Supplementary Information for

Thermogenic hydrocarbon biodegradation by diverse depth-stratified microbial populations at a Scotian Basin cold seep

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Supplementary Note 1

To understand the wider metabolic capabilities of these cold seep microbial communities, metabolomic analysis was combined with gene-centric analysis of quality-filtered reads, as well as genome-centric analysis of the 376 MAGs**.**

With respect to organotrophy, genes for the degradation of complex carbohydrates and peptides were prevalent. In total, ~7454 potential carbohydrate-active enzymes (CAZymes) were detected, most notably glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases[1]; CAZymes were numerous in bacterial lineages such as Planctomycetes, Bacteroidetes, and Armatimonadetes **(Supplementary Data 6)**. Annotated peptidases were more evenly distributed across bacteria and archaea, with the highest proportions found in genomes assigned to Patescibacteria, *Dehalococcoidia*, Aerophobetes, and Atribacteria **(Figure 3a and Supplementary Data 7)**. Consistent with these genomic observations, metabolites associated with carbohydrate and peptide hydrolysis were detected (e.g. glucose and sucrose) **(Figure 3b)**. Many community members also possess gene sets to convert hydrolysis products to pyruvate through the Embden-Meyerhof-Parnas (185 MAGs) and Entner-Doudoroff (51 MAGs) pathways **(Figure 3b and Supplementary Data 5)**. Accordingly, both glucose 6-phosphate and pyruvate were detected in the sediment metabolomes **(Figure 3b)**. Most genomes also encoded the potential to ferment pyruvate to formate (119 MAGs), acetate (270 MAGs), lactate (14 MAGs), and ethanol (94 MAGs), as well as the potential for hydrogen production via abundant [FeFe]-hydrogenases associated with fermentation or group 3 [NiFe]-hydrogenases[2] **(Figure 3a, Supplementary Figure 11 and Supplementary Data 5)**. The ability to degrade fatty acids and other organic acids via beta-oxidation was common to Lokiarchaeota, Chloroflexi, and Proteobacteria **(Supplementary Data 8)**, with associated metabolites also detected (e.g. 6-carboxyhexanoate, 10-hydroxydecanoate, and other carboxylic acids) **(Figure 3b)**.

The most prevalent carbon fixation pathways in these sediments are the Calvin-Benson cycle (68 MAGs), Wood-Ljungdahl pathway (114 MAGs), and 4-hydroxybutyrate cycle (4 MAGs) **(Figure 3a and Supplementary Data 5)**, consistent with the abundance of

corresponding key metabolic genes **(Supplementary Figure 14)**. The sediment microbial communities include various putative sulfide, thiosulfate, ammonia, nitrite, carbon monoxide, and hydrogen oxidizers **(Supplementary Data 5)**, with provision of these compounds potentially via biological or geological processes. Canonical genes for sulfide oxidation (sulfide:quinone oxidoreductase and flavocytochrome *c* sulfide dehydrogenase) were found in 38 MAGs from diverse lineages (e.g. Proteobacteria, *Anaerolineales*, and Heimdallarchaeota) **(Supplementary Figures 3-4)**. The oxidative bacterial-type DsrAB-type dissimilatory sulfite reductases[3] were detected in four genomes from *Alpha*- and *Gammaproteobacteria* potentially responsible for sulfide oxidation **(Supplementary Figure 5)**. Five MAGs from *Epsilon*- and *Gammaproteobacteria* encoded genes for the SoxB component of the periplasmic thiosulfate-oxidizing Sox enzyme complex **(Supplementary Figure 6).** Genes for ammonia oxidation to hydroxylamine (ammonia monooxygenase) were detected in four Thaumarchaeota genomes **(Supplementary Figure 7)**, while those associated with further oxidation of nitrite (nitrite oxidoreductase) were tentatively found in five MAGs affiliated to *Anaerolineales*, Acidobacteria, Actinobacteria, and Planctomycetes **(Supplementary Figure 8)**. Gene abundance profiles suggest that these nitrogen metabolisms mainly occur in 0-20 cmbsf surface sediments **(Supplementary Figure 14)**. Three MAGs belonging to Actinobacteria, Actinobacteria, and *Alphaproteobacteria* encoded carbon monoxide dehydrogenase large subunit (CoxL), a marker for carbon monoxide oxidation **(Supplementary Figure 9)**. Ten phyla (58 MAGs) encoded group 1 and group 2 [NiFe]-hydrogenases associated with hydrogenotrophic respiration, with certain lineages, including *Deltaproteobacteria*, Planctomycetes, *Dehalococcoidia*, Actinobacteria, and Aerophobetes, encoding subgroups (1a, 1b) that support hydrogenotrophic sulfate reduction and halorespiration[4, 5] **(Supplementary Figure 10)**. Additionally, 197 MAGs from 35 phyla encode cofactor-coupled group 3 [NiFe]-hydrogenases (**Supplementary Figure 11)** and energy-converting group 4 [NiFe]-hydrogenases (**Supplementary Figure 12)**, both of which are known to be physiologically reversible[2].

