

Supplementary Materials for

Phylogenetic and ecophysiological novelty of subsurface mercury
methylators in mangrove sediments

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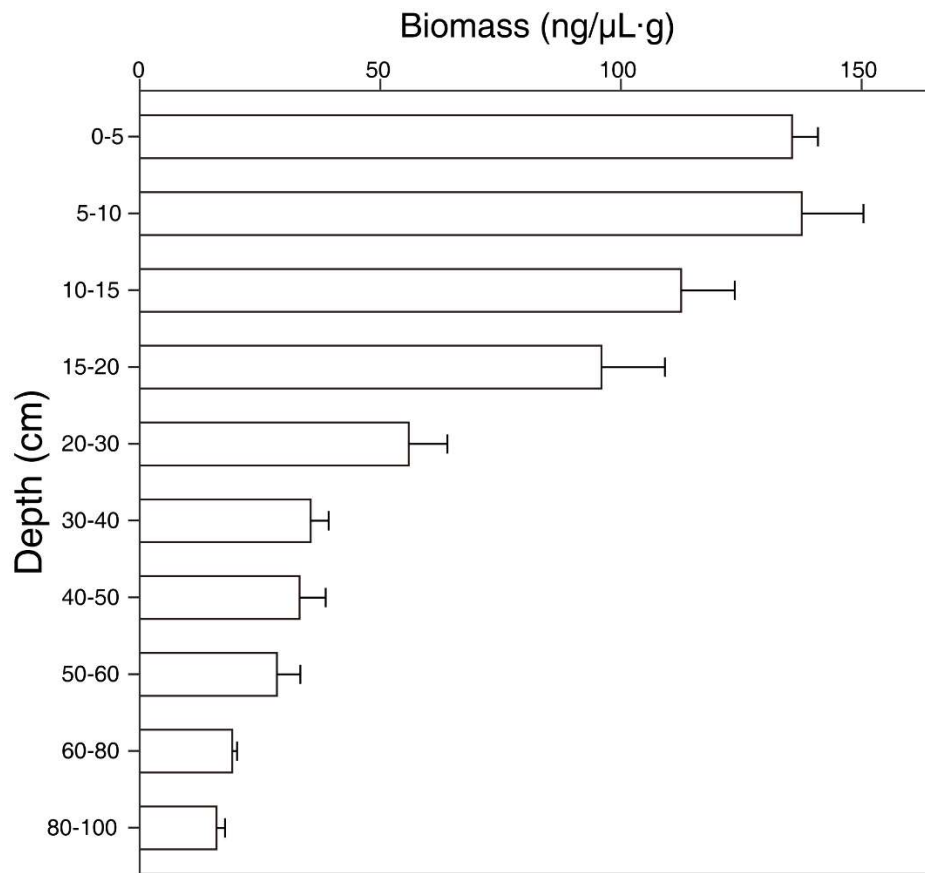


Fig. S1. Vertical profiles of microbial biomass across the mangrove sediment column.

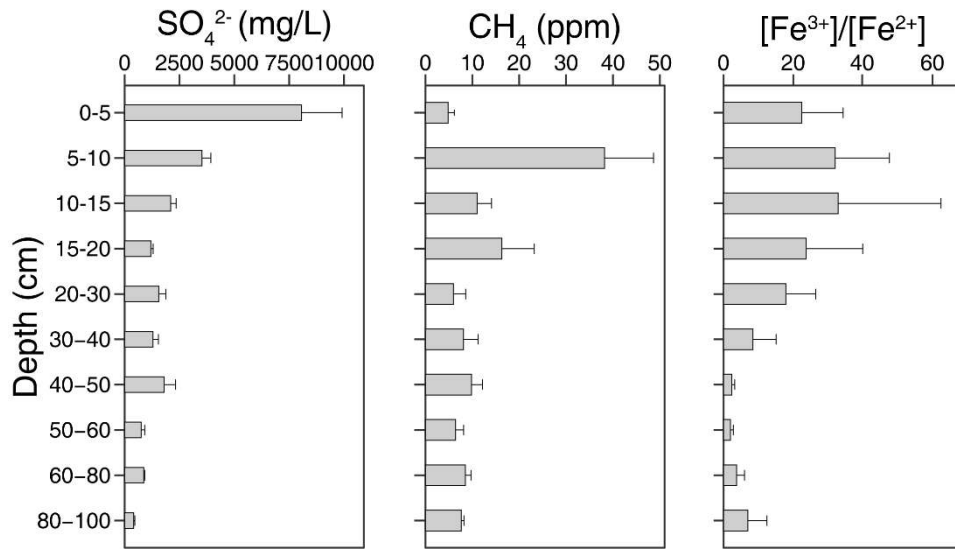


Fig. S2. Geochemical profiles in sampled mangrove sediments at discrete depths.

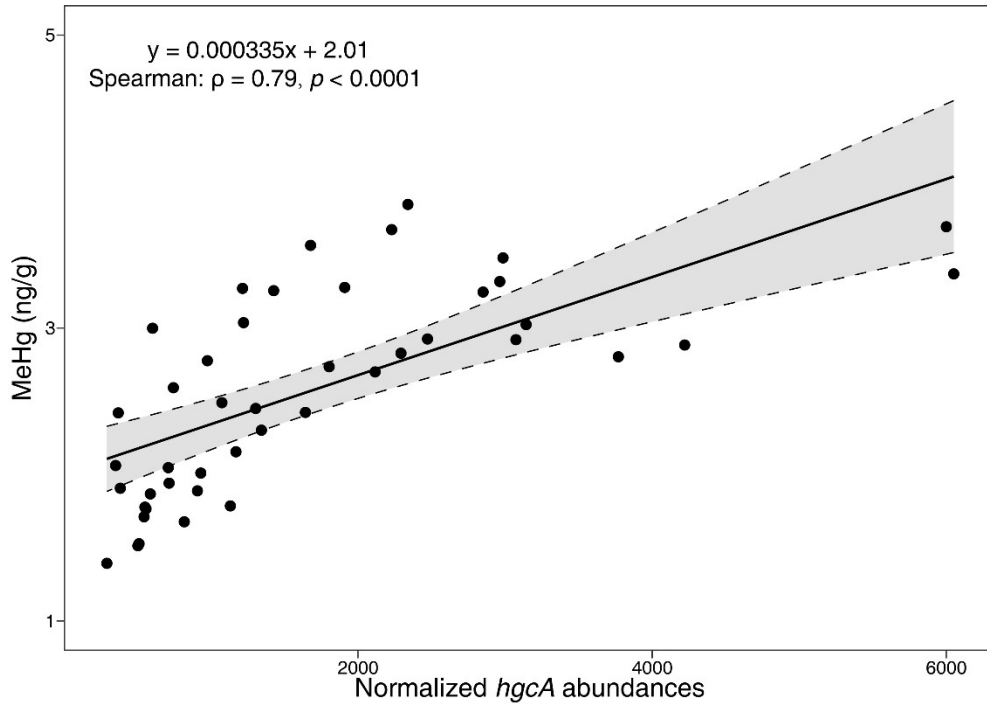


Fig. S3. Significant Spearman correlation between the normalized abundances of *hgcA* and the MeHg concentration across the mangrove sediment column. The gray shadow indicates the 95% confidence interval.

