1 **Diversity and biogeography of Woesearchaeota: A comprehensive** 2 **analysis of multi-environment data**

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25 **Abstract**

26 Woesearchaeota is a newly proposed archaeal phylum frequently detected in various
 27 environments. Due to the limited systematical study, little is known about their
 28 distribution, taxonomy, and metabolism. Here, we conducted a comprehensive study
 29 for Woesearchaeota with 16S ribosomal RNA (rRNA) gene sequencing data of 27,709
 30 samples and metagenomic whole genome sequencing (WGS) data of 1,266 samples.
 31 We find that apart from free-living environments, Woesearchaeota also widely
 32 distribute in host-associated environments. And host-associated environmental
 33 parameters greatly affect their distribution. 81 Woesearchaeota genomes, including 33
 34 genomes firstly reconstructed in this project, were assigned to 59 Woesearchaeota
 35 species, suggesting their high taxonomic diversity. Comparative analysis indicated
 36 that Woesearchaeota have an open pan-genome with small core genome. Metabolic
 37 reconstruction showed that particular metabolic pathway absence in specific
 38 environments, demonstrated the metabolic diversity of Woesearchaeota varies in
 39 differences environments. These results have placed host-associated environments
 40 into the global biogeography of Woesearchaeota and have demonstrated their genomic
 41 diversity for future investigation of adaptive evolution.

42

43 **Key words:** Woesearchaeota, metagenomics, pan-genome, metabolism, distribution

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45

1. Introduction

In the past few years, an increasing number of archaeal phyla have been proposed, which have greatly deepened our understanding for the ecological and evolutionary roles of Archaea domain¹⁻⁵. Woesearchaeota is an archaeal phylum proposed by Castelle *et al.* based on metagenomic analysis⁶. With the limited genomic data extracted from environmental samples, Woesearchaeota is considered as one of the most widely distributed archaea in DPANN superphylum^{7,8}. They have been detected in sediments, groundwater, soil, deep-sea hydrothermal vents, hypersaline lakes, wetland, permafrost, and human lung^{6,9,10}. However, how the geochemical settings define the distribution pattern of Woesearchaeota and their ecological function, especially on a global scale, remain unclear.

Moreover, members within Woesearchaeota phylum appear to have highly divergent, sometimes deficient, metabolic potentials, and this further hinders the identification and isolation of Woesearchaeota. For example, based on 16S rRNA gene sequences, 26 potential subgroups were detected, although the taxonomic documentation was ambiguous⁹. Reconstruction of metabolic pathways from metagenome-assembled genomes (MAGs) of Woesearchaeota showed the absence of certain core biosynthesis, suggesting a symbiotic or parasitic lifestyle^{6,7,9}. If this deficiency of independent living is a common phenomenon in the entire phylum, it would be particularly intriguing in the evolutionary point of view. Considering the lack of Woesearchaeota isolate, comparative genomics study based on large datasets of WGS should be a promising approach to access the genomic and metabolic feature of Woesearchaeota.

To date, with the accomplishment of several world-class microbiome projects, such as Earth Microbiome Project (EMP) and *Tara* Oceans Project¹¹⁻¹⁴, more data from various environments are available for a systematically investigation. To expand knowledge for distribution, taxonomy, and metabolism of Woesearchaeota, we conducted a comprehensive study with combination of two types of data. 16S rRNA

gene sequencing data were used to explore their distribution characteristic, meanwhile, metagenomic data were collected to investigate taxonomic and metabolic diversity of Woesearchaeota. This endeavor allowed us to construct a framework for distribution, taxonomy, and metabolism of Woesearchaeota, contributing to guidance to efficient cultivation and profound investigation.

2. Results

2.1 Biogeography of Woesearchaeota

Widely distribution of Woesearchaeota.

16S rRNA gene sequences from EMP were collected to explore the distribution characteristics of Woesearchaeota. A total of 27,709 samples were carefully analyzed in this study, and we got 23,428 qualified samples, among which 6,788 samples were identified as containing Woesearchaeota. These Woesearchaeota are widely distributed around the world, in both marine and inland environments (Fig. 1A). Further analysis revealed that Woesearchaeota not only present in free-living environments such as water, soil, and sediments, but also live in host-associated environments such as plant rhizosphere, biofilm, animal surface, and animal secretion (Fig. 1B). Apparently, Woesearchaeota are more often to be discovered in free-living environments rather than in host-associated environments. In free-living environments, Woesearchaeota are most widely distributed in the sediment, while in host-associated environment, Woesearchaeota are most extensively distributed in rhizosphere.