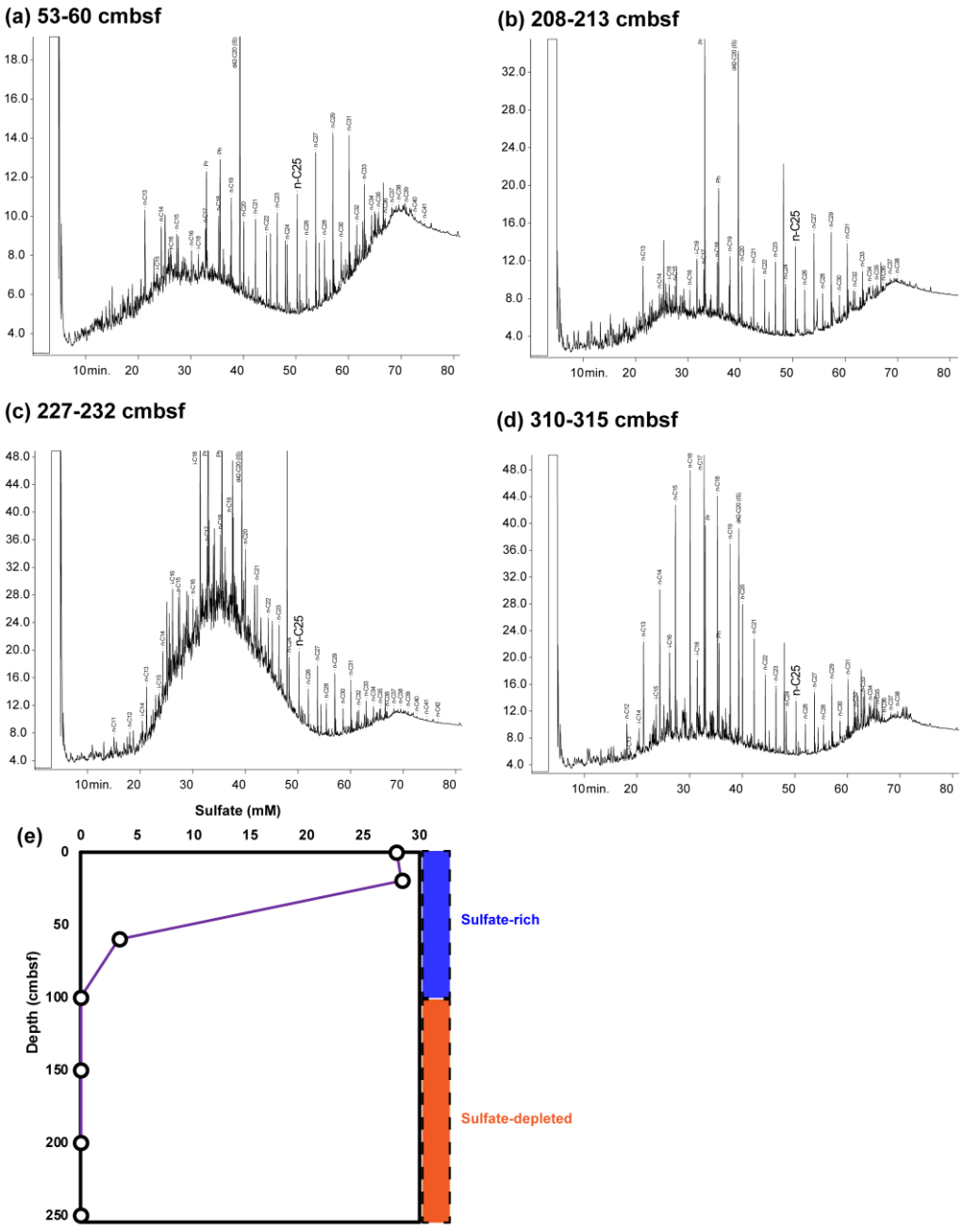
Metabolomics revealed organohalides as potential respiratory electron acceptors in the sediments, e.g. dichlorotoluene and dichlorodiphenyldichloroethane **(Figure 3b)**.