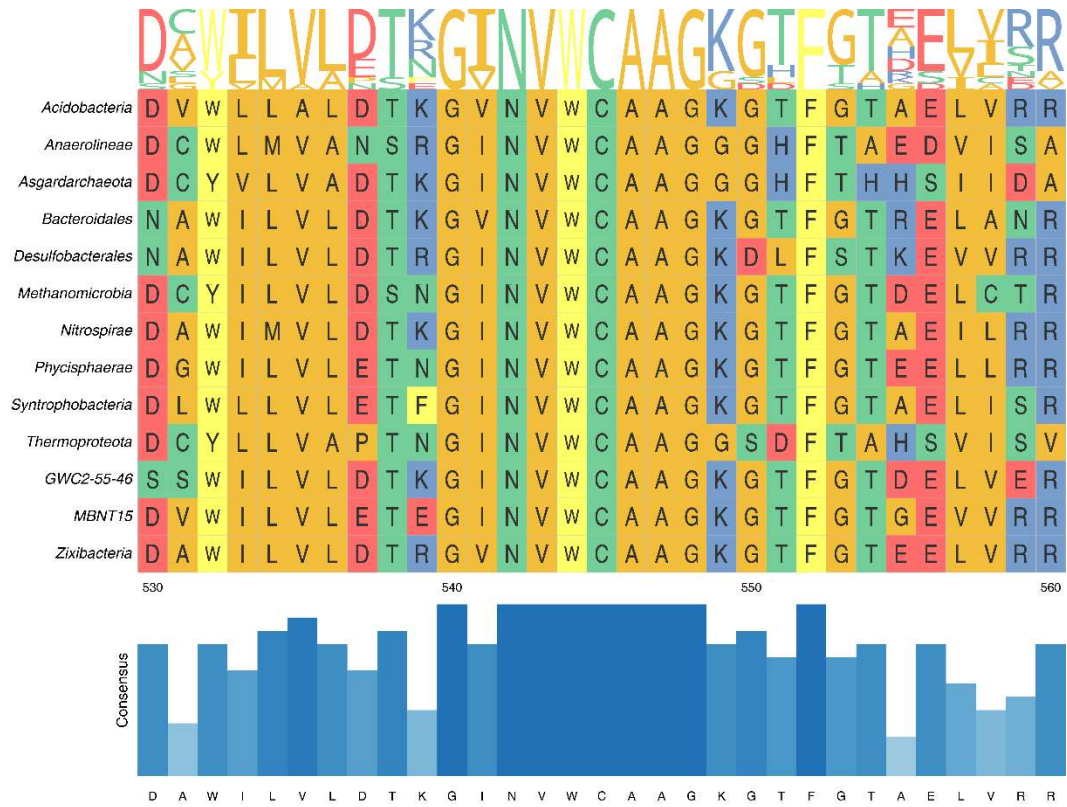


Fig. S4. Alignments of identified HgcA protein sequences from all Hg methylator taxa recovered from our sampled mangrove sediments.

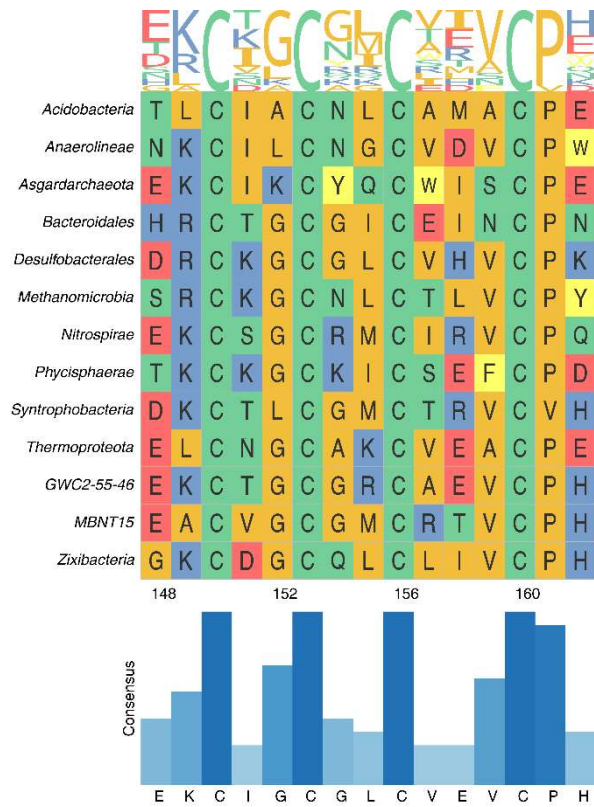


Fig. S5. Alignments of identified HgcB protein sequences from all Hg methylator taxa recovered from our sampled mangrove sediments.

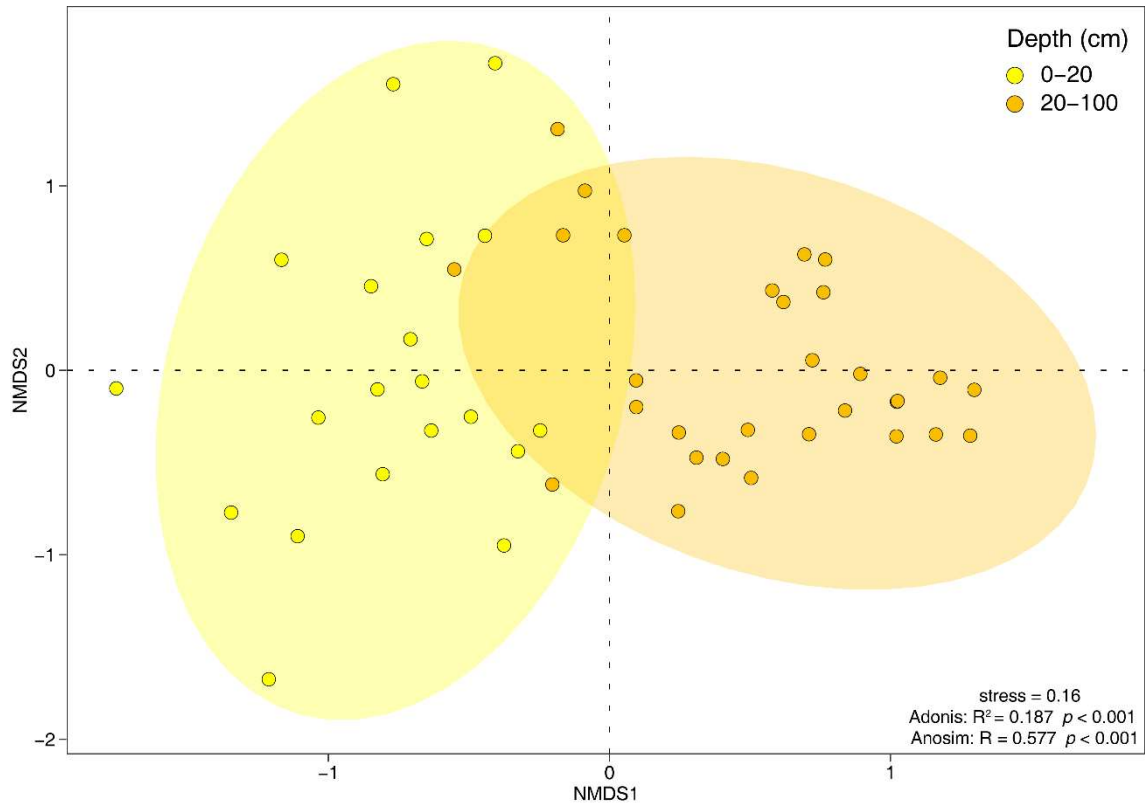


Fig. S6. NMDS ordinations representing the Bray–Curtis distances between Hg methylators across different depths (stress = 0.17).