Fig. 1 Distribution characteristics of Woesearchaeota. **A.** Global distribution of EMP samples showed the present/absence of Woesearchaeota. **B.** Distribution of Woesearchaeota in different types of environment (*Number of samples from hypersaline environment is relatively small compared to other environments, for all 13 samples, 10 of them contain Woesearchaeota). **C.** Importance of different environmental factors affecting the distribution for Woesearchaeota.

Impact of environmental parameters.

To assess how environmental parameters effect the distribution of Woesearchaeota, Random Forest classifier model¹⁵ was constructed, and 27 environmental parameters were taken as input feature vectors. The mean area under the curve (AUC) values of the model is 0.9467(10-fold cross validation). Feature importance is evaluated by Gini index (Fig. 1C). Among all the environmental factors, elevation and depth of the

109 samples are of great importance, affecting the distribution of Woesearchaeota. Besides,
110 6 features (Lifestyle_free, Oxic_unkw, Env_others, Salinity_unkw, Lifestyle_host,
111 Temp-med) related to free-living/host-associated lifestyle also matters, which
112 consistent with the finding that Woesearchaeota are mainly distributed in free-living
113 environments. For the remaining environment factors, oxygen conditions, temperature,
114 terrestrial/aquatic, and salinity show a decreasing importance, but they also affect the
115 distribution of Woesearchaeota. While altitude, host type (plant/animal) almost have
116 no effect on their distribution.

117

118 **2.2 Taxonomy of Woesearchaeota**

119 **Genome collection of Woesearchaeota.**

120 To explore the taxonomic characteristics of Woesearchaeota, genomes of
121 Woesearchaeota in public database were also collected. We got 48 high-quality
122 Woesearchaeota genomes (Supplementary Fig.1) after de-duplication and quality
123 control for all 105 candidate genomes of Woesearchaeota. Among all high-quality
124 genomes, nearly 90% of the genomes were from water sample including groundwater,
125 marine water, and hot spring water, while only 4 genomes from sediments and 1
126 genome from soil.

127

128 **Genome reconstruction and phylogeny of Woesearchaeota.**

129 We collected over ~35 terabyte metagenomic WGS data from dominant habitats of
130 Woesearchaeota, including samples from sea water, rhizosphere and sediment. After
131 trimmed all these data, de novo assembly and binning were then conducted, resulted
132 in the reconstruction of 74 high quality (>70% completeness and <5% contamination)
133 target archaeal genome bins. Phylogeny based on 16 concatenated ribosomal proteins
134 reveals that these archaeal bins belong to different archaeal clades (Fig. 2). And 33
135 genome bins belong to Woesearchaeota, among which 5 from sediments, 23 from sea
136 water, and 5 from host-associated environment rhizosphere (Supplementary Table 1).

137

138

139 **Fig. 2** Phylogeny of archaeal phyla. Each leaf represents an archaeal genome, grey dot on each
 140 branch represents bootstrap value, ranging from 0.6 to 1. and red range represent Woesearchaeota,
 141 leaves with colored dot mean the Woesearchaeotal genomes reconstructed in this study, different
 142 color represents different sample source. (dark blue dot: *Tara* Oceans Project; light blue dot:
 143 in-house data; orange dot: NCBI-SRA).

144

145 Taxonomic groups of Woesearchaeota.

146 For further investigation of Woesearchaeotal taxonomic characteristics, 48
 147 high-quality genomes of Woesearchaeota from public database were also used.
 148 Adding up 33 high-quality Woesearchaeota genome bins reconstructed in this study,
 149 we finally gathered 81 high-quality Woesearchaeota genomes for further
 150 study(Supplementary Fig.1). CheckM tool was used to evaluate genome quality, and
 151 among all these high-quality genomes bins, more than half of the genome bins have
 152 completeness higher than 90%. Moreover, all these Woesearchaeota genome bins are
 153 of small size (averagely 1.04 Mb), encoding 1,174 genes on average.

154 To accurately identify the taxonomic groups of Woesearchaeota genomes, we used
 155 whole genome sequences. Pairwise orthoANI (ANI, Average nucleotide identity)^{16, 17}
 156 calculation among all 81 genomes were conducted. Based on previous studies,
 157 orthoANI value takes a similar range of cut cut-off as ANI for species demarcation,
 158 which is approximately 95–96%¹⁸. The taxonomic identification results showed that
 159 all 81 genomes belong to 59 Woesearchaeal species (Fig. 3), and orthoANI values
 160 among most genome bins are ~63%, showing that the Woesearchaeota are of high
 161 taxonomic diversity at the species level. Meanwhile, 19 new species of
 162 Woesearchaeota have been discovered in our study, including 2 species first
 163 discovered from host-associated environments.

Fig. 3 Taxonomic diversity of Woesearchaeota. Each grid represents the OrthoANI value between two corresponding genomes.

