 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 compassing ~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

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47 The Archaea domain is an essential but usually rare, component of different ecosystems, not only

48 extreme ones as originally suggested [1-4], for instance, they are critical in hydrological systems

49 and their abundance and composition changes according to identifiable spatial and temporal scales

50 [5-7]. Archaea is represented in 26 phyla [8], most of which are novel and some lineages include

51 only one species of uncultivated strains that have been assembled through metagenomics [9, 10].

52 Indeed, a current challenge in their study is the isolation and cultivation of novel Archaea, since

53 they are usually restricted to extreme conditions. Traditional culture-dependent approaches have

54 underestimated abundance and diversity of Archaea, making it hard to achieve an accurate

55 understanding of their role in microbial communities in an ecological niche [11]. Nevertheless, a

56 successful co-cultivation of an Asgard archaeon associated with bacteria was reported after a long-

57 term methane-fed bioreactor culture of deep marine sediments, demonstrating that non-traditional

58 cultures of untapped environments are reservoirs of Archaea genetic and functional diversity yet to

59 be uncovered [12]. Another challenge is to fully comprehend their phylogenetic association, given

60 the limited genetic information available for some Archaea phyla.

61 In the last decade, our knowledge on the diversity and taxonomy of Archaea has

62 substantially improved [13] due to the rise of metagenomics and increasing available archaeal

63 genomes. The Archaea tree is being rapidly filled up with new branches, demonstrating that the

64 Archaea domain remains largely unexplored [14] along with a diverse metabolism [15, 16],

65 unveiling new processes and key features involving microbes and community structure.

66 Cuatro Cienegas Basin (CCB) is an endangered oasis within the northern zone of the

67 Chihuahuan Desert in Mexico, characterized by an extremely unbalanced nutrient stoichiometry of

68 the area (N:P = 159:1), similar to the conditions of the Precambrian sea [17-21]. Strikingly, despite

69   this nutrient deficiency CCB is considered a biodiversity hotspot for macroorganisms [22] and one

70   of the most diverse sites for microorganisms in the world [23-27]; this microbial biodiversity is

71   mirrored by its extreme diversity in virus [28]. CCB biodiversity is believed to have evolved as a

72   result of a long time environmental stability of a deep aquifer [29], as suggested by the marine

73   affiliations in many of the studied bacterial genomes [25, 30], virus [31], and probably, Archaea.

74   These observations led CCB scientists to raise hypothesis and propose CCB as a model of "*lost*

75   *world*", where extreme conditions favored the survival of ancestral marine lineages that in some

76   cases persist exclusively in this area [32]. Nevertheless, Archaea in CCB has been poorly studied

77   since they were underrepresented in previous metagenomic studies [24, 28, 33, 34].

78   During a late March 2016 field work, an atypical spring rain apparently dissolved the salty

79   crust of an unnoticed shallow pond, allowing the uniquely flexible and impermeable microbial mats

80   to arise from the ground building bubbles, or domes with a strong sulfur-like smell. Our hypothesis

81   was that gas production of methanogenic as well as other microorganisms associated to the sulfur

82   cycle were degassing creating a locally anaerobic atmosphere. Therefore, the anaerobic layer of the

83   microbial mats were creating the domes by raising the flexible and impermeable mat where

84   photosynthesis was evident given the purple, and green layers, creating an unique "alien" local

85   landscape (Figure 1). Based on the macroscopic morphology of the microbial mats, this locality in

86   the Pozas Azules ProNatura ranch (a private ecological reserve) was named by us the "Archean

87   Domes" (AD thereafter) (Fig. 1a).

88   These unusual structures in an small extremophilic and fluctuating pond, are by themselves a

89   singularity, within CCB unique nature and amazing microbial diversity that has fascinated scientists

90   with unexpected findings [19-21, 25, 27, 29, 30, 33, 35-43]. High salt concentration in dry

91   conditions, and the production of biogases along dome formation under wet conditions, suggested

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92   that this fluctuating evaporitic hypersaline microbial mat would be an ideal place to explore the

93   Archaea diversity in CCB.

94   Considering the taxonomic profiling of different ponds previously studied within the Cuatro

95   Cienegas system along 20 years [17, 28, 30, 33, 37-39, 41, 44-53], a working hypothesis was

96   proposed for the community composition of AD microbial mat: that each sample would be unique,

97   given the drastic environmental fluctuations. A null hypothesis would predict that the AD

98   community would constitute a unique community with a constant core community, able to construct

99   the domes given the right environmental conditions (heavy rain). In order to explore these

100   hypotheses for the Archaea domain, a whole genome metagenomic approach was applied to explore

101   the community shaping the AD microbial mat, expecting to achieve a coverage depth sensitive

102   enough for the rare biosphere. Sampling for AD was done in three seasons (dry-wet-dry).

103   As mentioned above, Archaea had not been observed to be diverse or abundant in CCB,

104   however, we found in this study that the Archaean Domes displayed an abundant and diverse

105   archaeal community. Even more, they represent one of the most diverse archaeal communities

106   known up to date in the world. Unexpectedly, this diversity is not only very large given the small

107   geographical scale of this site (at cm^2 level), but it is constant along all sampling seasons. This

108   novel window to the Archaea world will be a biodiversity resource that may provide us with paths

109   to understand the uniqueness that CCB *lost world* represents for ecology, evolution, systematics and

110   future bioprospecting studies.