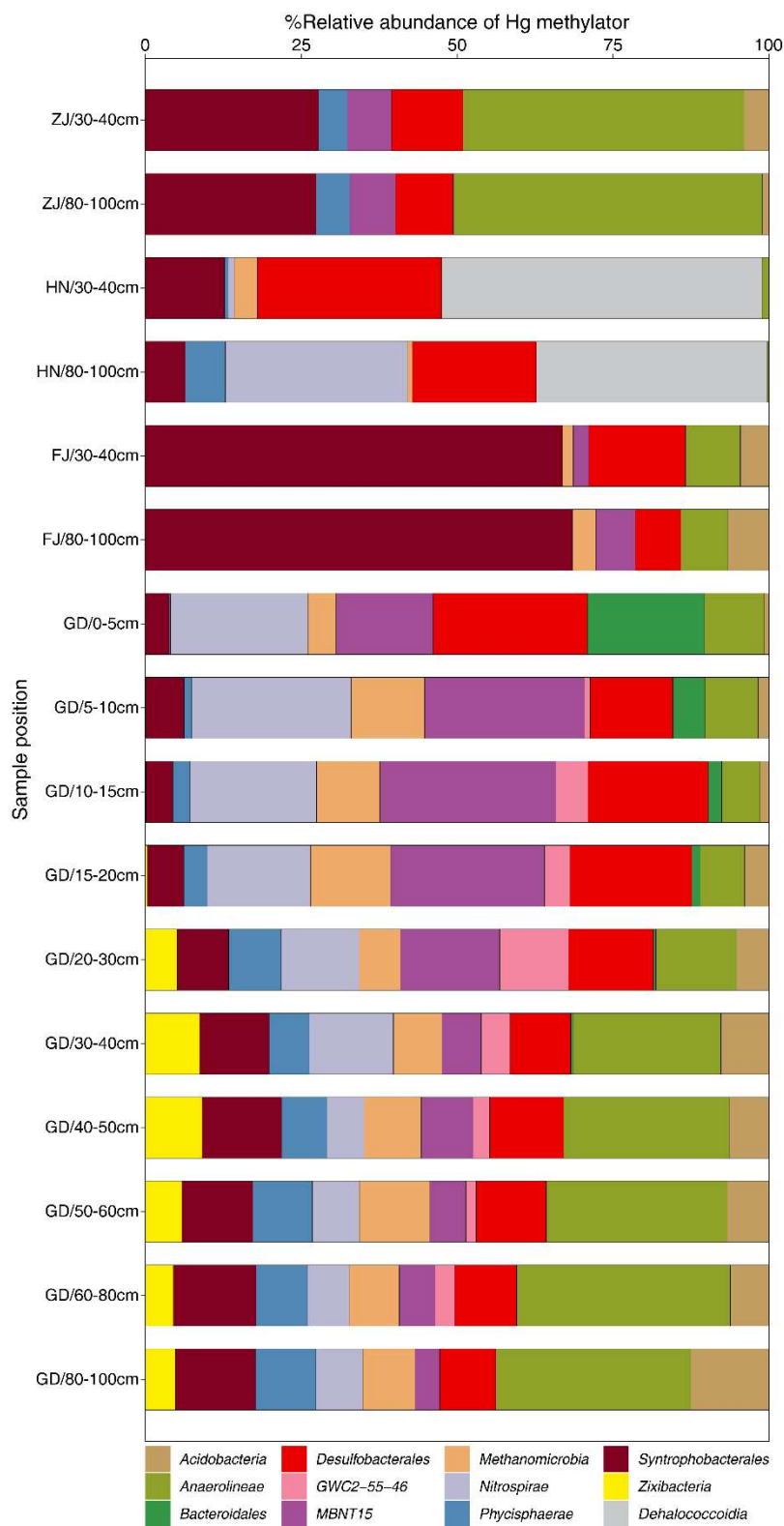


Fig. S7. Taxonomic profile of Hg methylator MAGs in the metagenomic data at four sampling mangrove sediments from Zhejiang, Hainan, Fujian, and Guangdong. Abbreviation: ZJ, Zhejiang; HN, Hainan; FJ, Fujian; GD, Guangdong. The abundance of Hg methylator MAGs is calculated by CoverM.

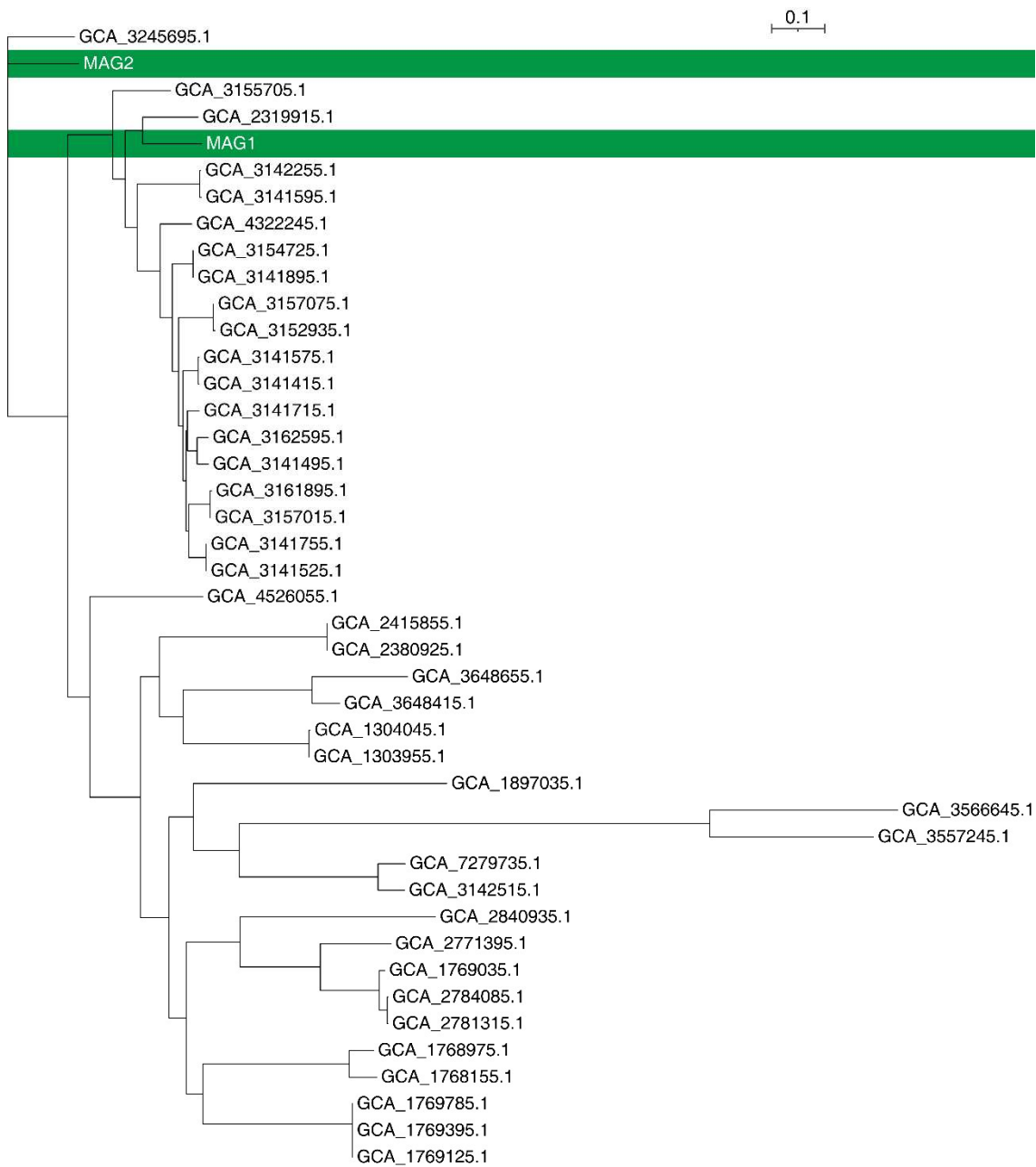


Fig. S8. Maximum likelihood tree (IQ-TREE 2, Q. yeast + F + R5) of specific marker proteins in *Bacteroidales* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in parentheses.

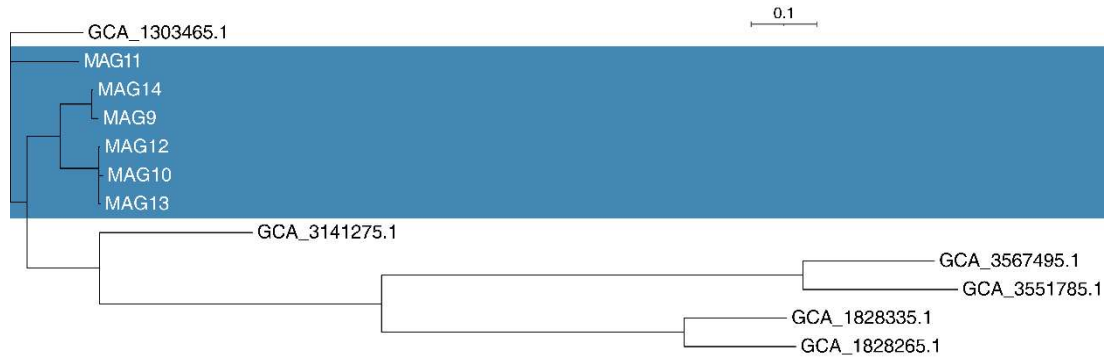


Fig. S9. Maximum likelihood tree (IQ-TREE 2, LG + G4) of specific marker proteins in *Phycisphaerae* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in parentheses.