2.3 Open pan-genome with limited core genome genes

Among all 81 high-quality Woesearchaeota genomes, 17 genome bins (Table 1; Supplementary Fig.1) are nearly-complete (completeness > 95%) with relatively low contamination (contamination < 2.2%). Thus, a comparative genomics analysis for Woesearchaeota was conducted by using these genomes. A total of 20,731 predicted protein-coding-genes were obtained, which were clustered into 15,109 orthologous

clusters. The power-law regression analyses indicated an open pan-genome for Woesearchaeota (Supplementary Fig.2). Besides, the contributions of core, accessory, and unique genes in Woesearchaeal pan-genome (Fig. 4 A) showed that they only contain a small core genome. On average, only 3.2% of genes in each Woesearchaeal genome are core genes, and the rest are accessory genes and unique genes, accounting for 47.3% and 49.5% genes in each genome, respectively. Moreover, proportion of accessory genes and unique genes varies in different Woesearchaeal genome, and the percentage of unique genes is higher than accessory genes in most genomes.

182

183 **Table 1. Genomic information of 17 nearly-complete Woesearchaeotal genomes**

No	Genome Id	Data Source	Completeness	Contamination	Biosample	Metagenome_source
01	GCA_005222965.1	NCBI	99.18	1.65	SAMN07236660	marine sediment
02	GCA_002867475.1	NCBI	98.9	2.2	SAMN07982670	enrichment from estuary sediment
03	GCA_000830295.1	NCBI	98.9	1.1	SAMN03202995	Rifle groundwater
04	GCA_002688315.1	NCBI	98.63	0	SAMN07982670	enrichment from estuary sediment
05	GCA_002762785.1	NCBI	97.8	0	SAMN06659469	Groundwater (2m)
06	ERR594345.116	In house	97.53	0	SAMEA2619974	sea water
07	ERR599062.136	In house	97.53	1.1	SAMEA2619970	sea water
08	ERR594345.48	In house	96.98	2.2	SAMEA2619974	sea water
09	SRR8464959.7	In house	96.89	0	SAMN10350739	Carex aquatilis--Peat Soil
10	GCA_002762985.1	NCBI	96.7	1.1	SAMN06659468	Groundwater (2m)
11	EPRCH7.83	In house	96.7	1.1	OEX003658	Black smokers
12	ERR594345.135	In house	96.43	1.1	SAMEA2619974	sea water
13	GCA_003695265.1	NCBI	96.15	1.1	SAMN10119972	hot springs metagenome
14	ERR594345.31	In house	96.15	2.2	SAMEA2619974	sea water
15	SRR8464960.7	In house	95.97	0	SAMN10350571	Carex aquatilis--Peat Soil
16	GCA_002498125.1	NCBI	95.6	1.1	SAMN06027228	soil metagenome
17	EPRCH7.420	In house	95.6	1.1	OEX003658	Black smokers

184

Further investigation revealed function profiles of Woesearchaeal pan-genome (Fig. 4 B). For core genes, most are assigned to “information storage and processing”, followed by “cellular processes and signaling”, which only make up a much smaller proportion. Compared to core genes, differences of function profiles are relatively small in accessory genes and unique genes. And the majority of accessory genes and

190 unique genes are assigned to “information storage and processing” and “metabolism”,
 191 respectively. Meanwhile, poorly characterized genes presented in all three groups, and
 192 functions of these genes are still not clear, over 25% of the unique genes are so-called
 193 “poorly characterized” genes.
 194

195
 196 **Fig. 4** Constituent and function of Woesearchaeotal Pan-genome. **A.** Relative abundance of
 197 core/accessory/unique genes in Woesearchaeotal genomes. **B.** Distribution of genes function.
 198

199 2.4 Metabolic capabilities of Woesearchaeota

200 To explore their metabolic capability, we used 17 nearly-complete genomes (Table 1)
 201 for further analysis. Metabolic reconstruction showed that most of the core
 202 biosynthetic pathways are incomplete in Woesearchaeota. For example, none of the
 203 Woesearchaeal genomes encodes the complete tricarboxylic acid cycle (TCA cycle).
 204 And most of the genes encoding respiratory-associated enzymes are absent in all these
 205 genomes. Besides, in a large proportion of these Woesearchaeal genomes, glycolytic
 206 pathway is incomplete because of the absence of few genes (Fig. 5).

Fig. 5 Metabolic pathways of Woesearchaeota. Pathways were constructed based on KEGG database, the soiled arrows mean related genes were presented in some genomes while the dotted arrows mean absence of corresponding genes in all genomes.

Carbon metabolism.