Accordingly, reductive dehalogenases were encoded in 54 MAGs from 13 phyla, including lineages such as Chloroflexi, Lokiarchaeota, Bathyarchaeota, and Proteobacteria **(Supplementary Figure 13)**. Organisms encoding these enzymes may conserve energy by coupling oxidation of H_2 and other electron donors to the reduction of organochloride compounds like the ones detected in the sediments. Full gene sets for dissimilatory reduction of sulfate to sulfide (*sat*, *aprAB* and *dsrAB*) were found in 12 genomes from *Deltaproteobacteria* and Zixibacteria **(Supplementary Figure 5)**. Thirty bacterial members of these sediment communities are predicted to use nitrate or nitrite as electron acceptors, though none encoded all of the genes necessary for complete denitrification or dissimilatory nitrate reduction to ammonia (**Supplementary Data 5)**. Terminal oxidases were also detected, but in line with oxygen only permeating the upper centimeters of marine sediments, these genes were mainly found in lineages such as Proteobacteria and Thaumarchaeota that dominated the upper sediment layers **(Supplementary Figure 14)**.

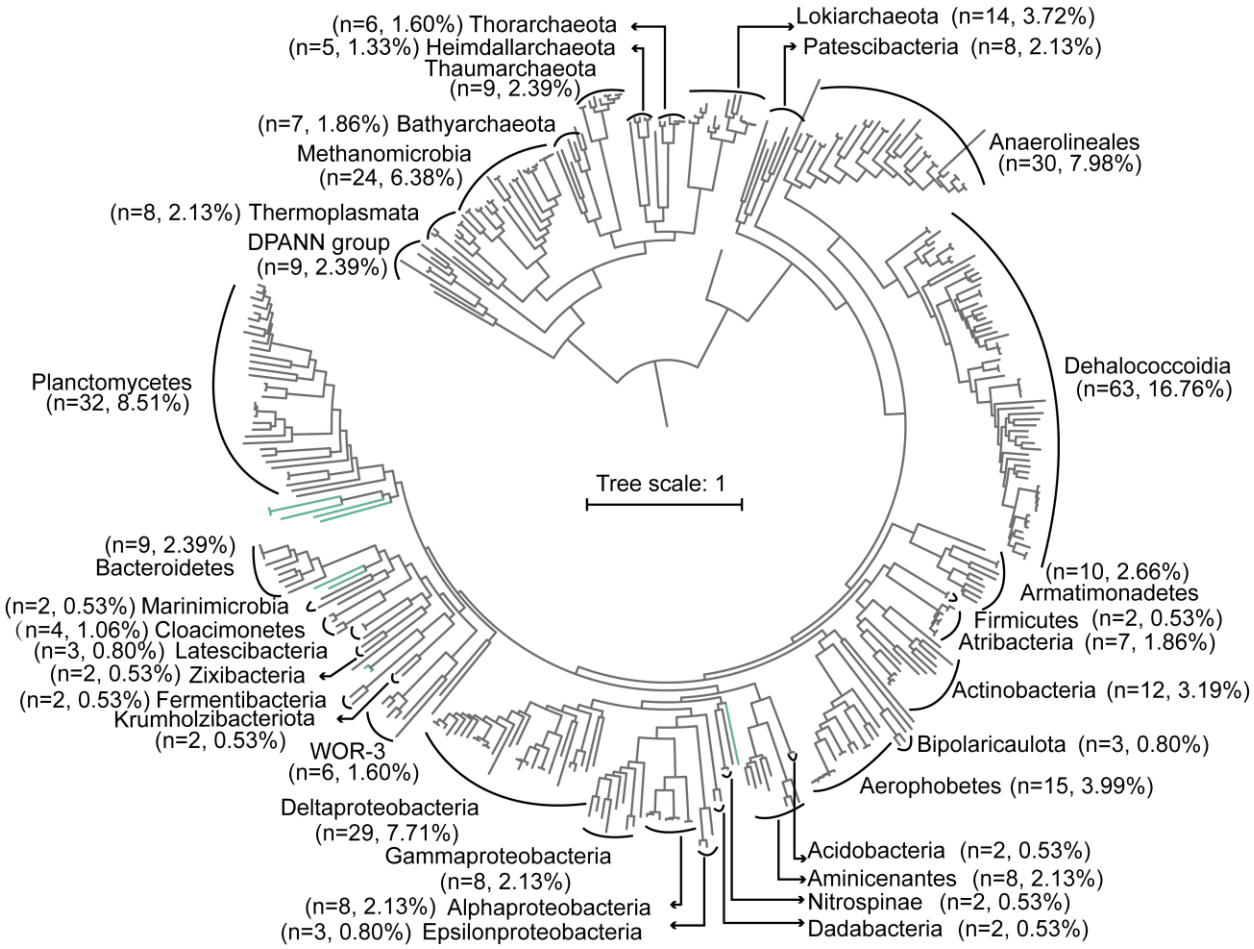
Supplementary Table 1 Extractable organic matter compositions of four sediment subsamples.

Depth (cmbsf)		53-60	208-213	227-232	310-315
TOC (%)		0.25	0.35	0.36	0.56
EOM (mg/kg rock)		104	361	177	168
wt% of EOM	SAT	47.5	52.1	35.0	24.6
	ARO	10.0	11.8	13.9	12.5
	POL	42.5	26.5	17.9	36.4
	ASP	0.0	9.6	33.2	26.4
δ^{13}C-Sat (‰)		ND	-31.5	ND	ND
δ^{13}C-Aro (‰)		ND	-29.6	ND	ND

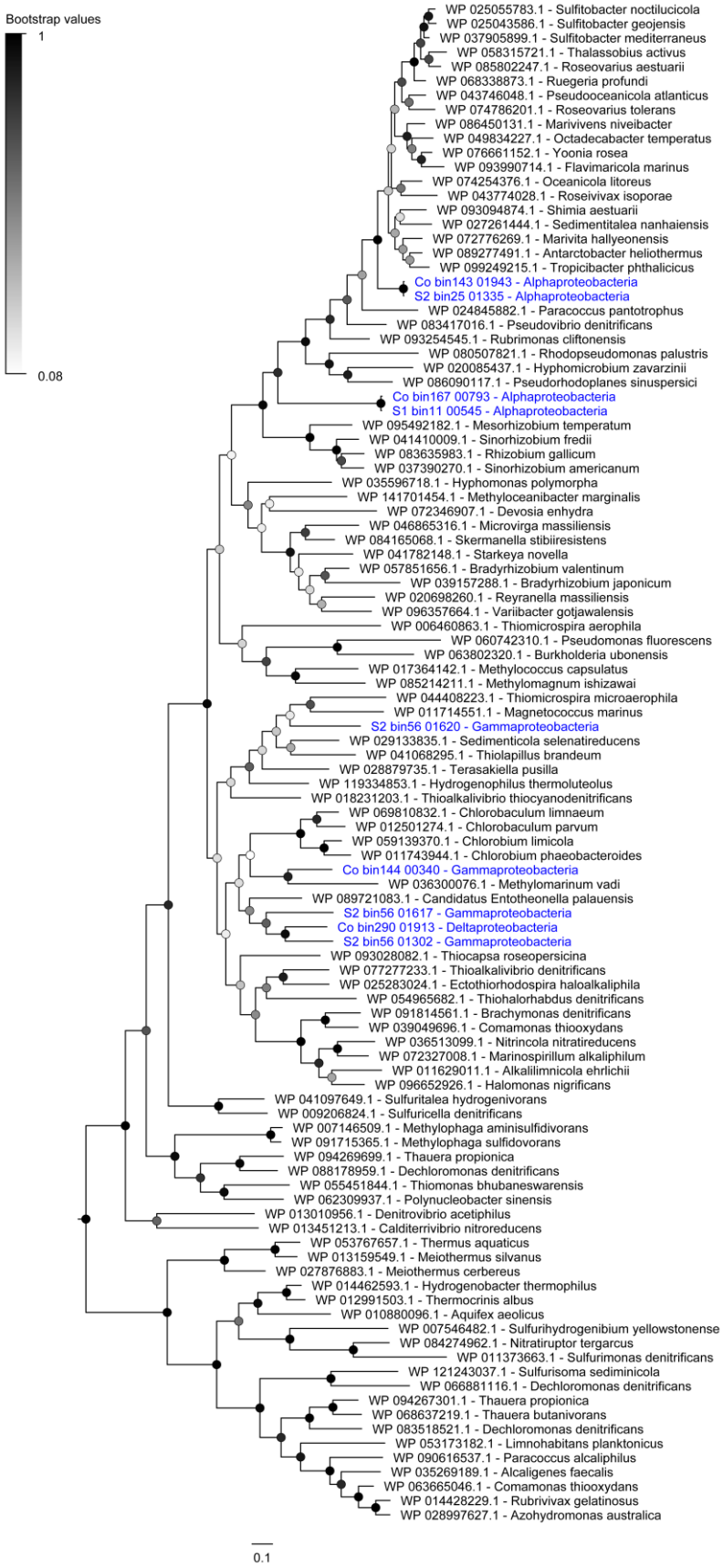
Abbreviations: TOC, total organic carbon; EOM, extractable organic matter; SAT, saturated hydrocarbons; ARO, aromatic hydrocarbons; POL, polars; ASP, asphaltenes; wt%, weight percentage; ND, not determined.



Supplementary Figure 1 Extractable organic matter from subsampled sediments and sulfate concentrations from porewater. GC-FID chromatograms showing unresolved complex mixture (UCM) humps for four different depths including (a) 53-60 cmbsf, (b) 208-213 cmbsf, (c) 227-232 cmbsf, and (d) 310-315 cmbsf; (e) porewater concentrations of sulfate along