"Sifarchaeota" a novel Asgard phylum capable of polysaccharide degradation and anaerobic methylotrophy

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Abstract

The Asgard superphylum is a deeply branching monophyletic group of Archaea, recently described as some of the closest relatives of the eukaryotic ancestor. The wide application of genomic analyses from metagenome sequencing has established six distinct phyla, whose genomes encode for diverse metabolic capacities and play important biogeochemical and ecological roles in marine sediments. Here, we describe two metagenome-assembled genomes (MAGs) recovered from deep marine sediments off Costa Rica margin, defining a novel lineage phylogenetically married to Thorarchaeota, as such we propose the name "Sifarchaeota" for this phylum. The two "Sifarchaeota" MAGs encode for an anaerobic methylotrophy pathway enabling the utilization of C1-C3 compounds (methanol and methylamines) to synthesize acetyl CoA. Also, the MAGs showed a remarkable saccharolytic capabilities compared to other Asgard lineages and encoded for diverse classes of carbohydrate active enzymes (CAZymes) targeting different mono-, di- and oligosaccharides. Comparative genomic analysis based on the full metabolic profiles of Asgard lineages revealed the close relation between "Sifarchaeota" and Odinarchaeota MAGs, which suggested a similar metabolic potentials and ecological roles. Furthermore, we identified multiple potential horizontal gene transfer (HGT) events from

23 different bacterial donors within “Sifarchaetoa” MAGs, which hypothetically expanded
24 “Sifarchaeota” capacities for substrate utilization, energy production and niche adaptation.

25 **Importance**

26 Deep marine sediments are the home of multiple poorly described archaeal lineages,
27 many of which have ecological and evolutionary importance. We recovered metagenome-
28 assembled genomes (MAGs) belonging to a novel Asgard phylum from the deep sediment of the
29 Costa Rica margin. We proposed the name “Sifarchaeota” to describe the members of this
30 phylum. Representative genomes of the “Sifarchaeota” showed remarkable saccharolytic
31 capacities extending the known metabolic features encoded by the Asgard lineages. We attribute
32 its ability to survive under the deep sediment conditions to its putative capacities to utilize
33 different (C1-C3) compounds commonly encountered in deep sediment environments via
34 anaerobic methylotrophy pathway. Also, we showed the importance of horizontal gene transfer
35 in enhancing the “Sifarchaeota” collective adaptation strategies.

36 **Introduction**

37 Deep marine sediments are the home of multiple poorly described archaeal lineages, most
38 of which are yet uncultured (1)(2). Recently, the discovery of Asgard archaea in benthic
39 environments has generated great interest in novel lineages from marine sediments. Additionally,
40 greater attention directed towards studying Asgard archaea is also in part to understanding
41 eukaryogenesis, as this superphylum harbors the most closely related archaeal group to
42 eukaryotes and their genomes encode for multiple homologs of eukaryotic proteins (3)(4). So far,
43 there are 6 established Asgard phyla: Lokiarchaeota, Thorarchaeota, Odinarchaeota,
44 Heimdallarchaeota, Helarchaeota and Gerdarchaeota (4)(5)(6). The number of novel lineages yet
45 to be found is at this point unknown. This raises the need for more genome resolved metagenome

46 surveys to recover genomes of these archaeal lineages, decipher their metabolic capacities and
47 place them in the context of microbial ecology. Previous studies targeting the deep sediment
48 from the Costa Rica margin subseafloor have shown the presence of diverse archaeal
49 communities (7)(8). Among these archaeal lineages, members of Asgard superphyla were highly
50 abundant at multiple depths, making up 17% of the archaeal communities present (7)(8). In this
51 study on the Costa Rica margin subseafloor, we employ genome resolved metagenomics to
52 describe the metabolic potential of the genomes belonging to a new Asgard phylum.
53 Phylogenomic analysis placed the sequences of the new Asgard genomes as a sister clade to the
54 sequences of Thorarchaeota and we propose the name "Sifarchaeota" to describe this new
55 Asgard phylum. Comparative analysis shows distinct differences between Sifarchaeota and
56 previously reported Asgard archaea in terms of substrate utilization, energy production and niche
57 adaptation strategies. Finally, we detect multiple potential horizontal gene transfer (HGT) events
58 from different bacterial donors expanding the substrate utilization, energy production and
59 secondary metabolite production capacities of the Sifarchaeota.

60 **Methods**

61 **Sample collection**

62 Samples were collected during International Ocean Drilling Program (IODP) Expedition
63 334, Site U1379B on the Costa Rican Margin. Details on sample location and sampling
64 methodology have been previously described (7)(9). Microbiology samples (whole-round cores)
65 were collected on board and frozen immediately at -80°C. They were shipped to the Gulf Core
66 Repository (College Station, Texas) on dry ice and stored at -80°C until shipping to the Biddle lab
67 (Lewes, Delaware) on dry ice and further storage at -80°C. Metagenome sequencing data were

68 generated from four silty clay sediment horizons (2H-1, 2H-2, 2H-5A, and 2H-5B) in the depth
69 interval of 2-9 mbsf, within the sulfate reduction zone (7).

70 **DNA extraction, library construction and sequencing**

71 DNA for metagenomic sequencing was extracted from ~7 g sediment (~0.7 g sediment in
72 10 individual lysis tubes) using PowerSoil DNA Isolation Kit (Qiagen) following the
73 manufacturer's instructions, except for the following minor modification: the lysing tubes were
74 incubated in water bath of 60°C for 15 min prior to beading beating on a MP machine at speed 6
75 for 45 seconds. The DNA extracts were iteratively eluted from the 10 spin columns into a final of
76 100 µL of double distilled H_2O for further analysis. Metagenomic libraries were prepared and
77 sequenced (150 bp paired-end) on an Illumina NextSeq 500 sequencer at the Genome Sequencing
78 & Genotype Center at the University of Delaware.

79 **Assembly and genome binning**

80 The raw sequencing data were processed with Trimmomatic v.0.36 (10) to remove Illumina
81 adapters and low quality reads ("SLIDINGWINDOW:10:25"). The quality-controlled reads from
82 the eight samples were de novo co-assembled into contigs using Megahit v.1.1.2 (11) with the k-
83 mer length varying from 27 to 117. Contigs longer than 1000 bp were automatically binned using
84 MaxBin2 (12) and Metabat2 (13), and the best quality ones were selected using DAS_Tool (14)
85 with the default parameters. The resulting MAGs were quality assessed using CheckM (15) and
86 taxonomically classified using GTDBTk v1.3.0 (16) using the default parameters. Genome bins of
87 >50% completeness were manually refined using the gbtools (17) based on the GC content,
88 taxonomic assignments, and differential coverages in different samples. Coverages of contigs in
89 each sample were determined by mapping trimmed reads onto the contigs using BBMap v.37.61
90 (18). Taxonomy of contigs were assigned according to the taxonomy of the single-copy marker

91 genes in contigs identified using a script modified from blobology (19) and classified by BLASTn.
92 SSU rRNA sequences in contigs were identified using Barrnap (Seeman 2015, Github), and
93 classified using VSEARCH with the SILVA 132 release (20) as the reference.

94 To improve the quality of the two novel Asgard archaea MAGs, we recruited quality-
95 controlled reads using BBMap from 2H-2, because the highest genome coverages of these two
96 MAGs were detected in this particular sample. The recruited reads were then re-assembled using
97 SPAdes v.3.12.0 (21) using default parameters. After removal of contigs shorter than 1 kb, the
98 resulting scaffolds were visualized and re-binned manually using gbtools (17) as described above.
99 The quality of the resulting Asgard archaea genomes were checked using CheckM v.1.0.7 (15)
100 with the "lineage_wf" option.