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2. Material and Methods

113   *2.1 Sampling site.* Water and sediment were obtained from the Archaean Domes (AD) in Rancho

114   Pozas Azules, from Pronatura Noreste (26º 49' 41.7" N 102º 01' 28.7" W) in CCB, under

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115   SEMARNAT scientific permit No. SGPA/DGVS/03121/15 (Figure 1). Sampling took place during

116   2016-2017 at the following times: April 2016 (dry, AD1), September 2016 (wet, AD2), February

117   2017 (dry, AD3); microbial mats and sediments (8 cm^2) were transferred to sterile conical tubes (50

118   mL) and used for metagenomic analysis.

119   *2.2 Metadata.*

120   Following the National Meteorological System data base for the Pozas Azules ranch weather station

121   (Supplementary material), we observed that in March 2016, there was an anomalous spring rain

122   peak (90 mm) that apparently allowed us to discover the AD. The 10th of April sampling occurred

123   when maximum air temperatures where ~ 30° C, and the domes were drying. In average,

124   precipitation in early fall, reached 48 mm in September 2016, during our wet sampling date, and no

125   rain were detected during February 2017, making it another dry sampling. The water

126   physicochemical conditions were measured in September 2016 sampling, using a Hydrolab MS5

127   Water multiparameter sonde (OTT Hydromet GmbH, Germany) (Supp. Table 1). Other sampling

128   dates were not tested due to the lack of water in the ponds (see Figure 1).

129   *2.3 Total DNA Extraction.* Extraction of total DNA from Archaean domes sediment was performed

130   in Experimental and Molecular Evolution Laboratory of the Ecology Institute, UNAM as reported

131   before [24] using a modification of Purdy et al. (1996) [54] protocol.

132   *2.4 Metagenomic Shotgun Sequencing.* Total DNA from sediment of the three seasoned samples

133   (AD1, AD2, and AD3) were sent to CINVESTAV- LANGEBIO, Irapuato, Mexico, for shotgun

134   whole genome sequencing using Illumina Mi-Seq 2x300 technology.

135   *2.5 Bioinformatic analysis for metagenomes.* Raw data from AD1, AD2 and AD3, was quality

136   checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Indexed adapter

137   and barcodes were removed and low quality sequences were discarded with Trimmomatic v0.36

138   using a sliding window of 4pb and an average quality per base of 25 [55], followed by the merging

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139   of the paired end reads using PEAR software [56]. Once reads were merged, both paired ends

140   assembled and unassembled reads were used to cover major accuracy to obtain relative abundance

141   for Eukarya, Bacteria and Archaean species using MetaPhlAn2 software [57].

142   *2.6 Diversity analysis and species accumulation curve.* Using normalized relative abundances of

143   Archaean taxa within each metagenome, α and β diversity were calculated using EstimateS 9.1.0

144   and Past Software, respectively [58, 59].

145   *2.7 Phylogenetic analysis.* Reference 16S rRNA sequences were downloaded from the NCBI- *Gen*

146   *Bank* and *RNA Central* Database according to identifications of MetaPhlAn2 hits. The sequences

147   were aligned with ClustalW [60] and trimmed using MEGA V 7.0 [61]. The phylogeny was

148   reconstructed with a Maximum Likelihood (ML) algorithm in MEGA and K-2 + G (Kimura 2-

149   parameter + gamma distribution) evolutionary model with 10,000 bootstraps. Also reference strains

150   were added to supply a robust interpretation.

151   2.8 *Identification number of Metagenomic Data.* The identification number for the metagenomic

152   data used in this study are available in MG-RAST with the following ID: AD 1: mgm4856917.3,

153   mgm4856915.3; AD 2: mgm4856913.3, mgm4856914.3; AD 3: mgm4856916.3, mgm4856918.3,

154   and also using the following link: https://www.mg-rast.org/linkin.cgi?project=mgp90438.

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162 *3. Results*

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164 *3.1. The "Archaean Domes" are hypersaline non-lithifying microbial mats.*

165 After obtaining the necessary collecting permits and adequate equipment, sample collection of the

166 AD was conducted in early April 2016. By then, the AD site was starting to dry, and the mat

167 exhibited a morphology in which a photosynthetic upper layer became evident in the dome-like

168 structure, while the interior of the domes maintained a wet black layer (Fig. 1b). Physicochemical

169 and environmental conditions of AD were registered on April 2016, September 2016, and February

170 2017, that is, during dry and wet conditions (Table 1 and Supp. Table 1). Further observations (not

171 shown) after four years revealed that during the dry seasons, AD microbial mats are active even if

172 they do not produce domes. Moreover, it is only after heavy rains dissolve the salts, that domes

173 emerge due to the elastic nature of the mats, producing its typical sulfur-like smell. We have

174 observed the same phenomena up to last exploration September 2019.

175   The AD mats sampled on April 10th, 2016, looked different from the original observation

176 (less than 3 weeks earlier). What firstly seemed like a shallow pond, was already drying up when

177 the first sample was collected for sequencing (AD1), and the dense salty liquid started to turn into a

178 salty-white-carbonate crust. As can be seen in Figure 1C, in, the second sampling (AD2, September

179 2016) represented a more wet environment, and the site looked similar to the one observed

180 originally (i.e., March 2016), although the domes were smaller. The last sample of this study was

181 collected in February 2017, by the end of the cold-dry season (AD3).

182   The microbial community of the AD is approximately 200 m away from the most described,

183 stromatolite-rich and iconic blue pond from Rancho Pozas Azules, but physicochemical conditions

184 found in the AD are extreme in salinity and higher in pH (Table 1). While Blue Pond pH is 7.9 [62,

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185   63], pH of AD almost peaks 10. Moreover, in the first sampling (AD1), salinity was 53%, and it

186   reached saturation later in that year, when the upper layer of the mat became a white salty crust,

187   which prevented measurement with the available equipment for consistent results. Considering all

188   the above, we can describe the AD as a hypersaline microbial mat [64].