depths. For (a)-(d), y-axis: detector response; x-axis: retention time (minutes). Additional parameters for the extractable organic matter of these samples are provided in **Supplementary Table 1**.



Supplementary Figure 2 Phylogenetic placement of 376 reconstructed metagenome-assembled genomes. A maximum-likelihood phylogenomic tree was built based on concatenated amino acid sequences of 43 conserved single-copy genes using RAxML with the PROTGAMMALG model. The scale bar represents 1 amino acid substitution per sequence position. Numbers in parentheses show total MAGs recovered. Phyla with only one detected MAG are not labelled. Ten MAGs corresponded to unclassified phylum-level lineages (green branches; unlabelled). The full tree file with references is available in **Supplementary Data 4**.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WP 068338873.1 - Ruegeria profundi
WP 043746048.1 - Pseudooceanicola atlanticus
WP 074786201.1 - Roseovarius tolerans
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WP 049834227.1 - Octadecabacter temperatus
WP 076661152.1 - Yoonia rosea
WP 093990714.1 - Flavimaricola marinus
WP 074254376.1 - Oceanicola litoreus
WP 043774028.1 - Roseivivax isoporae
WP 093094874.1 - Shimia aestuarii
WP 027261444.1 - Sedimentitalea nanhaiensis
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WP 089277491.1 - Antarctobacter heliothermus
WP 099249215.1 - Tropicibacter phthalicicus
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S2 bin25 01335 - Alphaproteobacteria
WP 024845882.1 - Paracoccus pantotrophus
WP 083417016.1 - Pseudovibrio denitrificans
WP 093254545.1 - Rubrimonas cliftonensis
WP 080507821.1 - Rhodopseudomonas palustris
WP 020085437.1 - Hyphomicrobium zavarzinii
WP 086090117.1 - Pseudorhodoplanes sinuspersici
Co bin167 00793 - Alphaproteobacteria
S1 bin11 00545 - Alphaproteobacteria
WP 095492182.1 - Mesorhizobium temperatum
WP 041410009.1 - Sinorhizobium fredii
WP 083635983.1 - Rhizobium gallicum
WP 037390270.1 - Sinorhizobium americanum
WP 035596718.1 - Hyphomonas polymorpha
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WP 046865316.1 - Microvirga massiliensis