A complete glycolytic pathway in Woesearchaeota was first discovered in this study. In previous studies, the gene *pfk* encoding phosphofructokinase was absent in all the Woesearchaeotal genomes. However, this gene was found in several genomes with the accumulation of nearly-complete Woesearchaeotal genomes. It can be inferred that most Woesearchaeota can convert glucose. Notably, we discovered gene *porA/B* in some Woesearchaeotal genomes, which has not been reported before. The *porA/B* gene encodes enzyme converting pyruvate to acetyl-CoA, meanwhile, some other Woesearchaeota accomplish the conversion by encoding pyruvate dehydrogenase.

Nitrogen metabolism.

Dissimilatory nitrate reduction pathway was first found in Woesearchaeota. And *narG* and *nirD* genes are discovered in Woesearchaeotal genome, encoding nitrate reductase and nitrite reductase respectively. These enzymes enable the transformation of Nitrite

226 to Ammonia. Moreover, genes encoding enzymes catalyzing denitrification were also
227 detected, including *narG*, *nirK*, and *norC* genes, while *nosZ* gene was not discovered
228 in our study.

229

230 **Sulfur metabolism.**

231 Only Assimilatory sulfate reduction pathways presented in Woesearchaeotal genomes.
232 In these genomes, sulfate is reduced to APS (Adenylyl sulfate) firstly, then reduced to
233 PAPS (3'-Phosphoadenylyl sulfate). Afterwards, *cysH* gene encodes the enzyme
234 catalyzing PAPS to sulfite. Finally, either gene (*ars/cysJ* gene) encode enzyme to
235 reduce sulfite to sulfide. *Ars* and *cysJ* genes present in different Woesearchaeotal
236 genomes, encoding anaerobic sulfite reductase and sulfite reductase respectively.
237 Additionally, we also found other sulfur metabolism related genes, including *doxD*,
238 *TST*, *phsA*, *cysK*, and *cysE*.

239

240 **Arsenic metabolism.**

241 Interestingly, several genes involved in arsenic metabolism were discovered in some
242 Woesearchaeotal genomes for the first time, such as *arsC2* and *ACR3* genes. In these
243 genome, *arsC2* gene encodes arsenate reductase, which reduces arsenate to arsenite,
244 and then arsenite is pumped out of the cells by using the transporter encoded by *ACR3*.
245 Moreover, *AS3MT* and *arsC* genes also presented in some Woesearchaeotal genomes,
246 indicating arsenic metabolism capability.

247

248 **2.5 Metabolic capability of Woesearchaeota varies in differences environments**

249 Metabolic reconstruction for Woesearchaeota from different environments shows that
250 metabolic capability of Woesearchaeota differs in various environments (Fig. 6). As
251 for nitrogen metabolism, most Woesearchaeotal genomes contain predicted genes for
252 dissimilatory nitrate reduction and denitrification, however, no genes related to
253 nitrogen metabolism presents in the genomes of Woesearchaeota from soil.

254 Meanwhile, metabolic reconstruction shows all Woesearchaeota genomes contain
255 sulfur metabolism related pathways, comparative analysis for their metabolic
256 capability reveals that none of Woesearchaeota from black smokers have genes
257 encoding enzymes that catalyze assimilatory sulfate reduction.
258

259
260 **Fig. 6** Metabolic capabilities of Woesearchaeota from different environments. Red grid means the
261 gene was detected in corresponding genome, while blue grid indicates the absence of the gene.
262 Numbers on the left represent genome ID in Table 1.

264 3. Discussion

265 In this study, a comprehensive analysis greatly expanded the knowledge of
266 Woesearchaeotal diversity in aspects of their distribution, taxonomy and metabolism.
267 Distribution analysis based on 16S rRNA gene sequencing data suggested that
268 Woesearchaeota also have a wide distribution in host-associated environments,
269 especially in plant rhizosphere, which greatly expanded our understanding for
270 distribution diversity of Woesearchaeota. The investigation of environmental
271 parameters revealed that apart from elevation and depth of these samples,
272 host-associated environmental parameters also play an important part in the
273 distribution of Woesearchaeota. Besides, oxygen condition is of greatest importance
274 among the remaining environmental parameters, which is consistent with previous
275 analysis⁹. The results suggest that the location rather than the geochemical conditions

276 plays a major role on driving the distribution pattern of Woesearchaeota. Meanwhile,
277 it is important to note that only elevation and depth are consecutive data, while most
278 parameters are discrete data due to the limitation of current data. With accumulation
279 of more environmental parameters, future work may develop a full picture of the
280 distribution characteristic of Woesearchaeota.