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190   *3.2. The Archaea domain in the Archaean Domes.*

191   The Illumina MiSeq run of 300-bp paired-ends delivered 28,859,454 reads for AD1, 24,772,053

192   reads for AD2 and 28,203,484 reads for AD3 of total DNA metagenomics, which were normalized

193   for bioinformatic analysis. The percentage for relative abundance of the Archaea domain in AD1,

194   AD2, and AD3, were 3.6%, 5% and 5% respectively. The binning of the metagenomic reads

195   showed that the archaeal community in the AD metagenomes was dominated by Euryarchaeota-

196   halophilic archaeal lineages. Sequences from Crenarchaeota, Thaumarchaeota, Korarchaeota, and

197   Nanoarchaeota phyla were also retrieved (Fig. 2). All five phyla comprise 15 classes dominated by

198   members of Halobacteria and Methanomicrobia classes (Fig. 2), both important groups of the

199   Euryarchaeota phylum. Additionally, 25 orders, 36 families, 93 genera, and 230 *species* (defined as

200   OTUs at 97% cutoff) were detected in the three metagenomes. It is noteworthy that from those 230

201   OTUs, a total of 24 could not be further classified (Supp. Fig.2).

202   To depict the extent of the biodiversity present within the AD metagenomes, we retrieved

203   from the marker databases the 16S rRNA sequences corresponding to the species identified by

204   MetaPhlAn2. We used these sequences to reconstruct a phylogeny (Fig. 3). Several sequences

205   belonging to the same genera were omitted to avoid redundancy; others without a full-length marker

206   sequence were also omitted. The phylogenetic reconstruction displays 213 sequences, 169

207   belonging to our mining and 44 are reference sequences. Five phyla were represented, that gave rise

208   to 11 major clades.

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

210   Compared to the fluctuations observed in several bacterial studies in CCB [65], the archaeal

211   diversity did not show drastic changes through sampling seasons (Table 2a). Moreover, 90% of the

212   total archaeal richness (OTUs at 97% cutoff level) is shared among the three samples. This is

213   confirmed by the Beta diversity estimates at species level (Table 2 b). Overall, richness evaluated at

214   the other different taxonomic levels (i.e., using the 15 classes, the 25 orders, and/or the 36 families)

215   of Archaea, remain similar in the three metagenomes.

216   In accordance with the Archaea's stable core hypothesis for the AD, the species

217   accumulation curve shows that the inventory for every sample (AD1, AD2, AD3) was very similar.

218   The expected diversity using the presence and absence for every identified OTUs in the

219   metagenomic analysis increased very little with the addition of each sample. Therefore, OTU

220   rarefaction curve with data from the 3 samples is asymptotic (Suppl. Fig. 1).

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222   *3.3 The Archaean Domes Rare Biosphere.*

223   Considering that 229 of the 230 identified archaeal OTUs are each present at relative abundances

224   below 0.1%, we applied a strict threshold of 0.01% of relative abundance [66] to identify what we

225   consider *strictly rare* archaeal taxa. Consequently, within the AD mats, 50 archaeal OTUs were

226   defined to be strictly rare (each covering less than <0.01% of the total reads). Interestingly, such

227   abundance profiles along the three sampling times show that 11 OTUs of the strictly rare Archaea

228   taxa seem to be *conditionally rare* [67], that is, these taxa maintain their lower abundance in only

229   one sample, and reach higher (while still rare <0.1%) abundances in other samples (Fig. 3A). This

230   suggests that while the rest of the Archaea community is relatively stable, the strictly rare biosphere

231   is dynamic and depends on the proper conditions to prosper. On the other hand, we found that 39

232   strictly rare Archaea OTUs were not detected in at least one metagenome (Fig. 3B). These taxa

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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.

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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 (cm^2), 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.

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250   *4.1 The Archaean Domes at CCB are a good model of the Archaean 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

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256   communities that cohesively survive despite the changing harsh abiotic factors, like those

257   experienced along their very long evolutionary trajectory [21]. We believe, that the microbial mat of

258   AD is not an exception in CCB, however, the observation that raised scientific interest to study

259   these particular mats was precisely its extreme conditions of pH and salinity, besides their

260   interesting "architectural" shape. Notwithstanding, analogue extreme environmental conditions

261   have been reported in other archaeal rich microbial mats before, for instance in the Desert of

262   Atacama or the human made salterns in Guerrero Negro, both stable environments (Table 1) show

263   much lower Archaea diversity [69-71]. A possibility is that the fluctuating environment in AD are

264   part of the community dynamics increasing possible niches, and it has been observed within CCB in

265   other fluctuating environments that harbor large diversity due to local adaptations to each condition

266   [37, 74]. However, the AD mat is peculiar because Archaea are more abundant and diverse than in

267   other fluctuating microbialites within CCB [62] (Table 1). Moreover, this is the first site where

268   there is a constant microbial core despite the environmental fluctuations among seasons. Not only

269   Archaea diversity within AD is relatively constant, it is also considerably large for a metagenomic

270   study using the Illumina platform [75].

271   On the other hand, targeted clone libraries for Archaean primers have revealed a high

272   abundance of Archaea (~10%) in stromatolites communities of the Hammelin Pool in SharkBay,

273   Australia, which reaches one of the highest archaeal abundances. However, such archaeal

274   community was represented by 27 archaeal clones, out of 176 total of 16S rRNA clone library from

275   a single sample point [76]. These numbers are hard to compare to our observed AD archaeal

276   diversity due to different experimental approaches. Other archaeal-rich hypersaline microbial mats

277   show relative abundances of Archaea that range from 4% in a mat in Camargue, Spain. [77], 9% in

278   Guerrero Negro hypersaline mat [78], 20% in some layers of the microbial mat in a shallow pond in

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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 (cm^2) 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

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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].