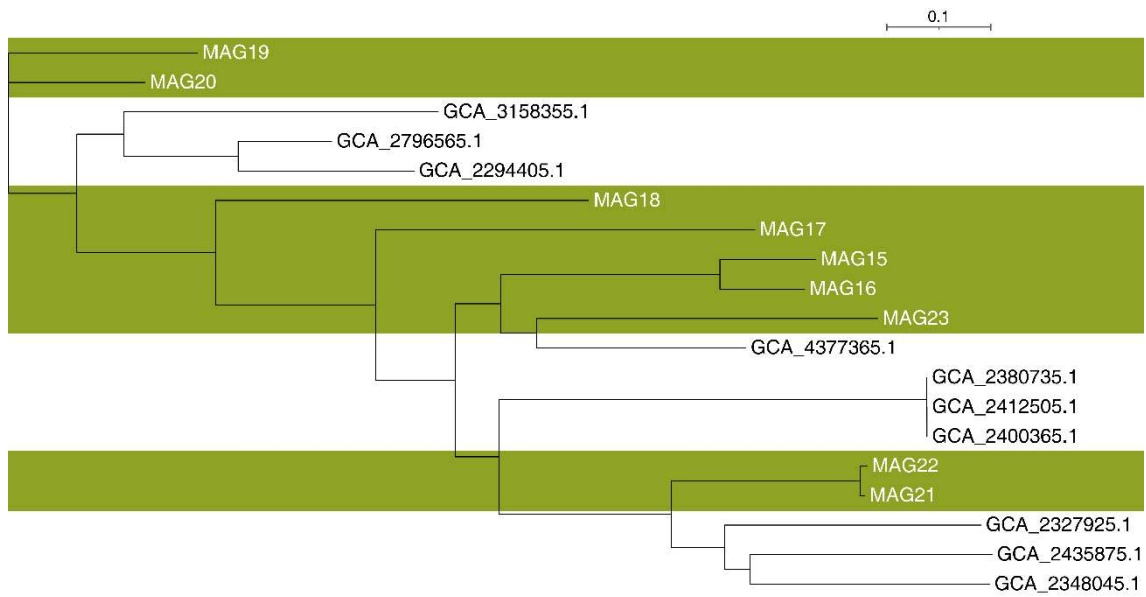


Fig. S10. Maximum likelihood tree (IQ-TREE 2, LG + F + I + G4) of specific marker proteins in *Anaerolineae* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in paratheses.

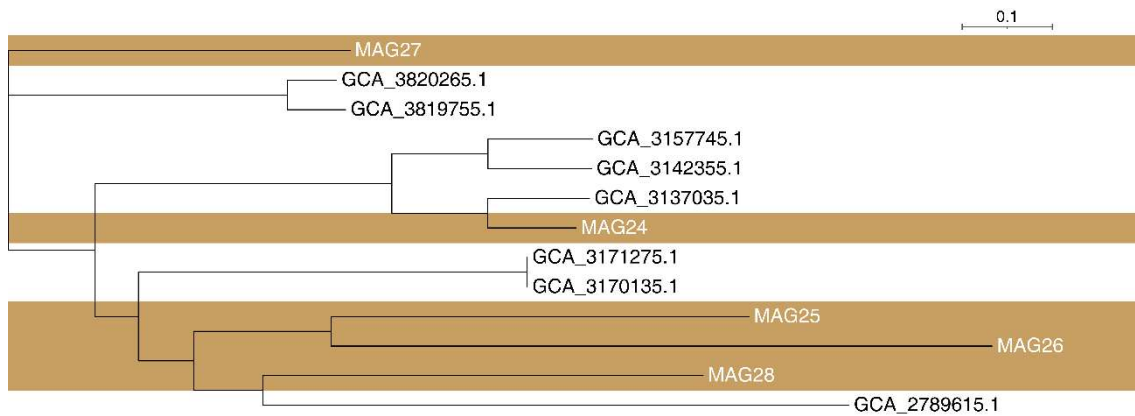


Fig. S11. Maximum likelihood tree (IQ-TREE 2, LG + F + G4) of specific marker proteins in *Acidobacteria* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in paratheses.

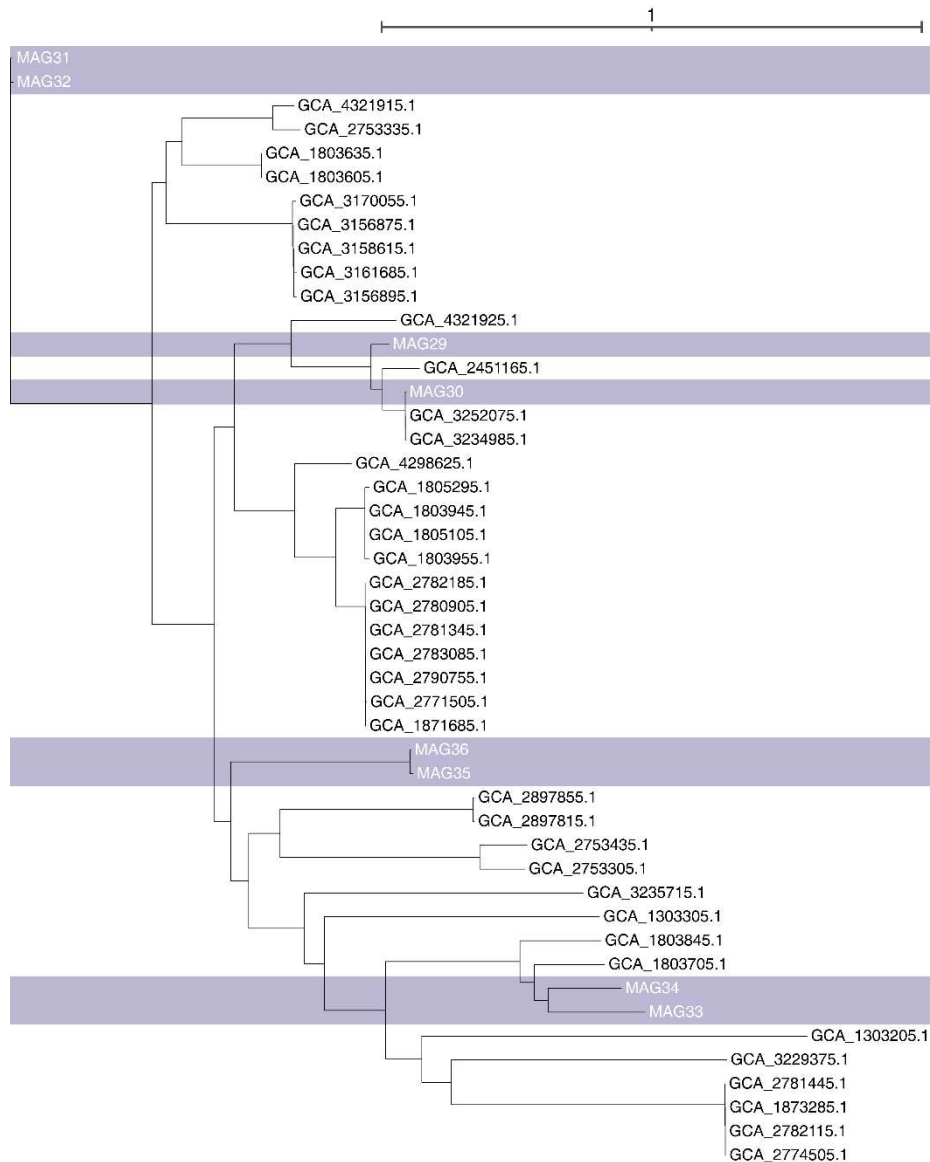


Fig. S12. Maximum likelihood tree (IQ-TREE 2, Q. yeast + F + I + I + R4) of specific marker proteins in *Nitrospirae* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in paratheses.