281 Besides, exploration of the taxonomic characteristic revealed that Woesearchaeota
282 are of high taxonomic diversity, with 81 genomes assigned to 59 species. Taxonomic
283 study for Woesearchaeota expanded the phylogenetic diversity of Woesearchaeota by
284 adding up 19 new species of Woesearchaeota, which account for 32% of all
285 Woesearchaeotal species, indicating analyses for metagenomic data from various
286 environments can greatly deepen our understanding for Woesearchaeota, and the
287 discovery of new Woesearchaeotal species will promote further taxonomic study.
288 Moreover, pan-genome studies for Woesearchaeota show that the core genome of
289 Woesearchaeota is small. Considering comparative analysis was on the phylum level,
290 the great distance among all genomes may account for the relatively small core
291 genome. Highly diversity and small core genome of Woesearchaeotal implies that
292 Woesearchaeota have strong ability in speciation, further research focusing on this
293 ability may reveal evolution mechanism of Woesearchaeota, which is also known as
294 fast-evolving archaeal taxa.

295 Meanwhile, with the extended dataset, Woesearchaeota is confirmed as with small
296 genome sizes with limited metabolic capabilities, suggesting a host-dependent
297 lifestyle. Metabolic reconstruction combined with pan-genome analyses provided a
298 framework to explore the metabolic diversity of Woesearchaeota. Woesearchaeota
299 have a large open pan-genome, of which accessory genes and unique genes make up a
300 large proportion. And over 30% of these genes are assigned to “Metabolism”,
301 suggesting unique metabolic way in different Woesearchaeotal genomes. These genes
302 play an important role in the diversity and adaptability of Woesearchaeota. Further
303 investigation for Woesearchaeota from various environments shows that Metabolic

304 capability of Woesearchaeota differs in different environments. Although some
 305 archaea were reported to have a significant impact on nitrogen cycles in soils¹⁹, the
 306 only environment where Woesearchaeota have deficiency in nitrogen metabolism was
 307 soil. Meanwhile, Woesearchaeota from black smokers are unable to conduct
 308 assimilatory sulfate reduction. It is known that black smokers are rich in
 309 sulfur-bearing minerals, they emit particles such as H₂S and FeS, which provide
 310 microorganism with energy by oxidation²⁰. Since sulfur plays an important role in this
 311 environment, it is vital to investigate whether the lack of specific genes is influenced
 312 by the environment. Lateral gene transfer (LGT) is an important driving force in the
 313 evolution of microorganisms²¹⁻²⁴, thus, it could conceivably be hypothesized that
 314 Woesearchaeota gained these genes by LGT from the environments to help them
 315 adapt to the environments. And future researches should be undertaken for hypothesis
 316 testing.

317

318 **4. Conclusions**

319 In summary, we performed a comprehensive study to investigate the distribution,
 320 taxonomy, and metabolism of Woesearchaeota. The distribution pattern suggested that
 321 Woesearchaeota are widely distributed in various biotopes, including host-associated
 322 environments. Then, 33 high-quality Woesearchaeotal genomes were reconstructed
 323 from metagenomic data, greatly expanded taxonomic group of Woesearchaeota. And
 324 these genomes are collected for further taxonomic study and revealed that
 325 Woesearchaeota are of high taxonomic diversity. Meanwhile, Comparative genomic
 326 analysis showed that Woesearchaeota have a large open pan-genome with small core
 327 genome. Metabolic reconstruction for Woesearchaeota implied their metabolic
 328 potential for Nitrogen, Sulfur, and Arsenic. Moreover, metabolic capacity of
 329 Woesearchaeota varies in different environments, suggested that they are of high
 330 metabolic diversity. This study greatly expanded our knowledge for distribution,
 331 taxonomy, and metabolism of Woesearchaeota. And it demonstrated great diversity of
 332 this archaeal phylum in different aspects. However, due to the limitation of current

data, our knowledge about the driving force of metabolic diversity in Woesearchaeota is limited. Thus, future researches are encouraged to explore this problem.

5. Data and methods

5.1 Data collection

16S rRNA gene sequencing data.

8,023,841 represented Operational taxonomic unit (OTU) sequences from 27,709 samples were collected from EMP^{11, 12}. Meanwhile, OTU composition and environmental parameters for all samples were gathered.

Woesearchaeotal metagenome-assembled genomes.

All metagenome-assembled genomes (MAGs) of Woesearchaeota from public database and previous studies were collected. These genomes were retrieved (by 8th October, 2019) from NCBI Assembly database (<https://www.ncbi.nlm.nih.gov/assembly>) matching the query string “(“Candidatus Woesearchaeota”[Organism] OR woesearchaeota[All Fields]) AND latest[filter]”. Furthermore, Woesearchaeotal genomes from prior studies were also gathered^{6, 9}. Then a custom perl script was used for de-duplication, and CheckM (version 1.0.13) was used to estimate the quality of these genomes. Only high-quality (completeness>70% and contamination<5%) genomes were used for further investigation.

Metagenomic WGS data.