101

102 **Concatenated ribosomal protein phylogeny**

103 To determine the phylogenetic affiliations of the two Sifarchaeota MAGs in the Archaea
104 domain, we performed a thorough phylogenomic analysis based on the concatenation of 16
105 ribosomal proteins (L2, L3, L4, L5, L6, L14, L15, L16, L18, L22, L24, S3, S8, S10, S17, and
106 S19). Reference genomes were selected from all the major archaeal phyla (3-5 for each) included
107 in the GTDB database (22), except for the Asgard superphylum for which all available genomes
108 were included. Ribosomal protein sequences were detected in Anvi'o (23) using the respective
109 HMM profiles, aligned using MUSCLE v3.8.31(24), and concatenated. The maximum likelihood
110 phylogenetic tree was reconstructed using IQ-Tree (v1.6.6) (25) (located on the CIPRES web
111 server) (26) with VT + F + R10 as the best-fit substitution model selected by ModelFinder (27),
112 and single branch location was tested using 1000 ultrafast bootstraps and approximate Bayesian
113 computation (28). Branches with bootstrap support >80% were marked by black circles. In

114 addition to phylogenomic analysis, we also calculated the average nucleotide identity (ANI) using
115 FastANI (29) and average amino acid identity (AAI) using CompareM
116 (https://github.com/dparks1134/CompareM) with default settings between these novel Asgard
117 MAGs and other public available ones (i.e., those included in the GTDB database), to further
118 explore the novelty of these MAGs.

119 **Metabolic reconstruction**

120 Amino acid sequences encoded by the Sifarchaeota MAGs were predicted using Prodigal
121 v2.6.3 (30) applying the default parameters and using translation table 11. The resulting amino
122 acid sequences were screened using HMMsearch tool (31) against custom HMM databases (32)
123 representing the key genes for specific metabolic pathways to understand the potential metabolic
124 capacities and their ecological roles of Sifarchaeota. The presence/absence profiles of the
125 metabolic pathways and their completion levels were further assessed through querying the
126 predicted amino acids against KEGG database using BlastKoala tool (33). Carbohydrate-active
127 enzymes encoded by the Sifarchaeota MAGs were analyzed using dbCAN-fam-HMMs (v6)
128 database (34). Proteases, peptidases, and peptidase inhibitors encoded by the MAGS were detected
129 via USEARCH-ublast tool (35) against the MEROPS database v12.1(36). Finally, the predicted
130 amino acid sequences were queried against the TCDB database (37) using USEARCH-ublast tool
131 (35) to identify the potential transporters.

132 **Genome centric comparative analysis for the Asgard superphylum**

133 We compared the total metabolic profiles of Sifarchaetoa MAGs with representatives from
134 other Asgard lineages including (Odinarchaetoa, Thorarchaeota, Lokiarchaeota and
135 Heimdallarcaheota) to identify the key metabolic differences between each of the phyla within the
136 Asgard superphylum. We queried the representative MAGs of each of the Asgard lineages against

137 KOfam database (KEGG release 94.1) via KofamKOALA webtool (38) and using evalue 10^{-3}.
138 Then, the Asgard MAGs were clustered based on the presence/absence profiles of the identified
139 KOfams, shared between at least 3 genomes, using clustergrammer web-based tool (39) and
140 applying the Euclidean distance and average linkage type.

141 **Horizontal gene transfer (HGT) analysis**

142 The HGT events were detected through querying Sifarchaeota predicted amino acids
143 against KEGG database (KEGG release 94.1) via GhostKoala web interface (33). Proteins with
144 KO annotation, non-Asgard hits, and a bit score >100 were considered as potential HGT candidate
145 proteins. Then, the candidate set of proteins were queried against (nr) and UniProtKB/swissprot
146 databases and all proteins showing Asgard hits as one of the top hits were removed from any
147 further analysis. Finally, each candidate protein is aligned to reference set of proteins collected via
148 Annotree (40) using the corresponding KO entry and the HGT events were confirmed through
149 creating approximately-maximum-likelihood phylogenetic tree for each candidate protein using
150 FastTree v2.1 (41). An outline for the HGT detection pipeline is illustrated in Supplementary
151 Figure 1.

152

153 **Results**

154 **MAG construction and phylogenomic analysis**

155 We reconstructed 3 MAGs belonging to a potentially novel Asgard phylum with moderate
156 completion levels (67-80%) and very low contamination levels (0.93-1.9%) (Table 1). The
157 taxonomic affiliations of these potentially novel Asgard MAGs were tested using 16 ribosomal
158 proteins phylogenomic tree and it showed that both MAGs clustered together and formed a sister
159 lineage to MAGs belonging to the phylum Thorarchaeota (Figure 1). The phylogenetic analysis

using a set of 122 archaea specific marker proteins implemented in GTDB-tk confirmed that these MAGs are belonging to a potentially novel Asgard archaeal lineage. To confirm the unique positions of these candidate novel Asgard lineage, we calculated the average nucleotide identities (AAI) and average amino acid identities (ANI) between the 3 novel Asgard MAGs and other MAGs representing the other Asgard lineages including (Lokiarchaeota, Thorarchaeota, Heimdallarchaeota and Odinarchaeota). On average, the 3 novel Asgard MAGs showed low AAI values when compared to the other Asgard MAGs (<50%) (Supplementary Tables 1 and 2).

Two of the new Asgard MAGs, bins 190 and 142, showed a very high similarity with AAI 98.81% and ANI 99.52%, as such, we focused all our subsequent analysis on two MAGs, bins 042 and 142, since they were the most complete and unique MAGs in this study.

Description of the taxa

Although the two Sifarchaeota MAGs, bin042 and bin142, shared many metabolic similarities, we could not describe them using one type strain due to the significant phylogenetic distances between the two MAGs (Figure 1) and the genomic differences as described using ANI and AAI values (Supplementary Tables 1 and 2). Therefore, we used two type strains to describe the two putative Sifarchaeota lineages.

Candidatus Sifarchaeotum costaricensis **(*costaricensis* of or from Costa Rica).** Type material is the genome designated as bin042 representing '*Candidatus* Sifarchaeotum costaricensis'.

Candidatus Sifarchaeotum subterraneus **(*subterraneus* latin name of subsurface).** Type material is the genome designated as bin142 representing '*Candidatus* Sifarchaeotum subterraneus'.

Based on these genera, we further propose the name of a new Asgard phylum, the phylum '*Candidatus* Sifarchaeota phylum nov.'

183 **Metabolic reconstruction of Sifarchaeota MAGs showed remarkable saccharolytic capacities**
184 **and potential anaerobic methylotrophy**

185 Previous reports showed that Asgard genomes encode for wide range of protein and fatty
186 acids degradation capacities (42). So far, most of the known Asgard genomes showed limited
187 saccharolytic capacities emphasized by their low genomic densities of CAZymes and sugar
188 transporters. However, Sifarchaeota showed high abundance and diversity of Carbohydrate Active
189 Enzymes (CAZymes) encoded by their MAGs specifically targeting sugars varying in
190 complexities from low (C1-C3) to moderate (C4-C6), including mono-, di- and oligo- saccharides
191 (Supplementary Figure 1, Supplementary Tables 3 and 5). Interestingly, CAZyme analysis
192 revealed the presence of different glycoside hydrolases (GH) families including cellulases and
193 endoglucanases (GH5 and GH9), cyclomaltodextrinase (GH13), α-glucosidase (GH63), β-1,4-
194 mannooligosaccharide phosphorylase (GH130) and β-L-arabinofuranosidase (GH142) targeting
195 cellobiose, maltose and maltooligosaccharides, alpha-glucosaccharides, glucose/mannose and
196 arabinosaccharides, respectively. We also identified dedicated sugar transporters mediating the
197 transfer of these sugars inside the Sifarchaeota cells, where the degradation and fermentation
198 processes take place (Supplementary Table 6). Metabolic reconstruction of Sifarchaeota MAGs
199 predict a general fermentative anaerobic life style with multiple anaerobic respiration capabilities
200 emphasized by the presence of incomplete reverse TCA cycle, presence of various fermentation
201 pathways capable of producing different fermentative products including acetate, acetoin and
202 butanediol under strictly anaerobic conditions as well as the potential capabilities to anaerobically
203 respire sulfate to sulfite and nitrite to ammonia. Notably, the degradation capacities for proteins,
204 peptides and amino acids as well as amino acid metabolism are limited in Sifarchaeota compared
205 to the other benthic archaea. Hence, Sifarchaeota may make up the short supply of fixed nitrogen

206 via reducing nitrite to ammonia and encoding for amidases to extract fixed nitrogen from nitrogen
207 containing amides like formamide.