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WP 012501274.1 - Chlorobaculum parvum
WP 059139370.1 - Chlorobium limicola
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WP 089721083.1 - Candidatus Entotheonella palauensis
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Co bin290 01913 - Deltaproteobacteria
S2 bin56 01302 - Gammaproteobacteria
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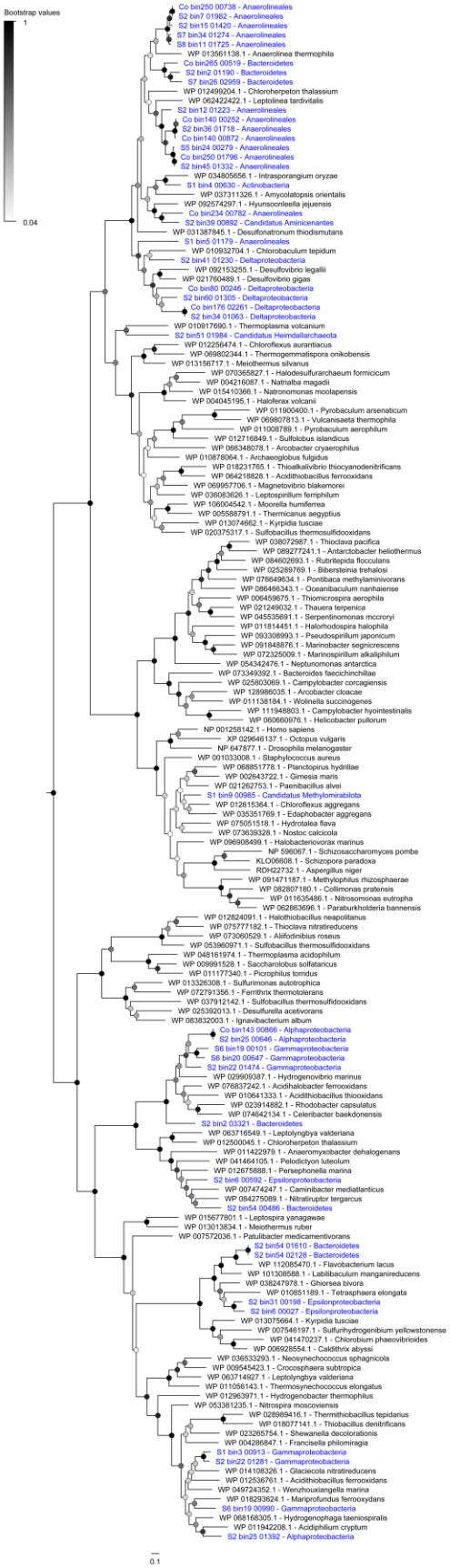
0.1

Supplementary Figure 3 Maximum-likelihood tree of amino acid sequences of flavocytochrome *c* sulfide dehydrogenase (FCC), a marker for sulfide oxidation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.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WP 013561138.1 - Anaerolinea thermophila
Co bin265 00519 - Bacteroidetes
S2 bin2 01190 - Bacteroidetes
S7 bin26 02959 - Bacteroidetes
WP 012499204.1 - Chloroherpeton thalassium
WP 062422422.1 - Leptolinea tardivitalis
S2 bin12 01223 - Anaerolineales
Co bin140 00252 - Anaerolineales
S2 bin36 01718 - Anaerolineales
Co bin140 00872 - Anaerolineales