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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

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324   environmental fluctuations) biosphere that exhibits variations. A similar response to cold versus

325   warm conditions have been noticed in other systems within CCB [37].

326   Among this rare biosphere, some taxa were found to be transiently rare (i.e., absent in one or

327   two of the samples) comprising 39 OTUs (Fig. 3B). We suggest that this type of rarity should be

328   driven at least in part by stochastic processes, such as passive dispersal of lineages temporarily

329   recruited from microbial seed bank, or due to immigration [88]. As an example, nine rare

330   unclassified taxa that were not present or were in very low abundances in the initial sample AD1,

331   became abundant in February sample (dry season) (Fig. 3; Suppl. Fig. 2). More metagenomic

332   studies are in their way to explore such pattern in a longer time frame.

333   It should not be discarded that the lowest-abundant taxa of AD could be undergoing

334   dormancy, a mechanism that maintains cells alive, but inactive and intermittently below detection

335   thresholds [89]. Indeed, Archaea can enter a cellular dormant state [89-91], thereby providing an

336   adaptive response to what would otherwise be a deleterious environmental perturbation. This has

337   been experimentally studied analyzing the thermoacidophile archaeon *Metallosphaera prunae* that

338   produces VapC toxins that drive cellular dormancy under uranium stress [92]. Another factor for

339   dormancy in Archaea is predatory organisms, specifically, virus, which are abundant in the AD

340   metagenomes (~28%). As an example, a research group showed that rare and even inactive viruses,

341   induce dormancy in the model archaeon *Sulfolobus islandicus* [90]. Also oligotrophy might be

342   playing a role in Archaea dormancy, as dormant bacteria taxa have been found to be enriched in

343   low phosphorus environment [89] and dormant microorganisms can also escape virus predation

344   [93]. Considering the extremely skew stoichiometry, virus may be the drivers of the community

345   structure, not only liberating immobilized nutrients to the system by lysing cells, but by maintaining

346   a large panoply of rare taxa coexisting and avoiding predation.

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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 sulforifontis, 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].

381

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 psycrophillic 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].

395   Both Crenarchaeota and Thaumarchaeota have in general terms similar metabolic

396   capabilities. PCR-amplified *amoA* genes from DNA of each sample (AD1, AD2, AD3; data not

397   shown) provided evidence of ammonia oxidation capabilities as potential energy source and

398   nitrification [105], suggesting a role for chemolithotrophy of these taxa in CCB, using a large

399   panoply of organic compounds as well as CO_2, iron, nitrogen and sulfur compounds as electron

400   acceptors, as for instance methanobacteria (Euryarchaeota) and hyperthermophilic Crenarchaeota

401   members use sulfur as an electron acceptor [106].

402   Other unexpected but interesting member of the phyla in the AD metagenomes were the

403   Nanoarchaeota *Nanoarchaeum equitans,* a small-sized archaeon and an ectoparasitic relationship

404   with the Crenarchaeota *Ignicoccus*, which was also detected in the sample [107]. Nanoarchaeaota

405   phylum has a widespread habitat distribution with diverse physicochemical features compatible

406   with hot springs and other mesophilic hypersaline environments [108].

407

408   *5. Perspectives and conclusions.*

409   The Archaean Domes microbial mats at the oasis Cuatro Ciénegas Basin, were recently discovered

410   as a hypersaline extreme site. In these mats we uncovered through metagenomic analysis, one of

411   the highest diversities registered so far in the Archaea domain, considering the site's geographic

412   small scale. Most of the 230 OTUs observed in this unusual small shallow temporal pond, are part

413   of the rare biosphere and form a stable core community. Within this core, we observed halophiles

414   and methanogens, but also spatially unexpected archaeal taxa, that thrive under high salt

415   concentrations. We also observed a transient rare biosphere that appears to be enriched under dry

416   environmental conditions, suggesting seasonal dynamics shaping community assemblage.

417   In order to explore this group of taxonomic the unclassified rare taxa more carefully, we are in the

418   process of analyzing more metagenomes in different seasons as well as manipulating the

18

419   environment using mesocosms experiments. More sequencing effort in deep sediment cores will

420   also help to look for the deep anaerobic biosphere, as well as eliminate blind spots in phylogeny of

421   unclassified Archaea, and this will require differential coverage binning approach [109] using all

422   available metagenomes from the AD to describe phylogenetic novelty within AD at CCB.

423   This highly diverse ecosystem within Cuatro Cienegas, Mexico, arises as an attractive novel

424   site for evolutionary, ecological, astrobiological and bioprospecting studies. The AD is, so far, the

425   most diverse microbial community found in CCB, despite its extreme conditions. Since this area is

426   being subjected to intense water exploitation by agricultural practices, and desiccation has become a

427   common occurrence in numerous ponds, it is priority for our research group to keep investigating

428   the ecology of the adaptation of this highly diverse archaeal-rich microbial communities to

429   fluctuating temperature and rainfall conditions, while working with shareholders on changes in

430   policy of water usage.

431   Now proven as an archaeal rich extreme site, CCB is once more attracting attention as an

432   astrobiological model [38, 41]. The Archaean Domes not only can take us further back into the "lost

433   world" but it is also a site that keeps providing evidences and new keys to understand how "life

434   cycle" could have been originated on Earth, or (will be) possible on, for example, Mars.