208 Sifarchaeota MAGs showed the capacity to utilize various C1 compounds including formate
209 and methanol. For example, methanol is metabolized using an anaerobic methylotrophy pathway
210 previously described (8), suggesting the potential widespread distribution of this pathway among
211 benthic marine archaea to metabolize methylated compounds. This potential anaerobic
212 methylotrophic capacity was inferred via detecting incomplete methylotrophic methanogenesis
213 pathway, where the key genes encoding for the methyl coenzyme reductase complex were
214 completely absent. Besides, genes for incomplete Wood Ljundahl (WL) pathway were identified,
215 where only the genes of the carbonyl branch were present and the genes of the methyl branch were
216 completely absent. Detecting these set of genes in Sifarchaeota MAGs suggests the presence of
217 anaerobic methylotrophic capability, enabling Sifarchaeota to recycle methyl groups within
218 methanol and other methylated compounds. Then, these methyl groups are transferred to
219 tetrahydrofolate complex replacing the function of the methyl branch of WL pathway and
220 ultimately producing acetyl CoA.

221 **Comparative genomic analyses between different Asgard lineages shows diverse metabolic**
222 **features and life style patterns**

223 To understand the key metabolic differences between the Asgard lineages, we conducted a
224 comprehensive genome-centric analyses. As described in the methods section, we parsed 13
225 different MAGs (2 MAGs obtained from this study and 11 publicly available MAGs) belonging
226 to 5 different Asgard phyla (Lokiarchaeota, Thorarchaeota, Heimdallarchaeota, Odinarchaeota and
227 Sifarchaeota) against KOfam database using an HMM search tool (Supplementary Table 7. The

228 analysis output focused on two aspects (1) exploring the range of life style diversity within the
229 Asgard superphylum and (2) comparing the metabolic capabilities of different Asgard lineages.

230 Overall, the comparative analyses grouped the Asgard superphylum into 4 distinct clusters
231 based on their whole metabolic profiles. Notably, all the MAGs within the Asgard superphylum
232 suggested a similar fermentative anaerobic life styles, where the core genes for reverse TCA cycle,
233 various fermentation pathways were present as well as the absence of oxidative phosphorylation
234 and oxygen tolerance related genes. Here we describe the clusters and the features that drive their
235 clustering.

236 The **first cluster** grouped the 2 Sifarchaeota MAGs (bin42 and 142), obtained from this
237 study, with Candidatus Odinarchaetoa archaeon LCB4. Sifarchaeota MAGs shared multiple
238 metabolic similarities with the Odinarchaeota MAGs including limited amino acid and fatty acids
239 metabolic potentials, evident saccharolytic activities, emphasized by high density of CAZyme
240 encoding genes targeting different mono-, di- and oligosaccharide. Also, we could only identify
241 genes encoding for the oxidative branch of the pentose phosphate pathway, producing
242 Phosphoribosyl pyrophosphate (PRPP), which eventually channeled to the purine and pyrimidine
243 metabolic pathways to be used for nucleotide and nucleic acid biosynthesis. Also, MAGs within
244 this cluster encoded for large number of genes involved in C1 metabolism including formate,
245 methylamines and methanol. Genes encoding for formate dehydrogenase, methanol and
246 methylamine specific corrinoid protein:coenzyme M methyltransferases were present, which
247 suggest the potential capability of both lineages to utilize formate, methylamines and methanol as
248 carbon sources, respectively.

249 The **second cluster** grouped Lokiarchaeota and Thorarchaeota MAGs together. Unlike the
250 previous cluster, MAGs belonging to that cluster are characterized by their proteins and peptide

251 degrading capabilities emphasized by the presence of high number of proteases and peptidases
252 encoding genes ranging in density from (126-193 proteins/Mbs) and belonging to different
253 families of serine and metalloproteases. Unlike other Asgard groups, Thorarchaeota and
254 Lokiarchaeota showed the capacities to synthesize different amino acids including nonpolar amino
255 acids (e.g. isoleucine, leucine, valine and alanine), aromatic amino acids (e.g. tryptophan and
256 phenylalanine) via the shikimate pathway and charged amino acids (e.g. glutamate and lysine).
257 Similar to other Asgard archaea, MAGs belonging to Thor- and Loki- archaeota encoded for genes
258 mediating the metabolism of C1 compounds (e.g. formate), however, the absence of
259 methyltransferases encoding genes excluded the use methylated compounds as one of the potential
260 substrates.

261 Finally, **the third and fourth clusters** included different Heimdallarchaeota MAGs. The
262 separation between Heimdallarchaeota based on their metabolic profiles suggests the presence of
263 fundamental metabolic differences between the members of Heimdallarchaeota phylum and
264 supports the previous findings that described Heimdallarcaheota as a polyphyletic group (3)(6).
265 This raises the need for wider sampling efforts targeting Heimdallarchaeota genomes to fully
266 resolve their phylogenetic position and evolutionary history. However, in this study we grouped
267 all the Heimdallarchaeota MAGs and treated them as one phylum and designed a model that
268 highlights the difference between them and the other Asgard lineages. Notably, all
269 Heimdallarcahetoa MAGs showed the capacity to utilize proteins and short chain fatty acids as
270 carbon sources, while polysaccharide degradation was less supported. Similar to Loki- and
271 Throarchaeota MAGs, Heimdallarcahetoa MAGs encoded for high number of peptidases with
272 coding densities (110-210 proteins/Mbs) and belonging to diverse families of proteases and
273 peptidases including serine, metallo, cysteine and threonine peptidases (Supplementary Table 3).

Also, Heimdallarcahetoa MAGs showed the capacity to metabolize and synthesize nonpolar amino acids (e.g. alanine, glycine, and threonine). Among all the Asgard MAGs included in this analysis, only Heimdallarchaeota MAGs encoded for enzymes mediating beta-oxidation pathway including acyl CoA dehydrogenase, enoyl CoA hydratase and hydroxyacyl CoA dehydrogenase, suggesting their potential to use short-chain fatty acids ~~(SCFA)~~ as carbon and energy sources. Similar to all other Asgards, Heimdallarchaeota encoded for incomplete WL pathway, which could have a role in metabolizing C1 compounds like formate and formaldehyde.

Due to the limited access to the surrounding microbial community composition and environmental conditions as well as the underrepresentation of the some of the lineages (only 1 MAG from Odinarchaeota), we could not assess the exact reasons for these diverse substrate preferences between the Asgard phyla (e.g. polysaccharides in Sifarchaeota, proteins in Thor- and Loki-archaeota and short-chain fatty acids in Heimdallarchaeota). Also, we are unable to conclude at this point if these findings could be generalized for all the members within each phylum or it is just limited to the lineages/MAGs included in the study.

Role of horizontal gene transfers (HGT) in enhancing Sifarchaeota metabolic capacities and niche adaptations

We investigated the role of HGT in expanding the metabolic capacities, substrate utilization and niche adaptation of the Sifarchaeota phylum. We traced the origin of each HGT event and identified the extent of the spread of each gene within the Asgard superphylum (Supplementary Figure 2). We successfully identified a total of 65 HGT events in Sifarchaeota, 12 (0.65% of the total proteins) and 53 (1.34% of the total proteins) events in bin042 and bin142, respectively. Most likely, the majority of the identified HGT events were lineage specific (58, 89.2%) and only few similar events were detected in other Asgard lineages (7, 11.8%). Also, we

297 identified the potential donors for most of the horizontally transferred genes, ~90% of which were
298 of bacterial origins. The major bacterial contributors for the horizontally transferred genes were
299 Firmicutes (~30%), Chloroflexi (~15%), Proteobacteria (~13%), Cyanobacteria (5%) and other
300 bacterial lineages (~30%). Only a small fraction (~10%) of the horizontally transferred genes were
301 of archaeal origin outside of the Asgard superphylum (Figure 5 and Supplementary Table 8).