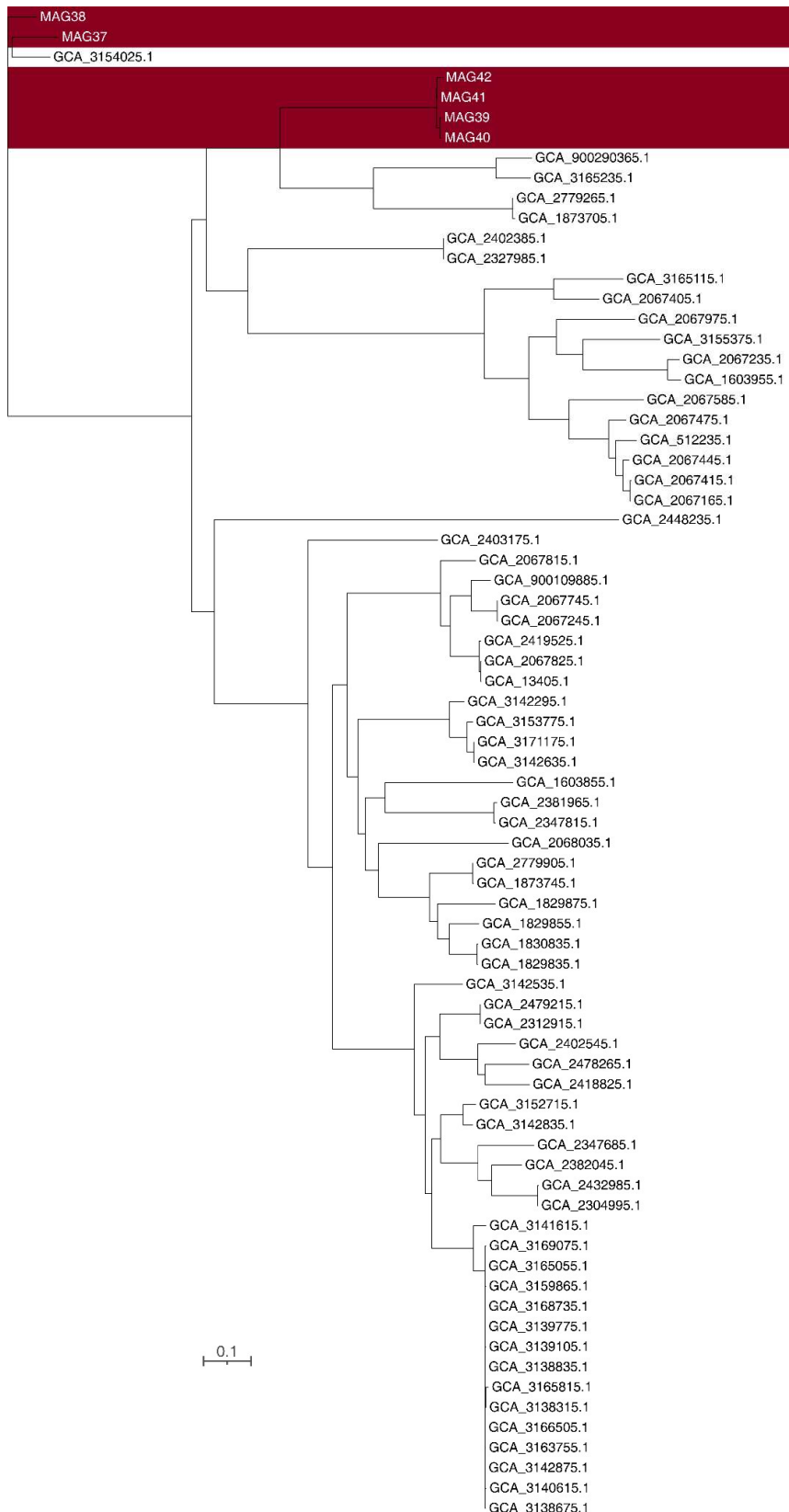


Fig. S13. Maximum likelihood tree (IQ-TREE 2, Q. yeast + F + I + I + R5) of specific marker proteins in *Syntrophobacterales* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in parentheses.

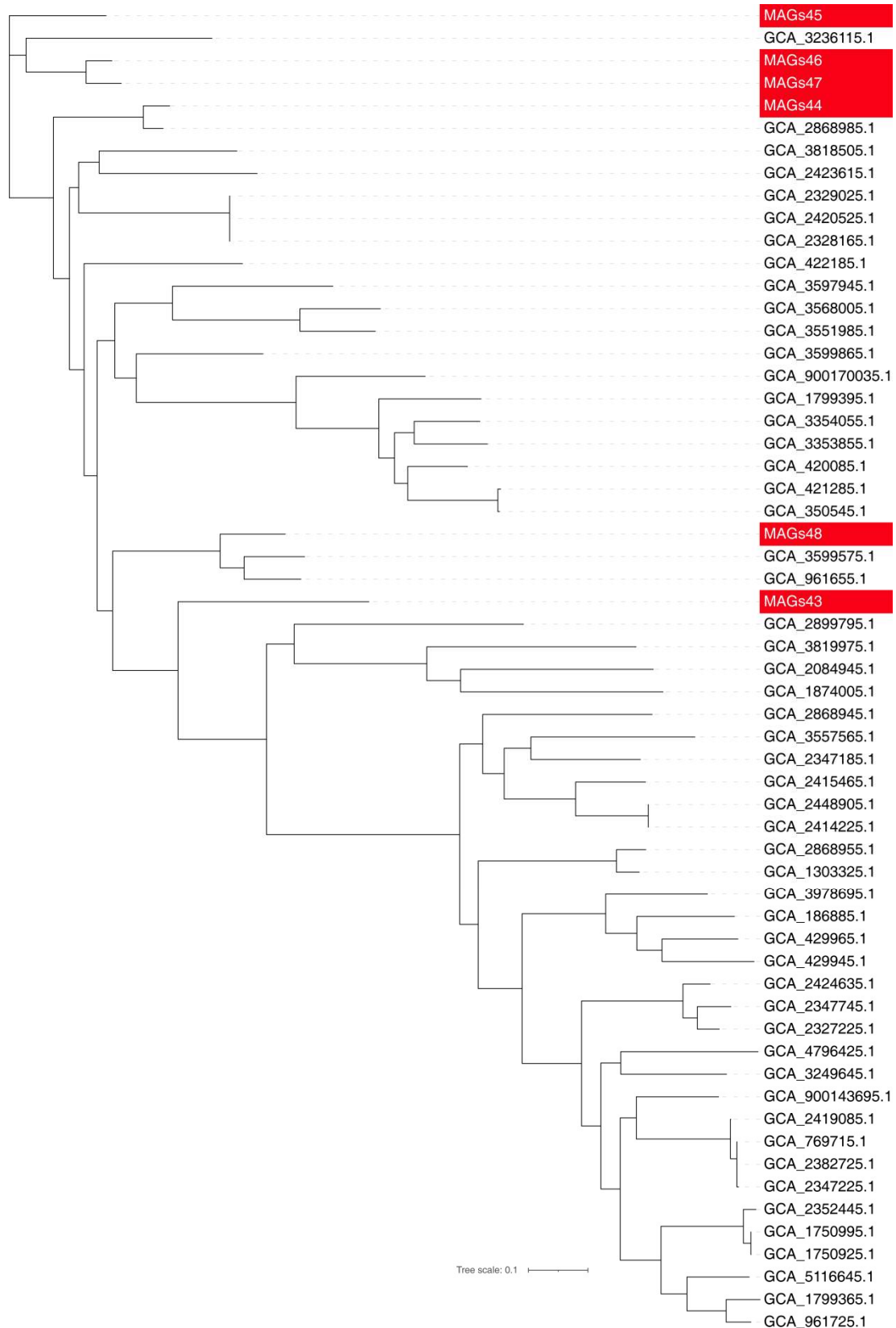


Fig. S14. Maximum likelihood tree (IQ-TREE 2, GTR20+F+R5) of specific marker proteins in *Desulfobacterales* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in paratheses.

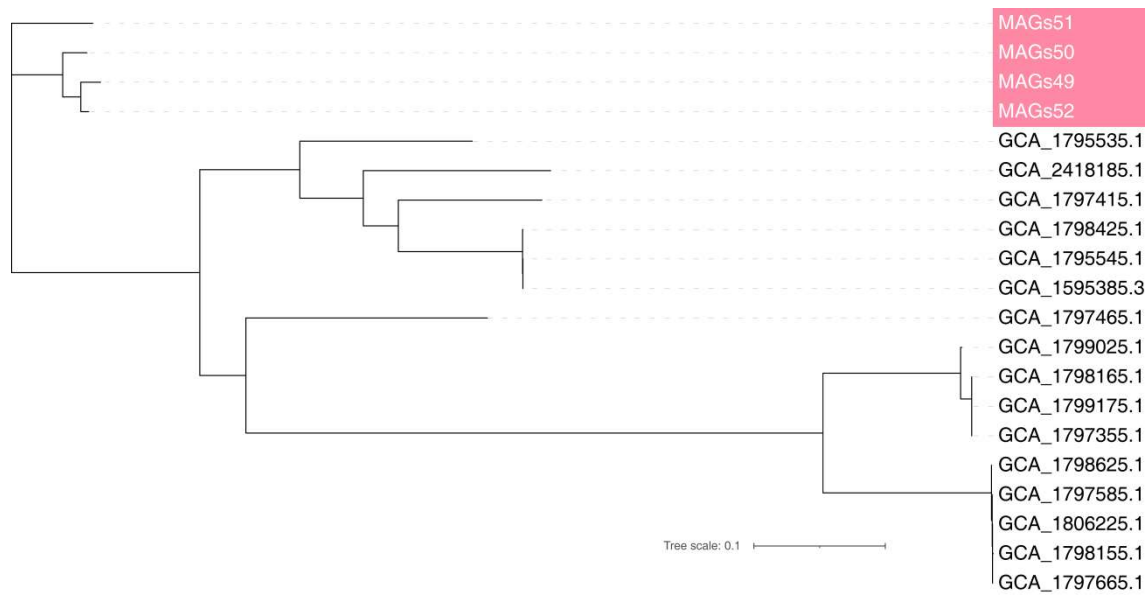


Fig. S15. Maximum likelihood tree (IQ-TREE 2, Q.insect+F+R3) of specific marker proteins in *GWC2-55-46* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in paratheses.