435

436   **Conflicts of interest**

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

438

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19

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447

448

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

Mats.

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Microbial Mat	Salinity %	Temperature Cº	pH	Archaea relative abundance %	Archaeal OTU´s (97%)	Sequencing approach	Reference
Archaean 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 *et al.*, 2018
Shark Bay, Australia	6.8%	24.8	8.13	<1%	13	Whole Genome-Shot Gun (Illumina MySeq)	Babilonia *et al*. 2018
Atacama, Chile	10.6-15%	23-27	7.65	50-66%	121	16s rRNA Tag (F515/R806) 454 pyrosequencing	Fernandez *et al*. 2016

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801 **Table 2. Diversity indexes of the Archaean Dome Metagenomes**. a) Alpha and b) beta estimator

802 indexes for specific Archaea diversity.

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	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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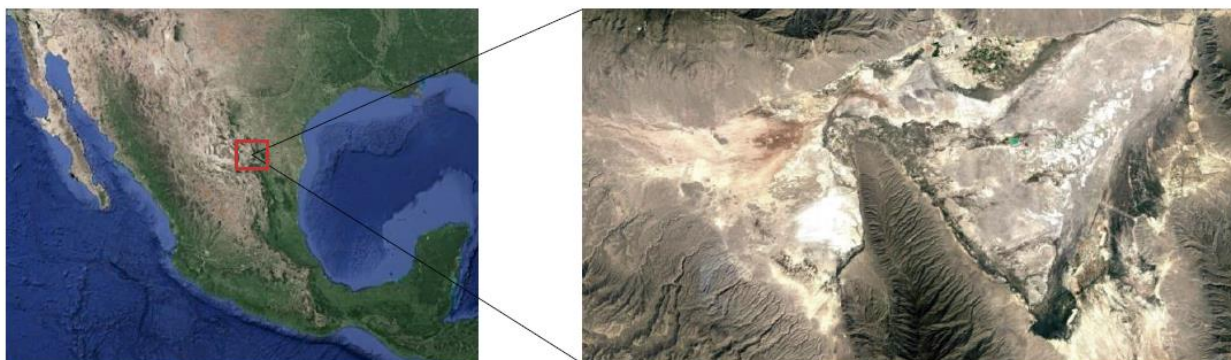
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AD1 (Apr.-2016)   AD2 (Sept.-2016)   AD3 (Feb.-2017)

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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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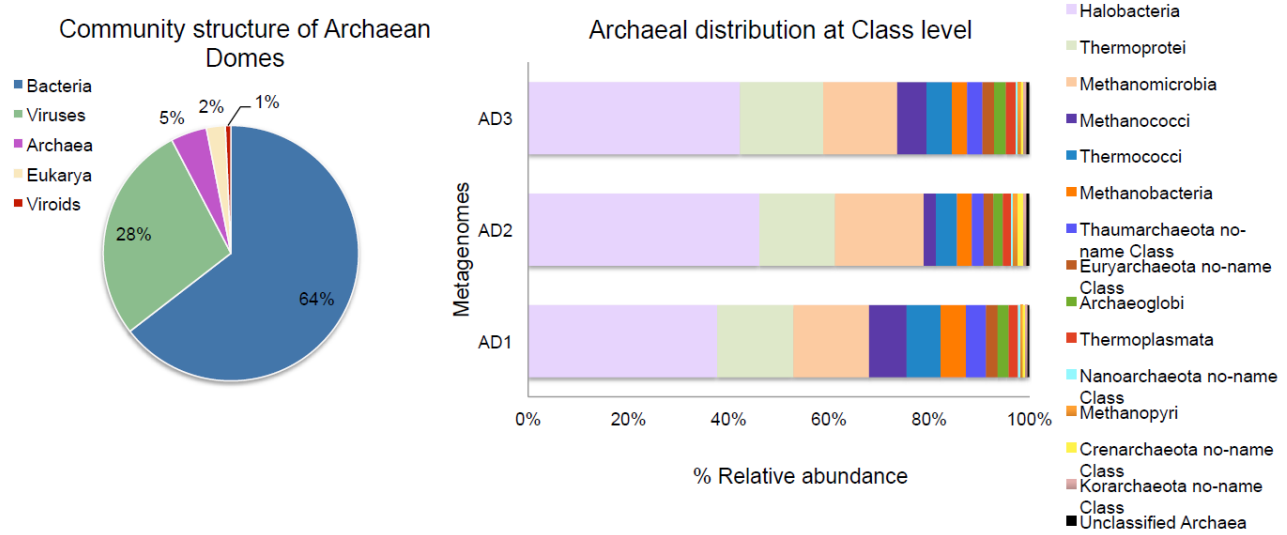
Figure 2. Metagenomic data of Archaean Domes. Mean Community composition (left) and Class-Level distribution (right) of Archaea in the three continuous season samples of Archaean Domes.