302 We classified HGT events based on the how widespread the transferred genes were among
303 the different Asgard phylum, as well as other archaeal lineages. Accordingly, the HGT events were
304 classified into lineage-specific**,** phylum-specific and domain-wide events. In the lineage specific
305 event, the closest relatives of the transferred genes were bacteria and the genes were exclusively
306 found in Sifarchaetoa MAGs and no orthologs were found in other Asgard or any other archaeal
307 phyla (e.g. butanediol dehydrogenase). In the phylum-specific event, the closest relatives of the
308 transferred genes were bacteria and the genes were found in multiple Asgard phyla (e.g. enoyl
309 CoA hydratase). In the domain wide event, the closest relatives of the transferred genes were
310 bacteria and the genes were found in different archaeal phyla (e.g. arsenate reductase *arsC*
311 thioredoxin) (Figure 5). In general, the functional annotation of the transferred genes showed that
312 these genes are involved in augmenting the Sifarchaeota metabolic repertoire. The majority of the
313 transferred genes fall within two metabolic modules: butanoate metabolism and biosynthesis of
314 secondary metabolites. In the butanoate metabolism, the majority of transferred genes were
315 encoding for butane diol dehydrogenases and glutaconate CoA-transferase subunits mediating the
316 key steps in pyruvate and acetate formation from butane diol and hydroxy glutaryl CoA,
317 respectively.

318 While the majority of the genes involved in the biosynthesis of secondary metabolites
319 were part of the porphyrin and chlorophyll metabolism and terpenoid biosynthesis (e.g.

320 anaerobic magnesium-protoporphyrin IX monomethyl ester cyclase and 1,4-dihydroxy-2-
321 naphthoate polyprenyltransferase). Interestingly, multiple genes involved in niche adaptation
322 may be acquired from the surrounding bacterial communities. These genes including
323 formamidase and MtaA/CmuA family methyltransferase which potentially enable Sifarchaeota
324 to utilize amide containing compounds and methylated compounds as nitrogen and carbon
325 sources, respectively. Also, a gene encodes for arsenate reductase was potentially acquired from
326 candidate division KSB1 bacterium, potentially allowing Sifarchaeota to use arsenate containing
327 compounds as final electron acceptors.

328 **Discussion**

329 In this study, we recovered three MAGs from deep Costa Rica sediments belonging to a new
330 Asgard phylum, forming a sister clade to MAGs belonging to Thorarchaeota. We propose the name
331 Sifarchaeota for this phylum, named after the Norse goddess, Sif, wife of Thor. Putative collective
332 metabolic profiles of the Sifarchaeota MAGs showed remarkable differences in the life style and
333 niche adaptations compared to the other Asgard members. We predict a saccharolytic,
334 fermentation-based life style with limited amino acid and fatty acid metabolism, whereas most of
335 the Asgard archaea identified before this study were known for their peptide degradation and short
336 chain fatty acid oxidation capacities (3)(5)(42). We detected genes encoding for incomplete
337 methanogenesis pathways coupled with the carbonyl branch of WL pathway suggesting the
338 capability of Sifarchaeota to perform anaerobic methylotrophy enabling the utilization of various
339 methylated compounds (e.g. methanol and methylamines). The widespread of anaerobic
340 methylotrophy in multiple benthic archaea highlights the importance of this pathway as an
341 effective strategy to utilize various methylated compounds commonly encountered in the marine
342 sediment niches (8)(43). On the other hand, Sifarchaeota MAGs shared similar potential

343 biogeochemical functions with other Asgard archaea including the presence of nitrite reductase
344 (*nirBD*) genes, putatively enabling Sifarchaeota members to reduce nitrite to ammonia as well as
345 genes encoding for sulfate adenylyltransferase (*sat*) and phosphoadenosine phosphosulfate
346 reductase (*cysH*), signifying their putative capability to perform assimilatory sulfate reduction and
347 sulfate activation. These shared characteristics between Asgard genomes confirm the significant
348 roles of different Asgard lineages in nitrogen and sulfur biogeochemical cycles in marine sediment
349 environments.

350 We also gauged the role of HGT in shaping the evolution of Sifarchaeaota. Our analysis
351 suggests that HGT events either added novel genes to the Sifarchaeota pangenome that impart new
352 functions e.g. butanoate metabolism and biosynthesis of secondary metabolites or enabled the
353 utilization of alternative non-organic compounds as electron source e.g. arsenate reductase.
354 Moreover, we explored the range of horizontally transferred gene donors and we concluded that
355 HGT is not limited to a specific phylogenetic group and probably acquired from the surrounding
356 bacterial communities, normally present in deep marine sediments. Finally, 91% of HGT events
357 are Sifarchaeota lineage specific and probably took place relatively recently during the course of
358 evolution, after the diversification of Sifarchaeota from the other Asgard lineages. We identified
359 only 9% of the events happened earlier to the full diversification of Sifarchaeota from other
360 Asgards, as well as other archaeal lineages. This could be a plausible explanation for the presence
361 of shared functions of non-archaeal origin between most of Asgard lineages.

362

363 **Acknowledgements**

364

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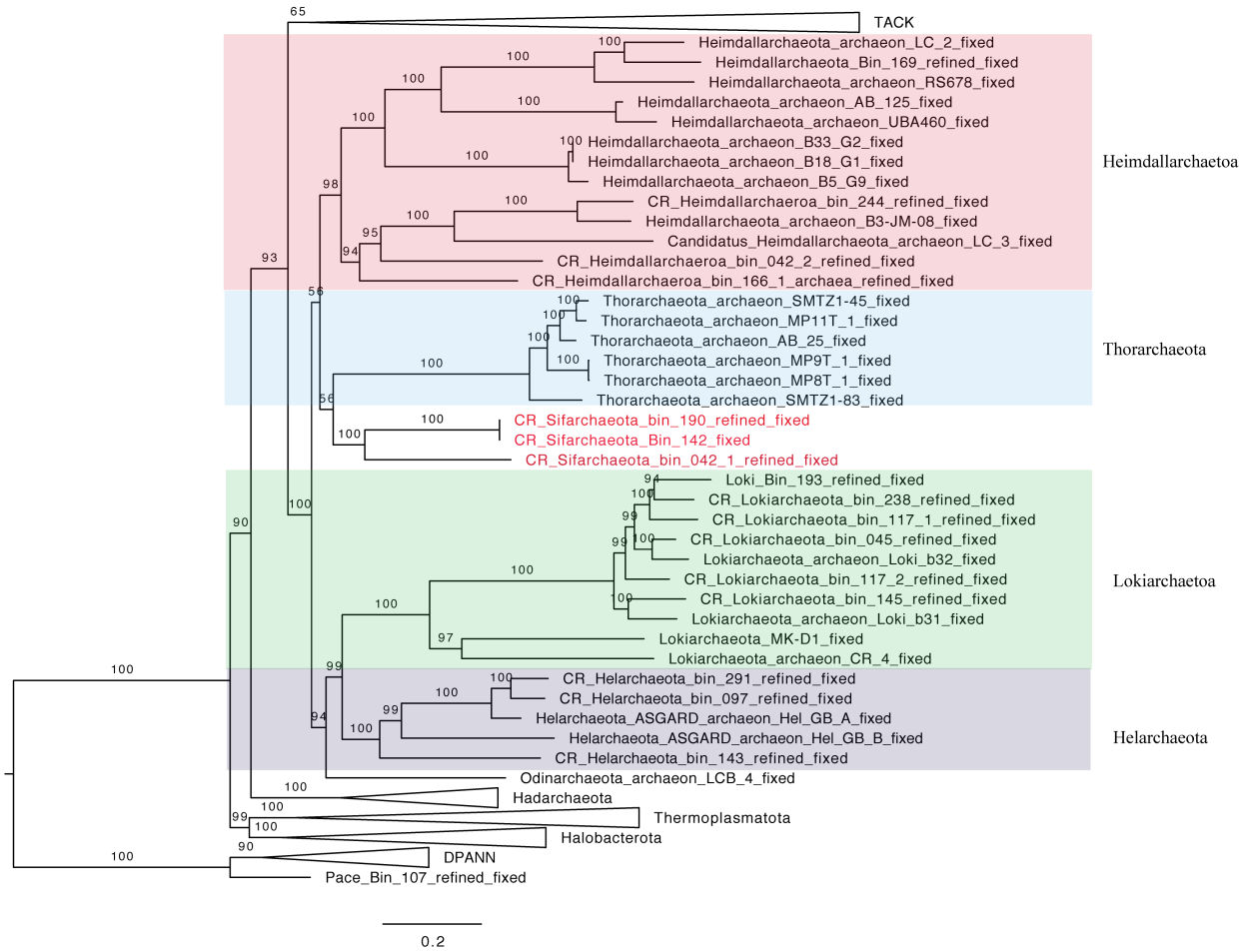