A total of ~35 terabyte metagenomic data were collected for metagenomic study, including samples from rhizosphere, sediment, and water. Based on our distribution exploration, among all host-associated environments, Woesearchaeota are most widely distributed in rhizosphere. Thus, metadata for metagenomes of 102 rhizosphere samples collected from NCBI-SRA (<https://trace.ncbi.nlm.nih.gov/Traces/sra/>). Meanwhile, only few genomes have been

reconstructed from sediment samples in public database, thus we used in-house marine sediments samples, including samples of black smoker and marine sediments (see **Data availability** for detail). Moreover, *Tara* Oceans Project provides a systematic sampling data for marine microbe^{13, 25}, and 1,158 water samples were collected from this project.

367

368 **5.2 Distribution characteristic exploration**

Taxonomic classification was conducted using BLAST+, all OTU sequences were BLASTed against SILVA SSU128^{26, 27}. For each OTU sequence, we filtered results with percent identity less than 0.80, and the OTU sequence will be annotated as Woesearchaeota while over 51% of the filtered results belonging to Woesearchaeota²⁸. Quality control for all samples (kept samples marked as qc_filtered == "TRUE" in EMP && counts>10,000 && no missing parameters) with OTU composition analysis identified samples containing Woesearchaeota (relative abundance of Woesearchaeotal OTU > 0.01%).

377

378 **Environmental parameter analysis.**

We used Random Forest¹⁵ to investigate the impact of environmental parameters on the distribution of Woesearchaeota. First, a label is assigned to each sample based on existence/absence of Woesearchaeota. Second, by utilizing the sample environment factors, such as altitude, depth, oxic/anoxic, salinity, etc., a feature vector containing all environmental parameters is generated for each sample. Third, a Random forest classifier is trained and 10-fold cross validation was implied for evaluation (R package 'randomForest'). Last, Gini importance index are calculated to estimate feature importance²⁹.

387

388 **5.3 Genome reconstruction from metagenomic data**

For all those metagenomic data, raw sequencing reads are trimmed by using

390 Trimmomatic (version 0.38; SLIDINGWINDOW:10:20 MINLEN:50)³⁰. Then
 391 trimmed short reads were assembled to long contigs using MEGAHIT (version 1.1.3)
 392 with default parameters³¹. Qualified reads were then mapped to contigs using BamM
 393 (version 1.7.3; <http://ecogenomics.github.io/BamM/>) to calculate the coverage
 394 information. Afterwards, genome binning was performed on contigs by MetaBAT2
 395 (version 2.12.1) with default parameters³², and the minimum size of a contig for
 396 binning is 2,500. CheckM (version 1.0.13) was used to estimate the completeness and
 397 contamination of all bins³³.

398

399 **5.4 Taxonomy analysis**

400 For phylogenetic analysis, representative archaeal reference genomes were
 401 downloaded from NCBI Assembly database. Then, CheckM was used to estimate the
 402 quality of these genomes, and high-quality reference genomes were collected.
 403 Meanwhile, target high-quality genome bins (Marker lineage annotated as
 404 “k__Archaea (UID2)” in CheckM) reconstructed from metagenomic data were also
 405 used for phylogenetic analysis. Gene prediction was performed using Prodigal
 406 (version 2.6.3) with “-p meta”³⁴. HMM models were used to identify 16 ribosomal
 407 proteins (L2, L3, L4, L5, L6, L14, L15, L16, L18, L22, L24, S3, S8, S10, S17, S19)
 408 from all archaeal genomes using HMMER (version 3.1b2) with “hmmsearch -E
 409 1E-5”^{6, 35, 36}. Genomes with less than 8 ribosomal proteins were not included in the
 410 analyses. Then, individual proteins were aligned with MUSCLE(version 3.8.3.1)³⁷,
 411 trimmed using trimAL (version 2.0) with “-automated1”³⁸. Maximum-Likelihood
 412 phylogeny of 16 concatenated proteins using both fasttree(version 2.1.0; -lg -gamma)
 413 and IQ-TREE (version 1.6.12; -st AA -m MFP -bb 1000 -nt 16)^{39, 40}.

414 Woesearchaeotal genomes reconstructed from metagenomic data combined with
 415 reference genomes belong to Woesearchaeota were collected for taxonomic
 416 identification(Supplementary Fig.3). OrthoANI value were calculated pairwise by
 417 using OrthoANI tool (<https://www.ezbiocloud.net/tools/orthoani>)¹⁸.