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A) Conditionally Rare Archaea Taxa through AD Temporals
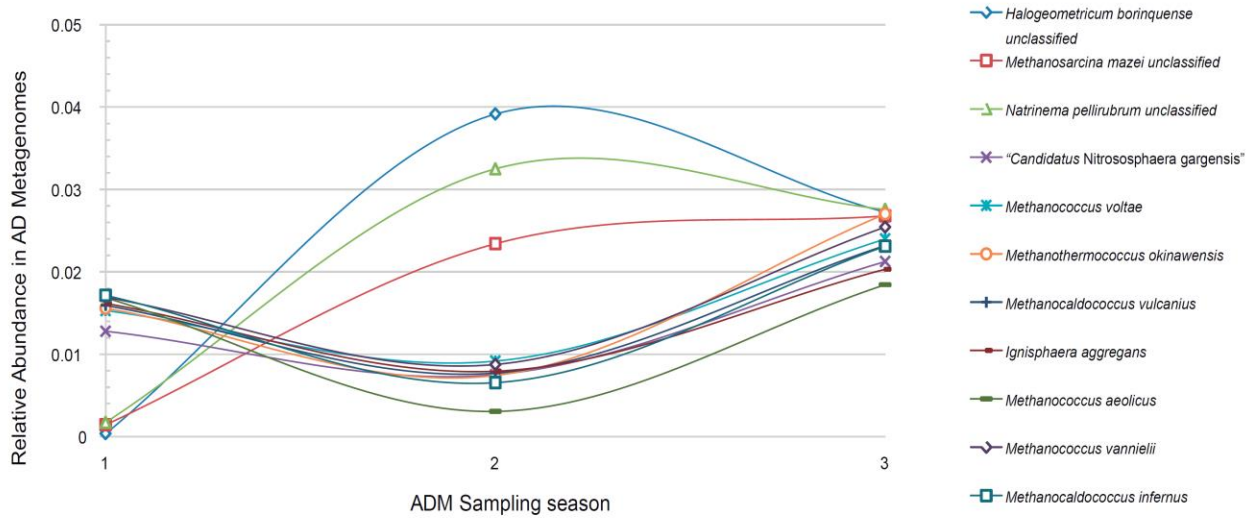
B) Transiently Rare Archaea Taxa through AD Temporals

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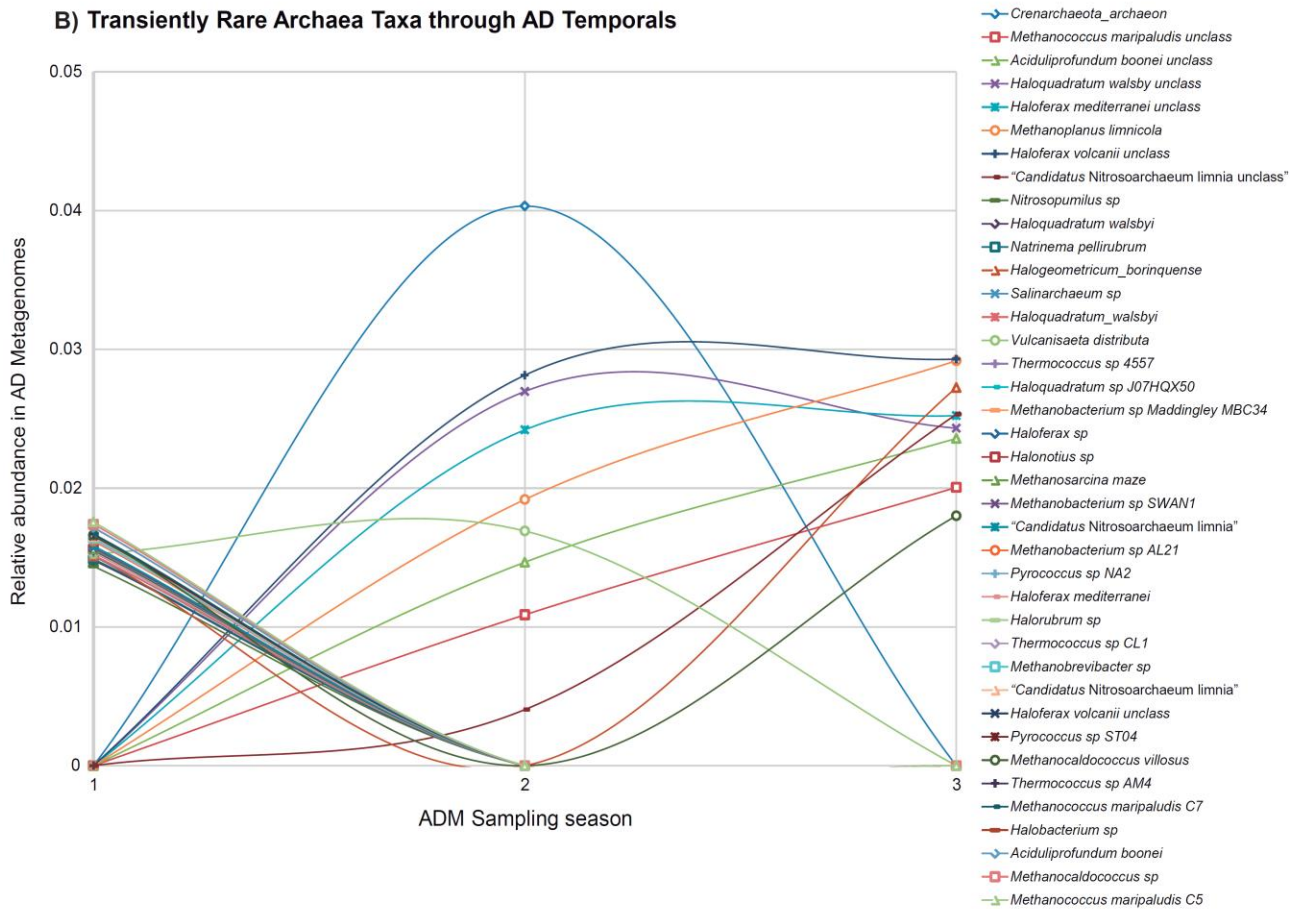
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845 **Figure 3: Archaean rare biosphere.** A) Relative abundance of Conditionally Rare archaeal taxa

846 through seasons in the Archaean Domes. B) Relative abundance of the Transiently Rare archaeal

847 taxa through seasons in the Archaean Domes.