Figure 1. Maximum-likelihood phylogenetic tree of archaea genomes based on concatenated 16 ribosomal proteins. This tree was inferred using IQ-TREE v1.6.10 with the LG+R7 model and 1000 ultrafast bootstraps. The Sifarchaeota MAGs recovered in this study is highlighted in red. Lineages of the Asgard superphylum are expanded, while the other linages were collapsed, if possible. The scale bar shows estimated sequence substitutions per residue.

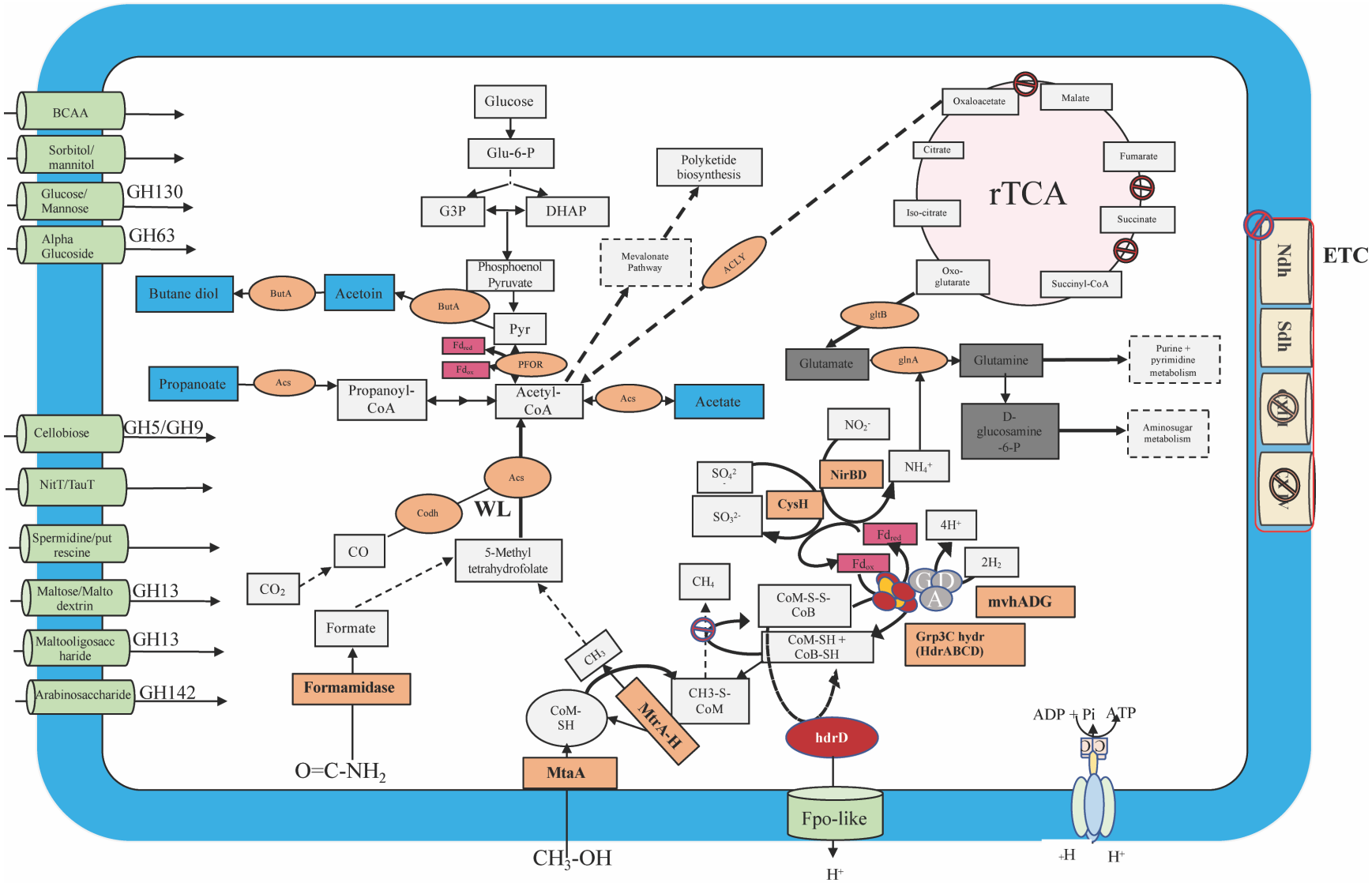

BCAA
Sorbitol/ mannitol
Glucose/ Mannose
GH130
Alpha Glucoside
GH63
Cellobiose
GH5/GH9
NitT/TauT
Spermidine/put rescine
Maltose/Malto dextrin
GH13
Maltooligosacc haride
GH13
Arabinosaccharide
GH142
Glucose
Glu-6-P
G3P
DHAP
Phosphoenol Pyruvate
Pyr
Butane diol
ButA
Acetoin
ButA
Propanoate
Acs
Propanoyl-CoA
Acetyl-CoA
Acs
Acetate
Fd red
Fd ox
PFOR
Mevalonate Pathway
Polyketide biosynthesis
ACLY
Acs
Codh
WL
CO
CO2
5-Methyl tetrahydrofolate
Formate
Formamidase
O=C-NH2
CH3
CoM-SH
MtrA-H
MtaA
CH3-OH
CH3-S-CoM
CH4
CoM-S-S-CoB
CoM-SH + CoB-SH
hdrD
Fpo-like
H+
Oxaloacetate
Malate
Citrate
Fumarate
Iso-citrate
rTCA
Succinate
Oxo-glutarate
Succinyl-CoA
gltB
Glutamate
glnA
Glutamine
Purine + pyrimidine metabolism
D-glucosamine -6-P
Aminosugar metabolism
NO2-
NH4+
NirBD
SO42-
SO32-
CysH
Fd red
Fd ox
4H+
2H2
C D A
mvhADG
Grp3C hydr (HdrABCD)
ADP + Pi
ATP
+H
H+
Ndh
Sdh
ETC

Figure 2. Metabolic reconstruction of the key metabolic pathways encoded by the Sifarchaeota MAGs. Central metabolic pathways are shown in gray boxes, carbon fixation pathways (WL and rTCA cycles) are shown in pink, electron transport chain (ETC) proteins are shown in yellow, fermentation products are shown in blue boxes, enzymes and enzyme complexes are shown in orange circles, energy carriers are shown in red, and metabolite and amino acid transporters are shown in light green.