418

419 **5.5 Pan-genome profiling**

420 Taken the reduced genomes of Woesearchaeota in consideration, quality estimation
 421 for all Woesearchaeotal genomes were conducted using CheckM with a refined
 422 marker set of Archaea. And those nearly complete genomes (completeness>95%,
 423 contamination <5%) were used for comparative genomics analysis. Protein sequences
 424 for each genome were predicted using Prodigal³⁴. USEARCH was used for
 425 orthologous clustering with 50% sequence identity taken as cut-off value. And the
 426 power-law regression model and exponential curve fit model were used to calculate
 427 the pan-genome size and core genome size, respectively. Then, we analyzed the
 428 distribution of core gene, accessory gene and unique gene in each Woesearchaeotal
 429 genome. In addition, function annotation for each orthologous protein cluster is based
 430 on protein BLAST against reference COG (Clusters of Orthologous Groups of
 431 proteins) and KEGG databases^{41, 42}. Protein clustering, pan-genome profile analysis,
 432 and function and pathway analysis are conducted using BPGA-pipeline⁴³
 433 (<https://iicb.res.in/bpga/downloads.html>).

434

435 **5.6 Metabolic prediction**

436 Nearly complete genomes of Woesearchaeota were collected to perform metabolic
 437 reconstruction. Prodigal was used to predict open reading frames (ORFs) from these
 438 genome bins. The ORFs were annotated by using eggno-mapper(v2)^{44, 45}, and
 439 resulting data contained Gene Ontology (GO) terms, KEGG Orthology (KO) and
 440 archaeal clusters of orthologous genes (arCOGs). KEGG metabolic pathways was
 441 reconstructed for each genome by using KO with KEGG mapper tool⁴². To infer
 442 metabolic capacities of Woesearchaeota from different environments, environmental
 443 factors are combined for a comparative analysis.

444

445 **Data availability**

446 Woese archaeotal high-quality genomes reconstructed in this study have been
447 deposited at NODE (<https://www.biosino.org/node/>) under accessions OEP000995.
448 Besides, other high-quality genomes reconstructed from *Tara* Oceans Project and
449 rhizosphere samples have been deposited at NODE under accessions OEP000994 and
450 accessions OEP000996, respectively.

451 Moreover, in-house metagenomic data used in this study have been deposited at
452 NODE under the project ID OEP000957, and the experiment ID are
453 OEX003653~OEX003658. These data are available under from the corresponding
454 author on reasonable request.

455 Meanwhile, metagenomic data from *Tara* Oceans Project used in this study are
456 under project ID PRJEB1787, PRJEB1788, PRJEB4352, PRJEB4419. Besides,
457 accession numbers of rhizosphere metagenomic data are provided in Supplementary
458 Table 2. And EMP data is available on <https://earthmicrobiome.org/>.

459

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466

467 **Competing interests**

468 The authors declare no competing interests.

469

470 **Author contribution statement**

471 RXZ and YZ conceived and designed the project. Each author has contributed
472 significantly to the submitted work. JX and YZ drafted the manuscript. WNC, YBX,
473 RZ, LWT, YQW, YDZ, XX and RXZ revised the manuscript. All authors read and

474 approved the final manuscript.

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476 **References**

- 477 1. Castelle, C.J. & Banfield, J.F. Major New Microbial Groups Expand Diversity
478 and Alter our Understanding of the Tree of Life. *Cell* **172**, 1181-1197 (2018).
- 479 2. Spang, A., Caceres, E.F. & Ettema, T.J.G. Genomic exploration of the
480 diversity, ecology, and evolution of the archaeal domain of life. *Science (New*
481 *York, N.Y.)* **357** (2017).
- 482 3. Xiao, J. et al. Archaea, the tree of life, and cellular evolution in eukaryotes.
483 *Science China Earth Sciences* **62**, 489-506 (2019).
- 484 4. Fan, L. et al. Phylogenetic analyses with systematic taxon sampling show that
485 mitochondria branch within Alphaproteobacteria. *Nature ecology & evolution*
486 (2020).
- 487 5. Baker, B.J. et al. Diversity, ecology and evolution of Archaea. *Nature*
488 *microbiology* **5**, 887-900 (2020).
- 489 6. Castelle, C.J. et al. Genomic expansion of domain archaea highlights roles for
490 organisms from new phyla in anaerobic carbon cycling. *Current biology* **25**,
491 690-701 (2015).
- 492 7. Dombrowski, N., Lee, J.H., Williams, T.A., Offre, P. & Spang, A. Genomic
493 diversity, lifestyles and evolutionary origins of DPANN archaea. *FEMS*
494 *microbiology letters* **366** (2019).
- 495 8. Rinke, C. et al. Insights into the phylogeny and coding potential of microbial
496 dark matter. *Nature* **499**, 431-437 (2013).
- 497 9. Liu, X. et al. Insights into the ecology, evolution, and metabolism of the
498 widespread Woese archaeal lineages. *Microbiome* **6**, 102 (2018).
- 499 10. Koskinen, K. et al. First Insights into the Diverse Human Archaeome: Specific
500 Detection of Archaea in the Gastrointestinal Tract, Lung, and Nose and on
501 Skin. *mBio* **8**, e00824-00817 (2017).
- 502 11. Gilbert, J.A., Jansson, J.K. & Knight, R. The Earth Microbiome project:
503 successes and aspirations. *BMC Biol* **12**, 69 (2014).
- 504 12. Thompson, L.R. et al. A communal catalogue reveals Earth's multiscale
505 microbial diversity. *Nature* **551**, 457-463 (2017).
- 506 13. Zhang, H. & Ning, K. The Tara Oceans Project: New Opportunities and
507 Greater Challenges Ahead. *Genomics, proteomics & bioinformatics* **13**,
508 275-277 (2015).
- 509 14. Parks, D.H. et al. Recovery of nearly 8,000 metagenome-assembled genomes
510 substantially expands the tree of life. *Nature microbiology* **2**, 1533-1542
511 (2017).
- 512 15. Breiman, L. Random forests. *Machine learning* **45**, 5-32 (2001).
- 513 16. Goris, J. et al. DNA-DNA hybridization values and their relationship to
514 whole-genome sequence similarities. *Int J Syst Evol Microbiol* **57**, 81-91