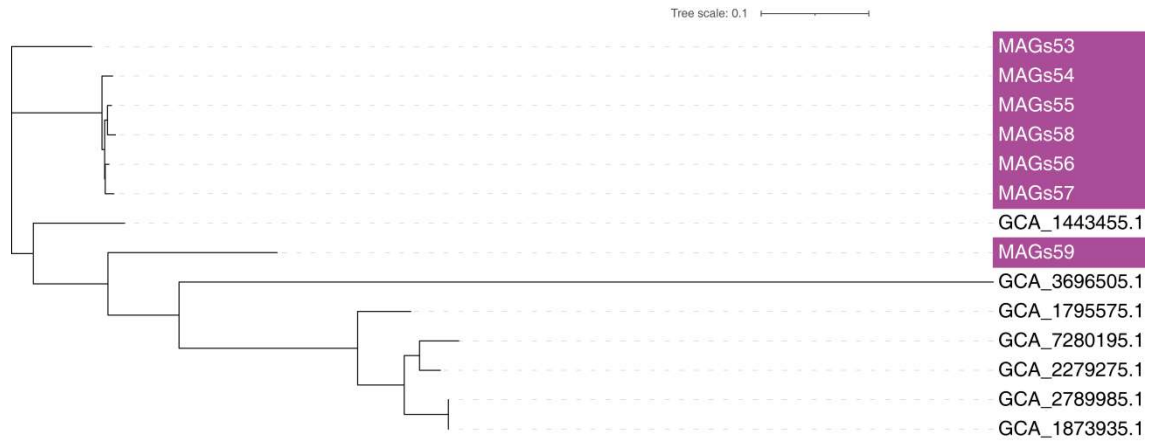


Fig. S16. Maximum likelihood tree (IQ-TREE 2, Q.insect+F+G4) of specific marker proteins in *MBNT15* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in parentheses.

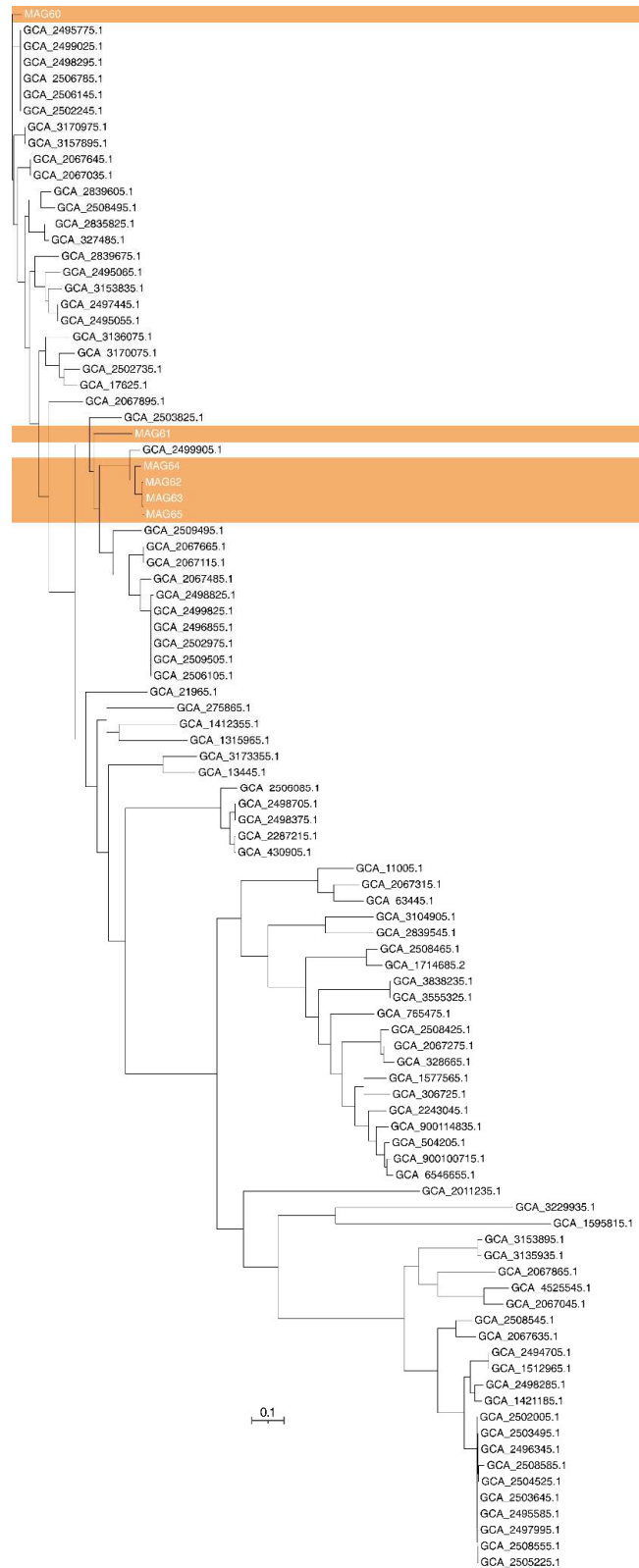


Fig. S17. Maximum likelihood tree (IQ-TREE 2, Q. insect + F + I + I + R2) of specific marker proteins in *Methanomicrobia* from *hgcA*-carrying MAGs and reference genomes from Hg-MATE-Db [1]. Names in white correspond to MAGs identified in this study. Genome names in black were pulled from Hg-MATE-Db and have the NCBI accession ID in parentheses.