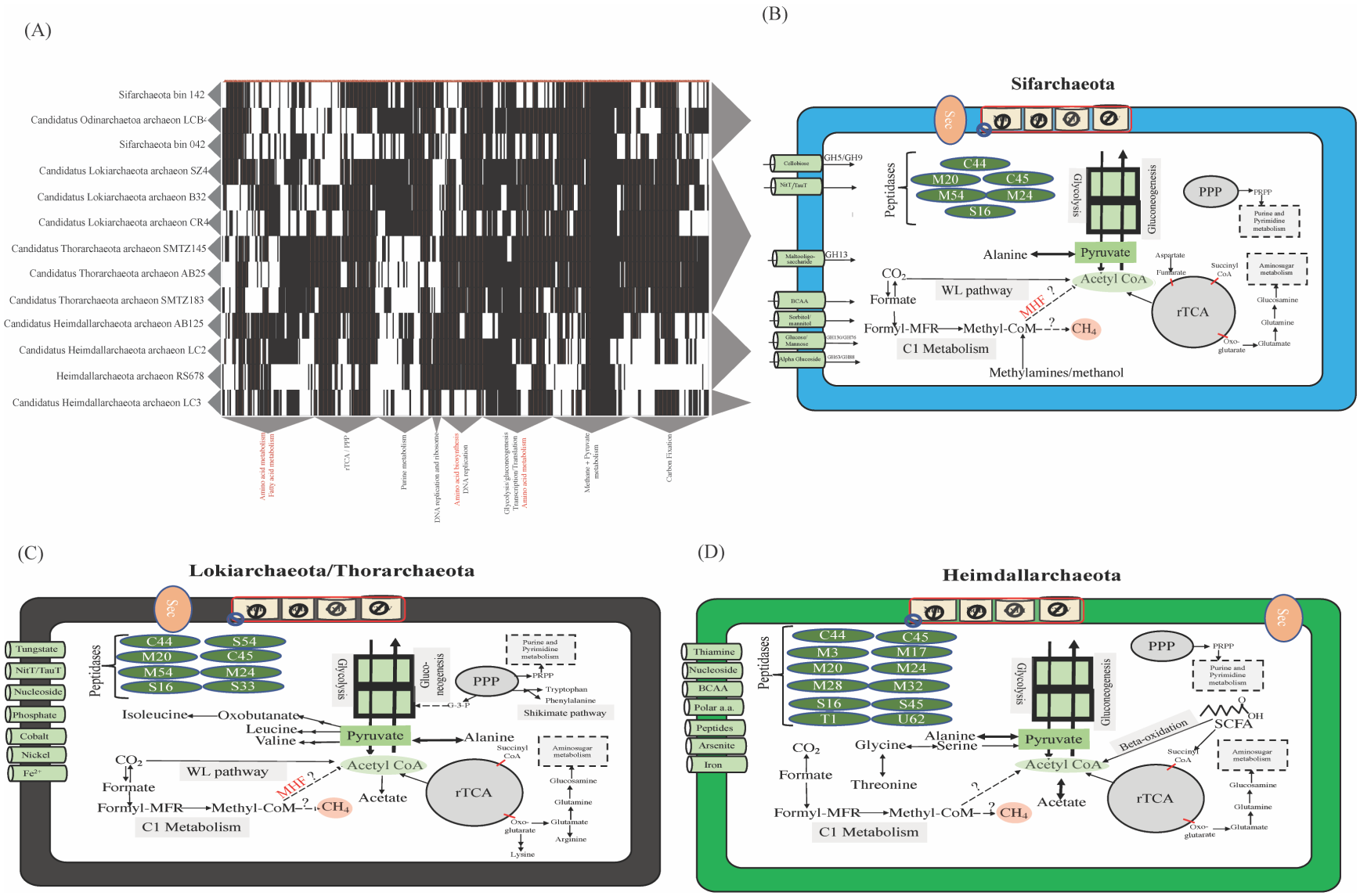

(A)
Sifarchaeota bin 142
Candidatus Odinarchaetoa archaeon LCB4
Sifarchaeota bin 042
Candidatus Lokiarchaeota archaeon SZ4
Candidatus Lokiarchaeota archaeon B32
Candidatus Lokiarchaeota archaeon CR4
Candidatus Thorarchaeota archaeon SMTZ145
Candidatus Thorarchaeota archaeon AB25
Candidatus Thorarchaeota archaeon SMTZ183
Candidatus Heimdallarchaeota archaeon AB125
Candidatus Heimdallarchaeota archaeon LC2
Heimdallarchaeota archaeon RS678
Candidatus Heimdallarchaeota archaeon LC3
Amino acid metabolism
Fatty acid metabolism
rTCA / PPP
Purine metabolism
DNA replication and ribosome
Amino acid biosynthesis
DNA replication
Glycolysis/ gluconeogenesis
Transcription/Translation
Amino acid metabolism
Methane + Pyruvate metabolism
Carbon Fixation
(B)
Sifarchaeota
Sec
Cellobiose
GH5/GH9
NitT/TauT
Peptidases
C44
M20
C45
M54
M24
S16
Glycolysis
Gluconeogenesis
PPP
PRPP
Purine and Pyrimidine metabolism
Maltooligo-saccharide
GH13
Alanine
Pyruvate
Aspartate
Fumarate
Succinyl CoA
Aminosugar metabolism
CO_2
Acetyl CoA
WL pathway
Formate
MHF
rTCA
Glucosamine
BCAA
Sorbitol/ mannitol
Glucose/ Mannose
GH13/GH70
Alpha Glucoside
GH63/GH88
Formyl-MFR
Methyl-CoM
CH_4
C1 Metabolism
Glutamine
Oxo-glutarate
Glutamate
Methylamines/methanol
(C)
Lokiarchaeota/Thorarchaeota
Sec
Tungstate
NitT/TauT
Nucleoside
Phosphate
Cobalt
Nickel
Fe^{2+}
Peptidases
C44
S54
M20
C45
M54
M24
S16
S33
Glycolysis
Gluco-neogenesis
PPP
Purine and Pyrimidine metabolism
PRPP
G-3-P
Tryptophan
Phenylalanine
Shikimate pathway
Isoleucine
Oxobutanate
Leucine
Valine
Pyruvate
Alanine
Succinyl CoA
Aminosugar metabolism
CO_2
WL pathway
Acetyl CoA
Formate
MHF
Acetate
rTCA
Glucosamine
Formyl-MFR
Methyl-CoM
CH_4
Glutamine
C1 Metabolism
Oxo-glutarate
Glutamate
Arginine
Lysine
(D)
Heimdallarchaeota
Sec
Thiamine
Nucleoside
BCAA
Polar a.a.
Peptides
Arsenite
Iron
Peptidases
C44
C45
M3
M17
M20
M24
M28
M32
S16
S45
T1
U62
Glycolysis
Gluconeogenesis
PPP
PRPP
Purine and Pyrimidine metabolism
Beta-oxidation
SCFA
Alanine
Serine
Glycine
Pyruvate
Succinyl CoA
Aminosugar metabolism
CO_2
Acetyl CoA
Formate
Threonine
Acetate
rTCA
Glucosamine
Formyl-MFR
Methyl-CoM
CH_4
Glutamine
C1 Metabolism
Oxo-glutarate
Glutamate

Figure 3. Comparative analysis between MAGs representing different Asgard members (A) Heatmap clustering the different Asgard MAGs (X-axis) based on their total metabolic profiles as predicted by Kofam database (Y-axis). The clustering was performed using Euclidean distance and complete linkage methods. (B) Metabolic model illustrates the key metabolic features identified in Sifarchaeota cluster. (C) Metabolic model illustrates the key metabolic features identified in Lokiarchaeota and Thorarchaeota cluster. (D) Metabolic model illustrates the key metabolic features identified in Heimdallarchaeota clusters.