(2007).

17. Teske, A. & Sorensen, K.B. Uncultured archaea in deep marine subsurface sediments: have we caught them all? *The ISME journal* **2**, 3-18 (2008).
18. Lee, I., Ouk Kim, Y., Park, S.-C. & Chun, J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *International Journal of Systematic and Evolutionary Microbiology* **66**, 1100-1103 (2016).
19. Xie, W. et al. The response of archaeal species to seasonal variables in a subtropical aerated soil: insight into the low abundant methanogens. *Applied microbiology and biotechnology* **101**, 6505-6515 (2017).
20. Kato, S. et al. Metabolic Potential of As-yet-uncultured Archaeal Lineages of Candidatus Hydrothermarchaeota Thriving in Deep-sea Metal Sulfide Deposits. *Microbes Environ* **34**, 293-303 (2019).
21. Zhaxybayeva, O. & Doolittle, W.F. Lateral gene transfer. *Current Biology* **21**, R242-R246 (2011).
22. Popa, O. & Dagan, T. Trends and barriers to lateral gene transfer in prokaryotes. *Current opinion in microbiology* **14**, 615-623 (2011).
23. Jain, R., Rivera, M.C. & Lake, J.A. Horizontal gene transfer among genomes: the complexity hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3801-3806 (1999).
24. Daubin, V. & Szollosi, G.J. Horizontal Gene Transfer and the History of Life. *Cold Spring Harb Perspect Biol* **8**, a018036 (2016).
25. Sunagawa, S. et al. Tara Oceans: towards global ocean ecosystems biology. *Nature reviews. Microbiology* (2020).
26. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. Basic local alignment search tool. *Journal of molecular biology* **215**, 403-410 (1990).
27. Glockner, F.O. et al. 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J Biotechnol* **261**, 169-176 (2017).
28. Bokulich, N.A. et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2 's q2-feature-classifier plugin. *Microbiome* **6**, 90 (2018).
29. Qi, Y. in Ensemble machine learning 307-323 (Springer, 2012).
30. Bolger, A.M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120 (2014).
31. Li, D., Liu, C.M., Luo, R., Sadakane, K. & Lam, T.W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **31**, 1674-1676 (2015).
32. Kang, D.D. et al. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* **7**, e7359 (2019).
33. Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. & Tyson, G.W. CheckM: assessing the quality of microbial genomes recovered from isolates,

single cells, and metagenomes. *Genome research* **25**, 1043-1055 (2015).

34. Hyatt, D. et al. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC bioinformatics* **11**, 119 (2010).

35. Johnson, L.S., Eddy, S.R. & Portugaly, E. Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC bioinformatics* **11**, 431 (2010).

36. Hug, L.A. et al. A new view of the tree of life. *Nature microbiology* **1**, 16048 (2016).

37. Edgar, R.C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* **32**, 1792-1797 (2004).

38. Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972-1973 (2009).

39. Price, M.N., Dehal, P.S. & Arkin, A.P. FastTree 2--approximately maximum-likelihood trees for large alignments. *PloS one* **5**, e9490 (2010).

40. Nguyen, L.-T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution* **32**, 268-274 (2015).

41. Tatusov, R.L., Galperin, M.Y., Natale, D.A. & Koonin, E.V. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* **28**, 33-36 (2000).

42. Kanehisa, M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research* **28**, 27-30 (2000).

43. Chaudhari, N.M., Gupta, V.K. & Dutta, C. BPGA- an ultra-fast pan-genome analysis pipeline. *Scientific reports* **6**, 24373 (2016).

44. Huerta-Cepas, J. et al. Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Molecular biology and evolution* **34**, 2115-2122 (2017).

45. Huerta-Cepas, J. et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res* **47**, D309-D314 (2019).