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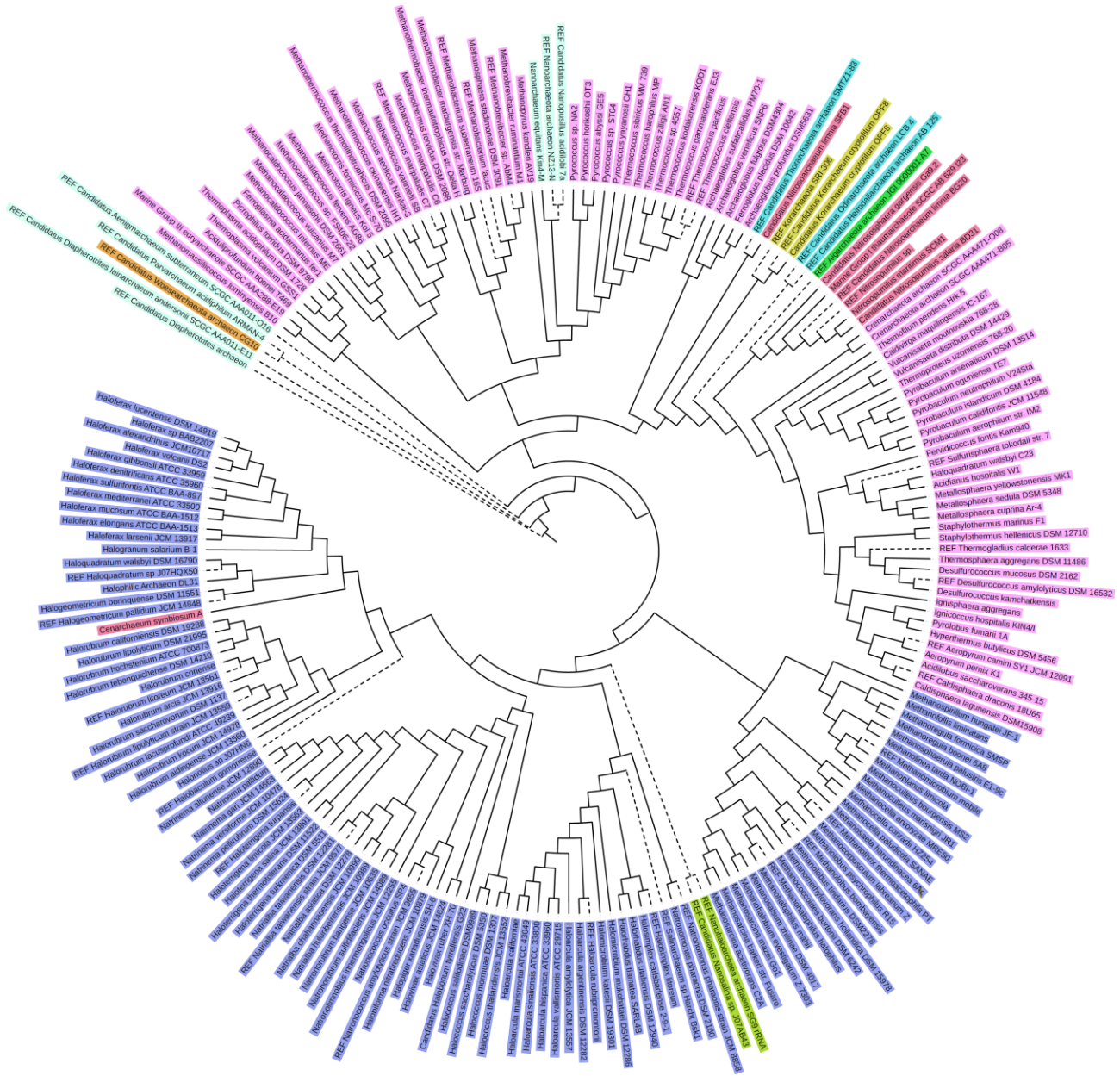
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871 **Figure 4.** Phylogenetic tree of the Archaea domain species found in AD1, AD2, AD3, though

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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885   **SUPPLEMENTARY MATERIAL**

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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

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892   **Supp. Table 1. Physicochemical parameters measured for Archaean Domes on April**

893   **2016.** IBV (volts); Temp: temperature ºC; SPC: specific conductance (μS/cm); Salinity (psu);