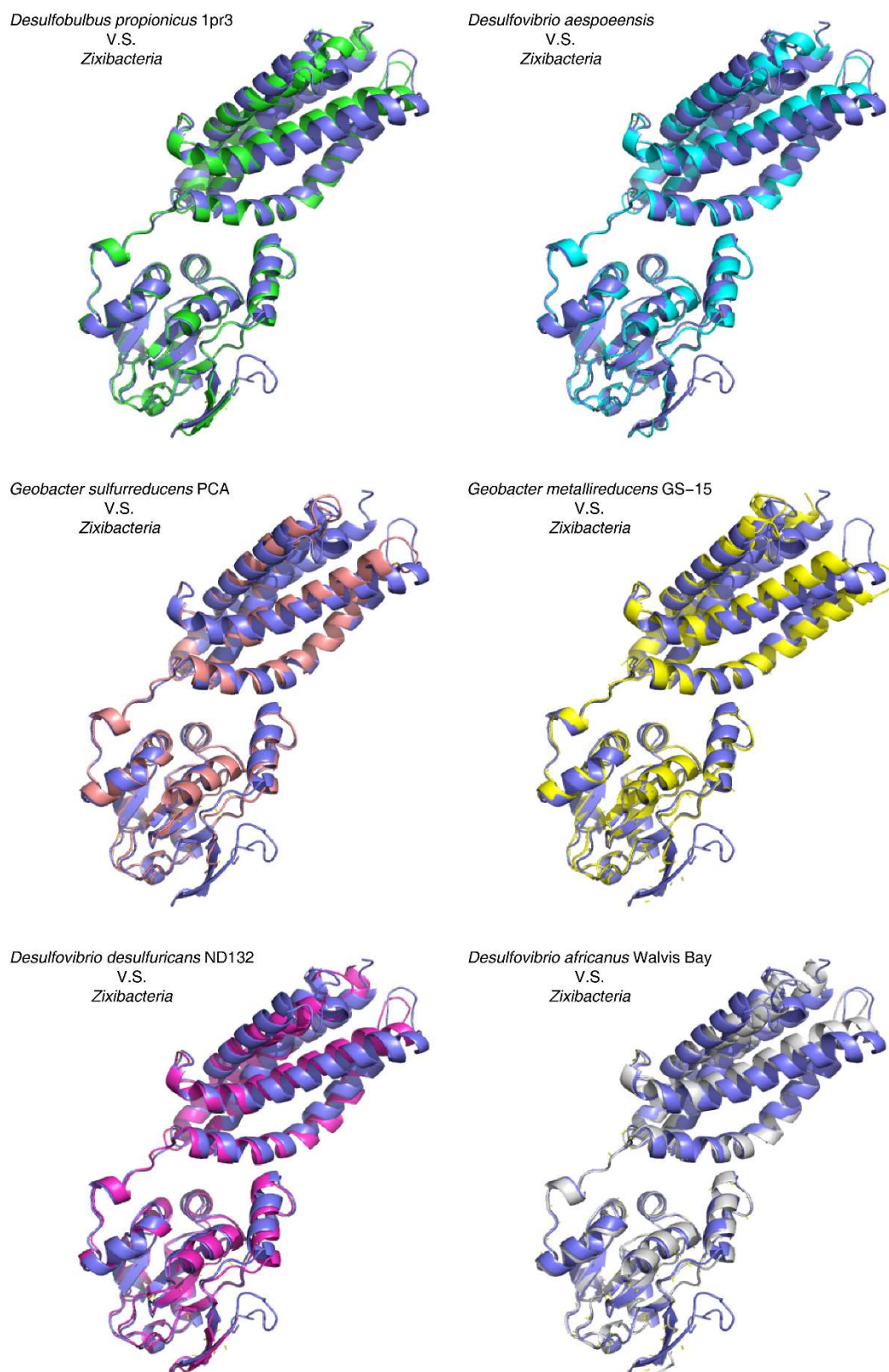


Fig. S18. Alignments between HgcA AlphaFold2 models of *Zixibacteria* and six confirmed Hg-methylating bacterial strains.

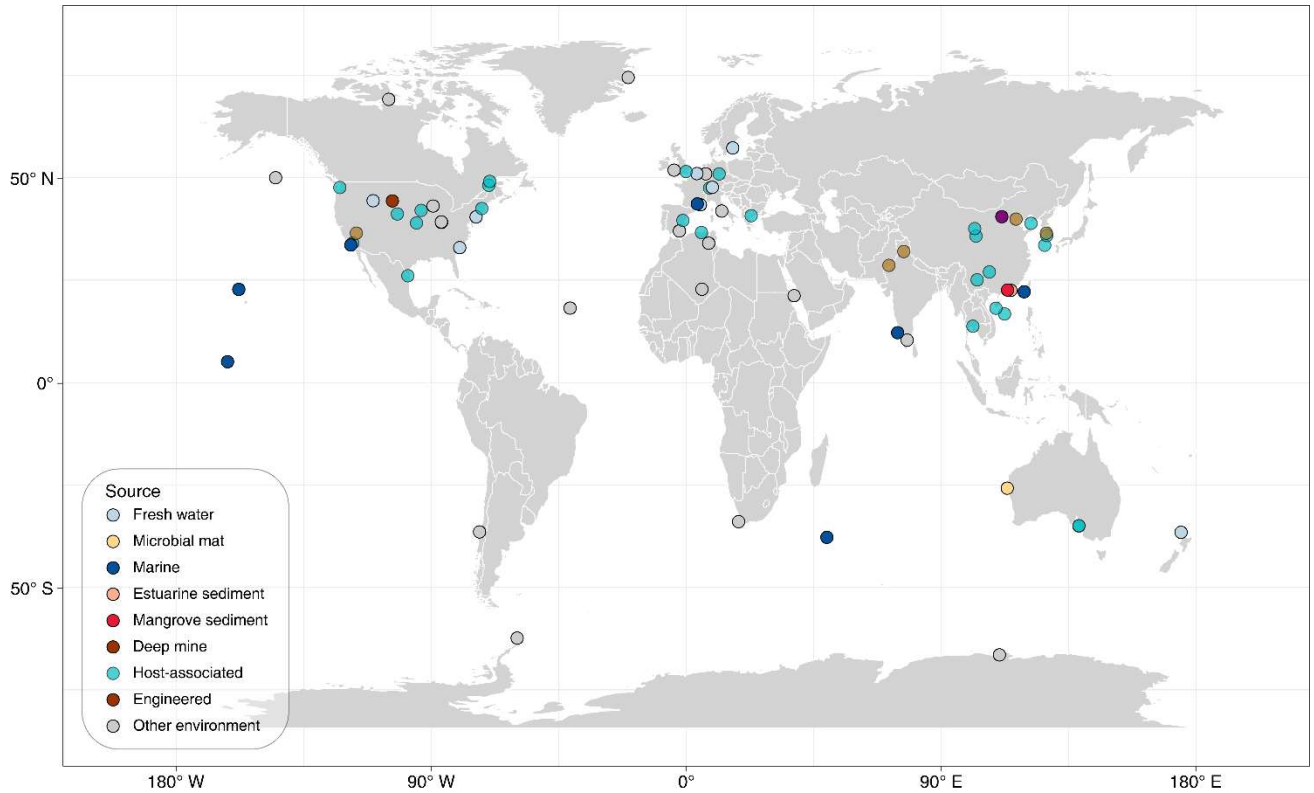


Fig. S19. Schematic map representing the global distribution of *Zixibacteria*. The dataset used corresponds to metagenome-derived 16S rRNA gene sequences homologous to *Zixibacteria*. Different colors represent the geographic location of the respective metagenomic samples.

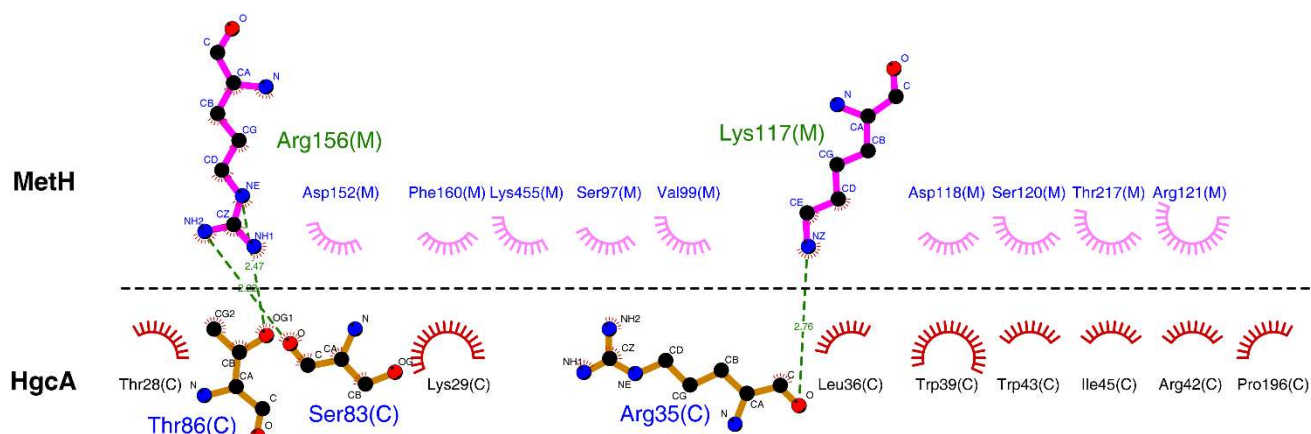


Fig. S20. Schematic representation of HgcA-MetH interaction in the 2D structure. Below the black dashed line is the HgcA protein, and above it is the MetH protein. Carbon atoms are depicted in black, oxygen atoms in red, and nitrogen atoms in blue. Green dashed lines represent hydrogen bonds, with the green numbers indicating the bond lengths. Red dashed lines represent salt bridges, while amino acid residues engaged in hydrophobic interactions are denoted by semicircles.