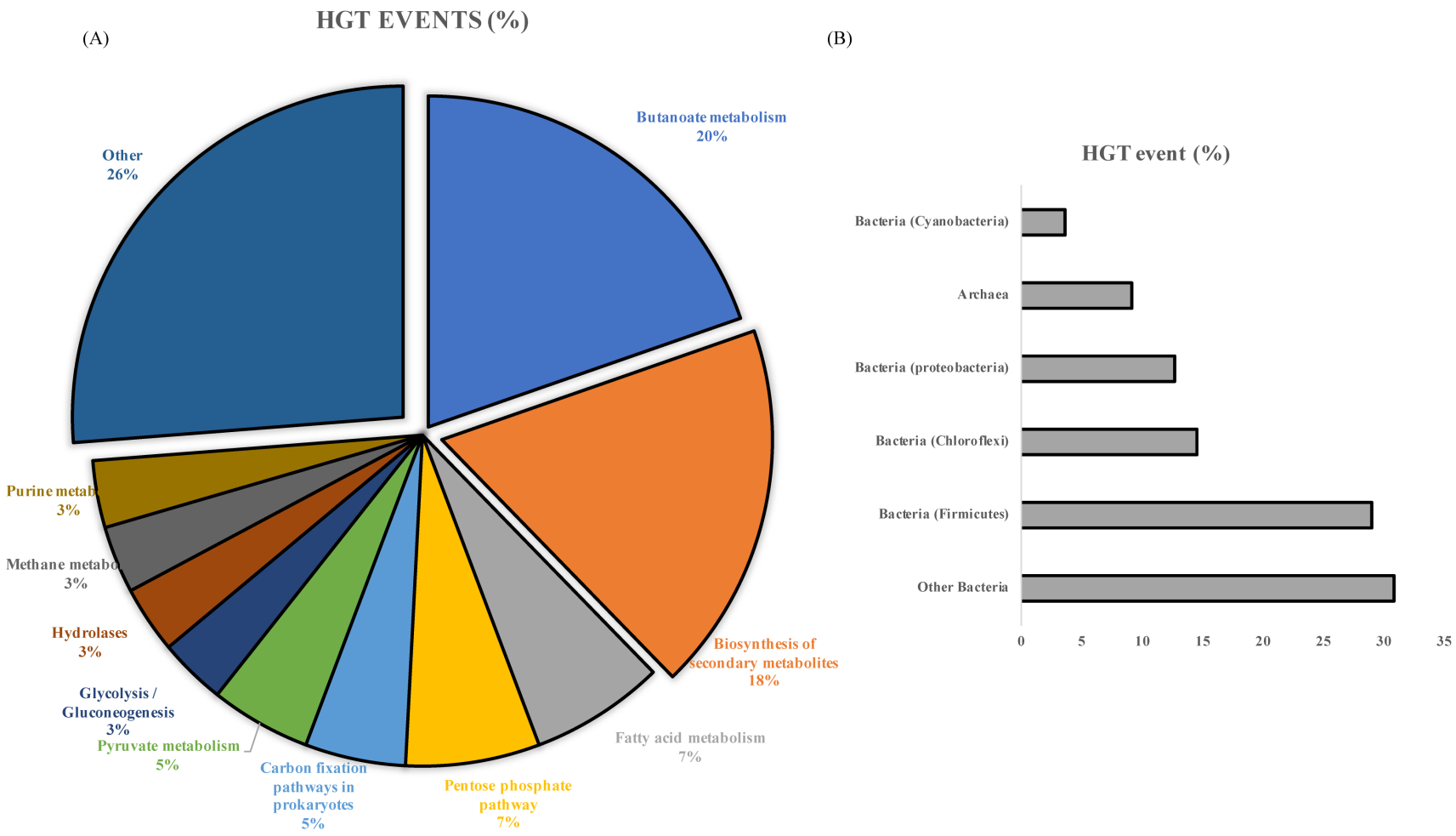
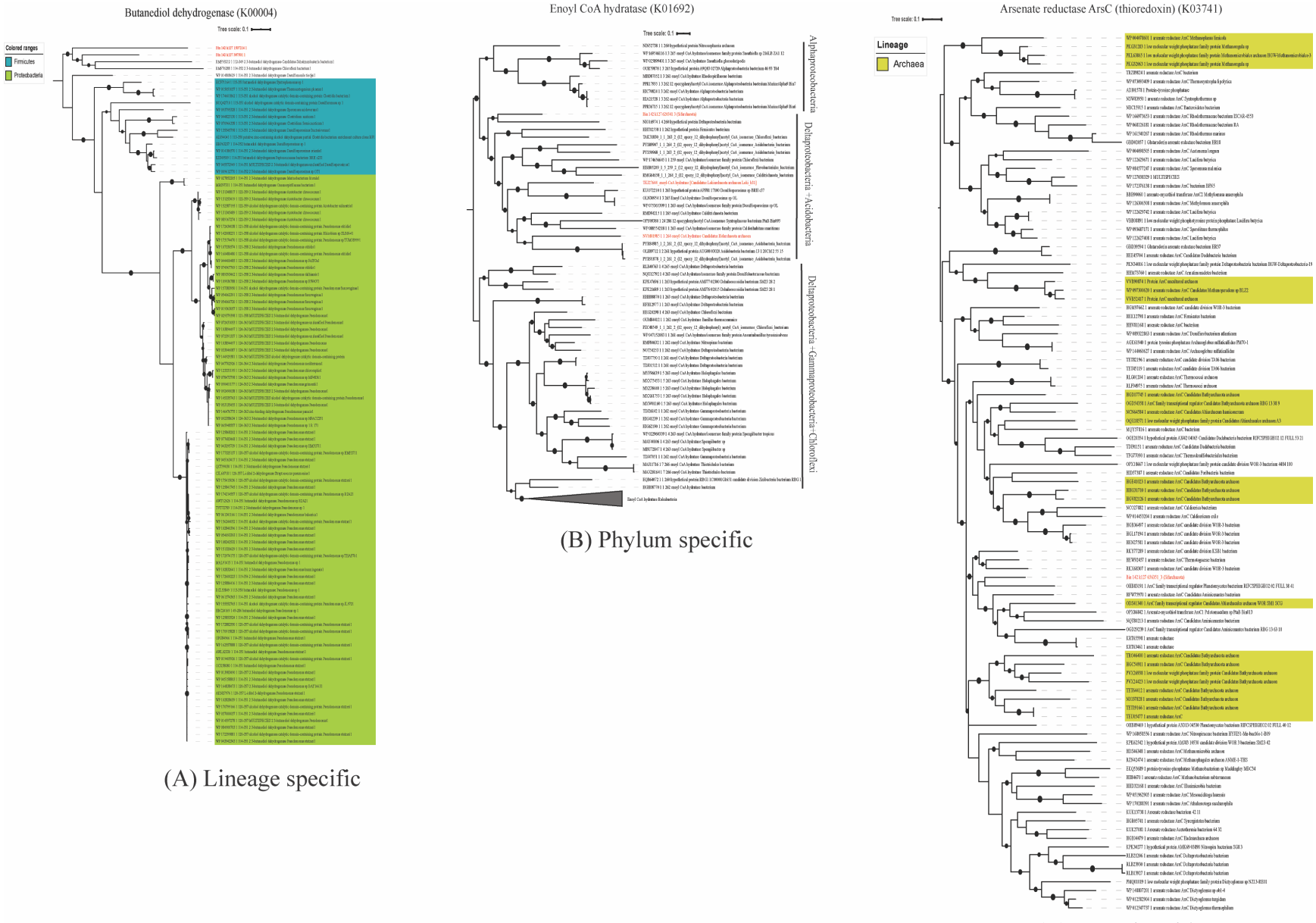


Figure 4. Summary of HGT events detected in the Sifarchaeota MAGs. (A) Different functional modules of horizontally transferred genes. (B) Major donors of the horizontally transferred genes.



(A) Lineage specific

(B) Phylum specific

(C) Domain wide event

Figure 5. Examples for horizontally transferred genes in Sifarchaeota MAGs. (A) Lineage specific HGT event. Maximum likelihood phylogenetic tree of butanediol dehydrogenase gene sequences. Shaded areas correspond to the potential source organims. The tree was constructed on the basis of butanediol dehydrogenase gene sequences using FastTree. Reference sequences were obtained using AnnoTree (K00004). (B) Phylum specific HGT event. Maximum likelihood phylogenetic tree of enoyl CoA hydratase gene sequences. The tree was constructed on the basis of enoyl CoA hydratase gene sequences using FastTree. Reference sequences were obtained using AnnoTree (K01692). (C) Domain wide HGT event. Maximum likelihood phylogenetic tree of arsenate reductase ArsC thioredoxin gene sequences. The tree was constructed on the basis of arsenate reductase ArsC thioredoxin gene sequences using FastTree. Reference sequences were obtained using AnnoTree (K03741).

Table 1. Details of the Sifarchaeota MAGs analyzed in this study.