894   TDS: total dissolved solids (g/L); pH (0-14); LDO: dissolved oxygen (mg/L); CH4 (μg/L)
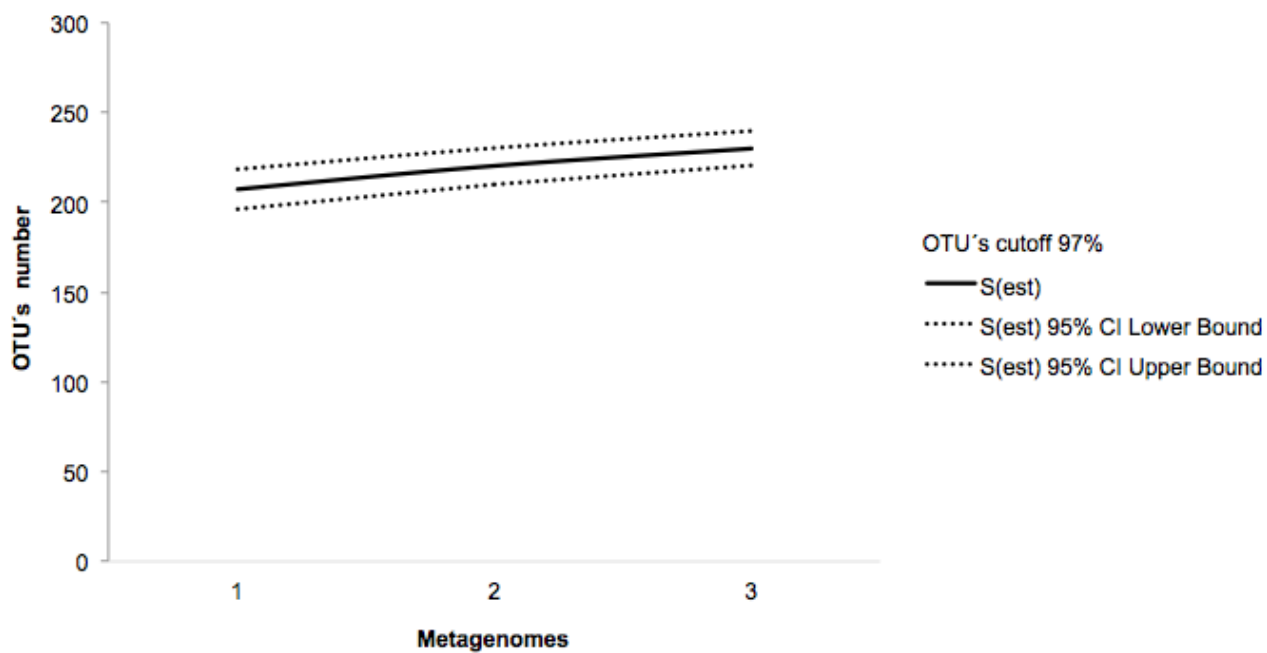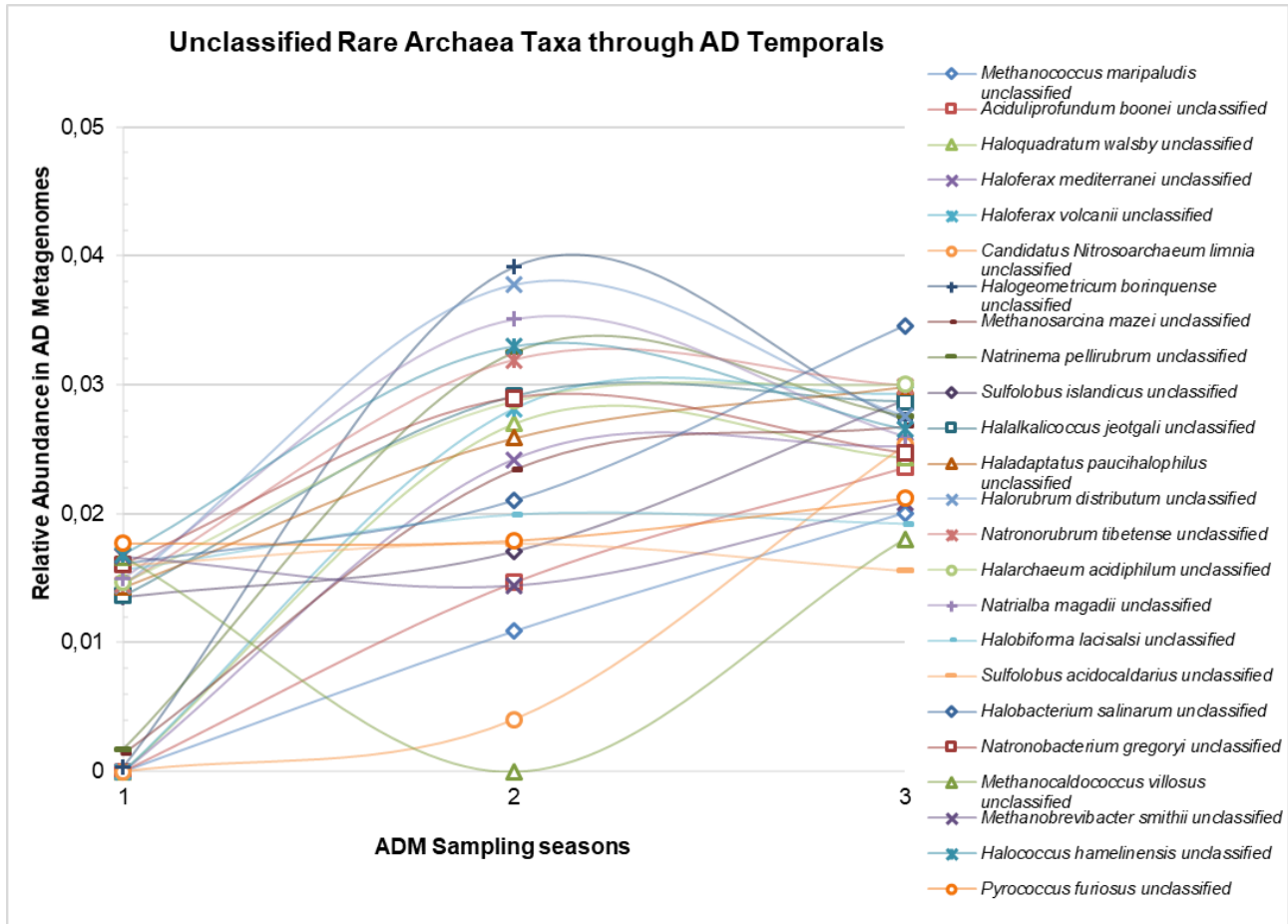
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904   Supp. Fig. 1. **Rarefaction curve to estimate the richness of Archean Domain in the different**

905   **season samples.**

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