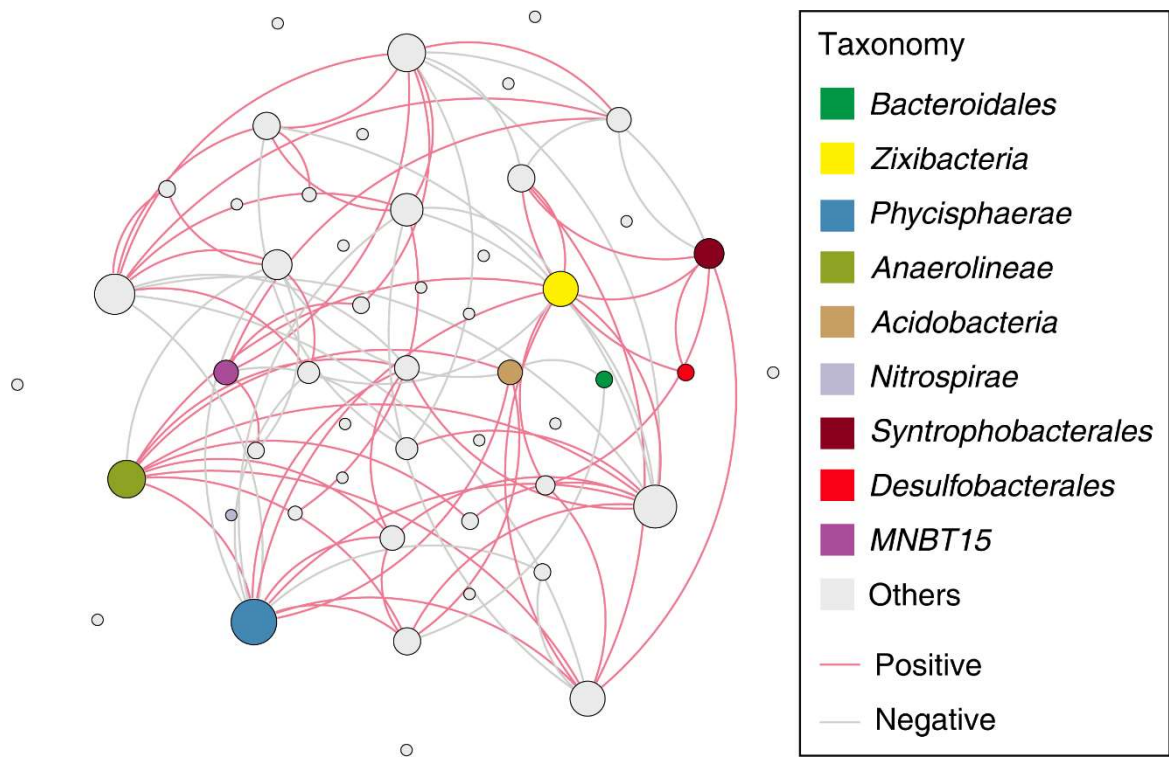


Fig. S21. Co-occurrence between syntrophic *Syntrophobacterales* and *Zixibacteria* revealed by 16S rRNA gene-based microbial network inference.

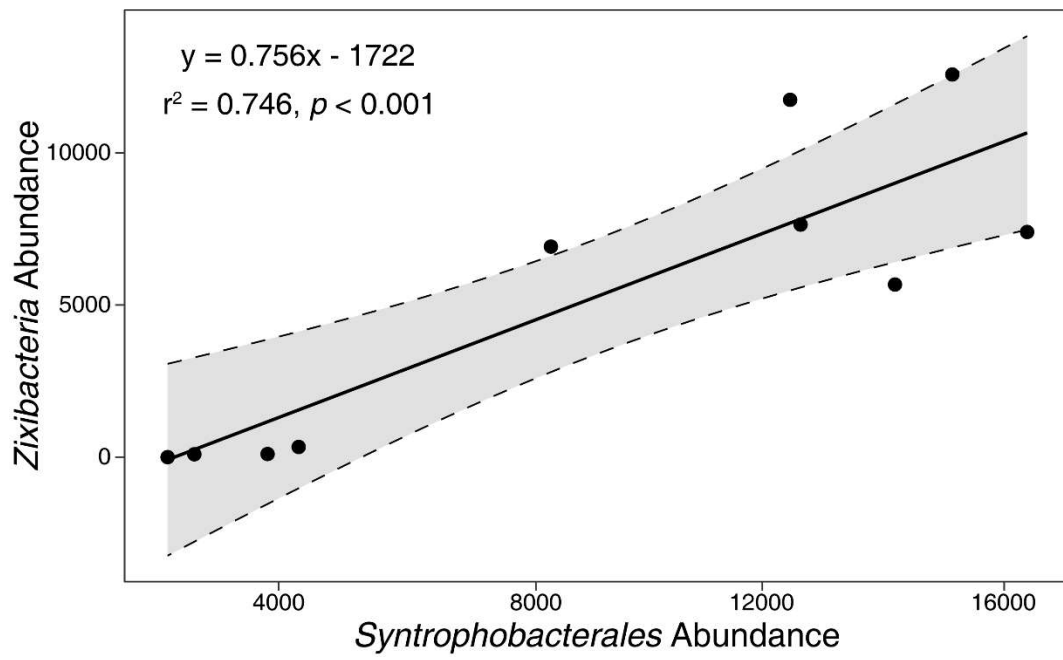


Fig. S22. Relationship between syntrophic *Syntrophobacterales* and *Zixibacteria* in abundance across the mangrove sediment column.

Table S1. General patterns in Hg isotope fractionation according to previous study [2].

Process	$\Delta^{199}\text{Hg}/\delta^{202}\text{Hg}$ ratio
Photochemical reduction from snow	-3.5
Xenon lamp reduction with thiol ligands	-0.8
Aqueous photochemical reduction	1.2
Aqueous photochemical demethylation	2.4
Equilibrium evaporation	0.1
Thiol-ligand binding /iron oxide sorption	0
Microbial methylation	0
Microbial reduction/demethylation	0

Table S2. Isotopic compositions of Hg in mangrove sediment.

Sample	Depth (cm)	$\delta^{202}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$
0-5-1		-0.99	0.05	0.02	-0.06
0-5-2	0-5	-1.24	-0.05	0.07	-0.05
0-5-3		-1.42	-0.03	0.06	-0.05
40-50-1		-1.20	0.01	0.03	-0.02
40-50-2	40-50	-1.52	-0.11	-0.06	-0.09
40-50-3		-1.31	-0.10	0.00	-0.11
60-80-1		-1.05	-0.18	-0.09	-0.20
60-80-2	60-80	-0.71	-0.09	0.04	-0.18
60-80-3		-0.67	-0.18	-0.02	-0.20

Table S3. The habitat-specific distribution of Hg-methylating lineage.

Microbial group	Mangrove sediments	Paddy soils	Lake water	Lake sediments	Brackish water	Reservoir sediments
<i>Euryarchaeota</i>	Present	Present	Present	Present	Absent	Present
<i>Chloroflexi</i>	Present	Present	Present	Present	Present	Present
PVC	Present	Present	Absent	Absent	Present	Present
<i>Bacteroidetes</i>	Present	Present	Present	Present	Absent	Present
<i>Acidobacteria</i>	Present	Present	Absent	Absent	Absent	Present
<i>Firmicutes</i>	Absent	Present	Present	Present	Present	Absent
<i>Spirochaetes</i>	Absent	Present	Present	Present	Present	Absent
<i>Deltaproteobacteria</i>	Present	Present	Present	Present	Present	Present
<i>Nitrospirae</i>	Present	Present	Absent	Absent	Absent	Present

Table S4. Confirmed Hg-methylating bacterial strains with completely sequenced genomes.

<i>Organism name</i>	Goldstamp ID¹	Culture collections	Isolation source
<i>Desulfovibrio desulfuricans</i> ND132	Gi03061	-	Mesohaline Chesapeake Bay sediments
<i>Desulfovibrio aespoeensis</i>	Gc01651	DSM 10631	Deep groundwater
<i>Desulfovibrio africanus</i> Walvis Bay	Gi03062	ATCC 19997, NCIB 8397	Marine sediments, Walvis Bay
<i>Desulfobulbus propionicus</i> lpr3	Gc01599	DSM 2032, ATCC 33891	Freshwater mud
<i>Geobacter sulfurreducens</i> PCA	Gc00166	DSM 12127, ATCC 51573	Ditch surface sediment
<i>Geobacter metallireducens</i> GS-15	Gc00314	DSM 7210, ATCC 53774	Freshwater sediment

¹ Genomes OnLine Database – GOLD

References

1. E. Capo, B. D. Peterson, M. Kim, D. S. Jones, S. G. Acinas, M. Amyot *et al.*, A consensus protocol for the recovery of mercury methylation genes from metagenomes. *Mol Ecol Resour.* **23**, 190-204 (2023).
2. J. D. Blum, Applications of stable mercury isotopes to biogeochemistry. *Handbook of Environmental Isotope Geochemistry: Vol I*, 229-245 (2012).