Bin ID	Completion	Contamination	Genome Size (Mbps)	Estimated Genome Size (Mbps)	Strain heterogeneity	# contigs
Bin.042_1	67.76	0.93	1.8	2.39	0	301
Bin_190	74.4	1.9	1.9	2.38	0	786
Bin_142	81.4	1.9	2.01	2.39	0	300

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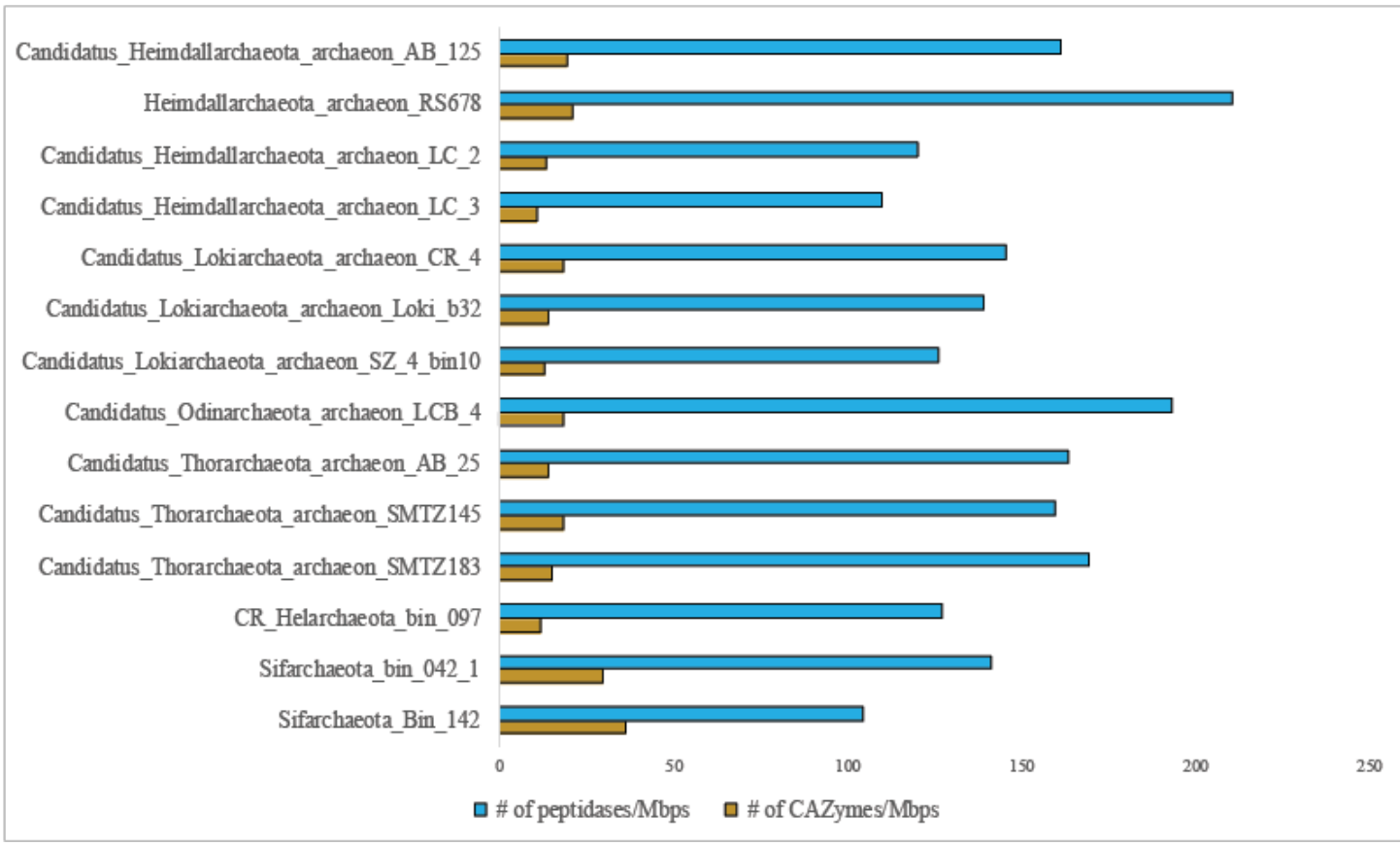
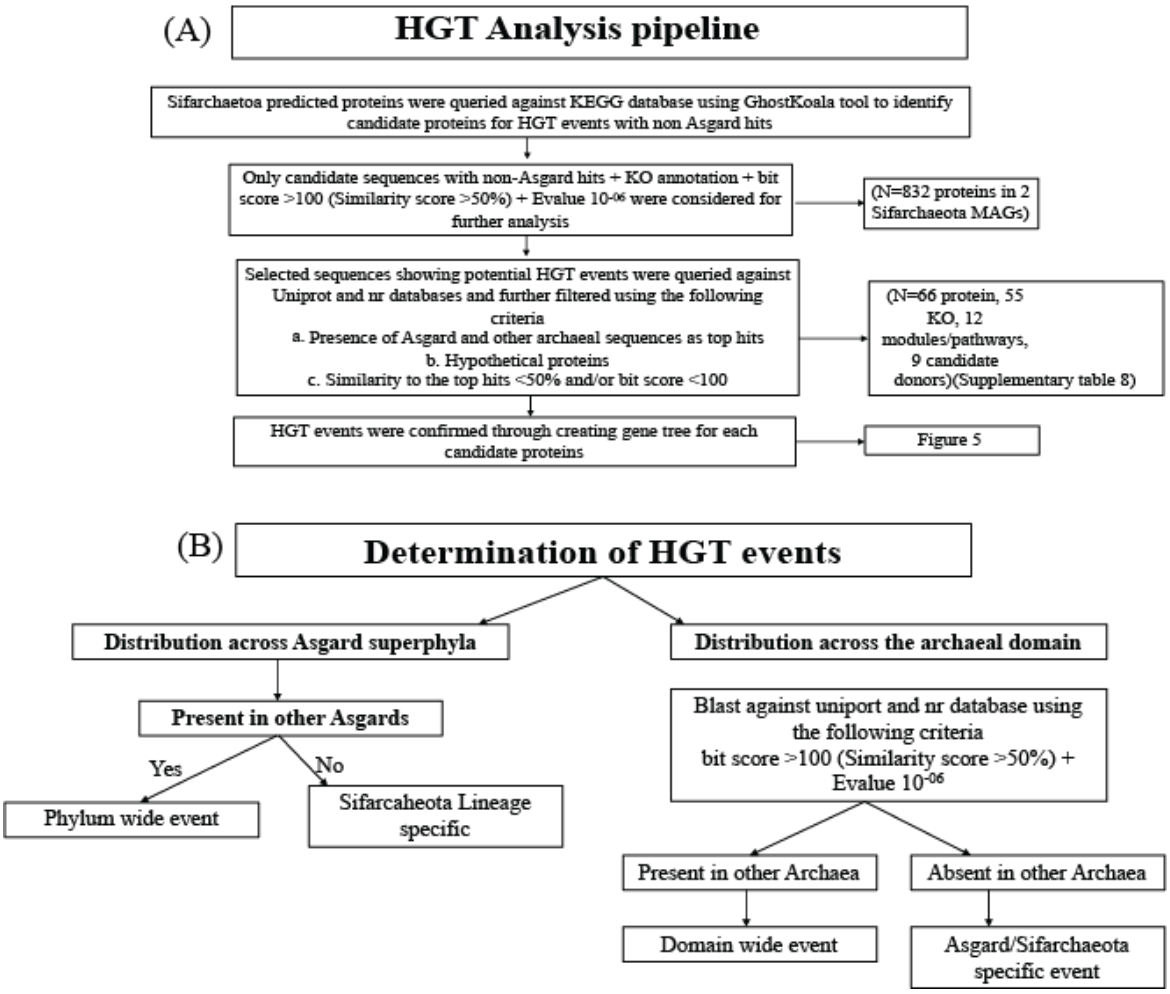


Supplementary Figure 1. Relative densities (numbers per 1 Mb) of peptidases and CAZymes encoded by the different Asgard MAGs.



Supplementary Figure 2. Workflow diagram describes the steps followed to identify HGT events in Sifarchaeota MAGs.