S5 bin24 00279 - Anaerolineales
Co bin250 01796 - Anaerolineales
S2 bin45 01332 - Anaerolineales
WP 034805656.1 - Intrasporangium oryzae
S1 bin4 00630 - Actinobacteria
WP 037311326.1 - Amycolatopsis orientalis
WP 092574297.1 - Hyunsoonleella jejuensis
Co bin234 00782 - Anaerolineales
S2 bin39 00892 - Candidatus Aminicenantes
WP 031387845.1 - Desulfonatronum thiodismutans
S1 bin5 01179 - Anaerolineales
WP 010932704.1 - Chlorobaculum tepidum
S2 bin41 01230 - Deltaproteobacteria
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WP 021760489.1 - Desulfovibrio gigas
Co bin80 00246 - Deltaproteobacteria
S2 bin60 01305 - Deltaproteobacteria
Co bin176 02261 - Deltaproteobacteria
S2 bin34 01063 - Deltaproteobacteria
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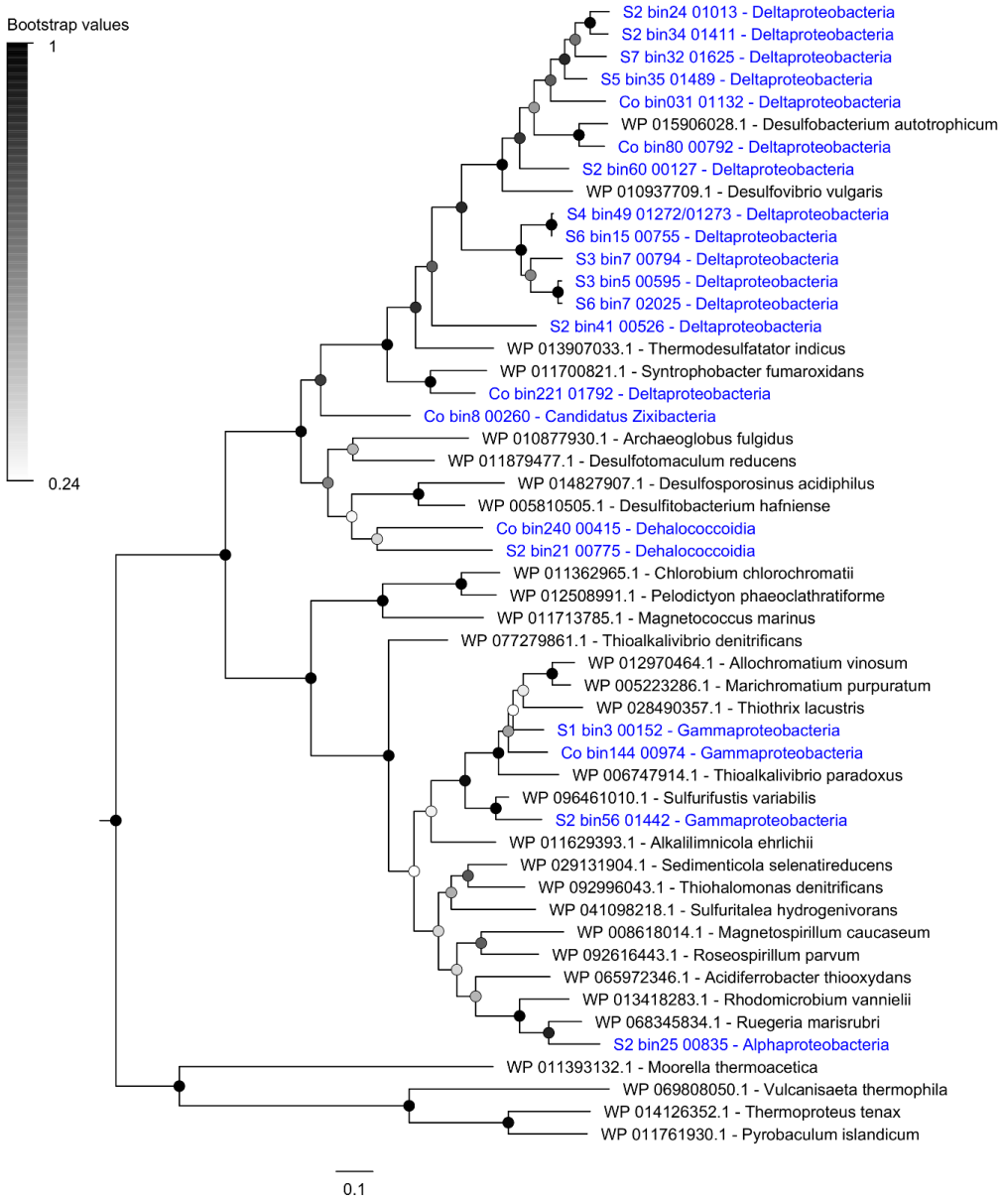
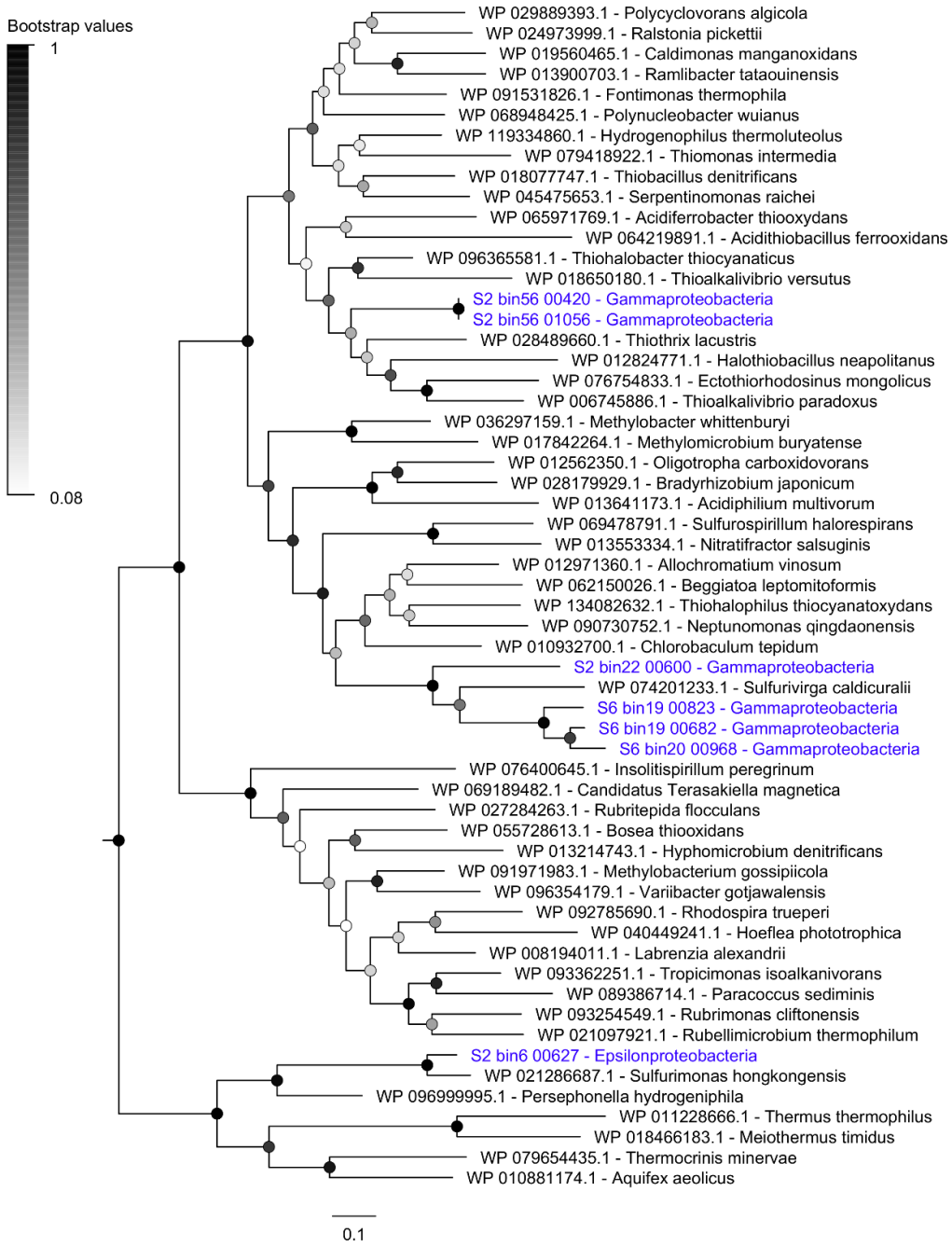
WP 018293624.1 - Mariprofundus ferrooxydans
S6 bin19 00990 - Gammaproteobacteria
WP 068168305.1 - Hydrogenophaga taeniospiralis
WP 011942208.1 - Acidiphilium cryptum
S2 bin25 01392 - Alphaproteobacteria
0.1

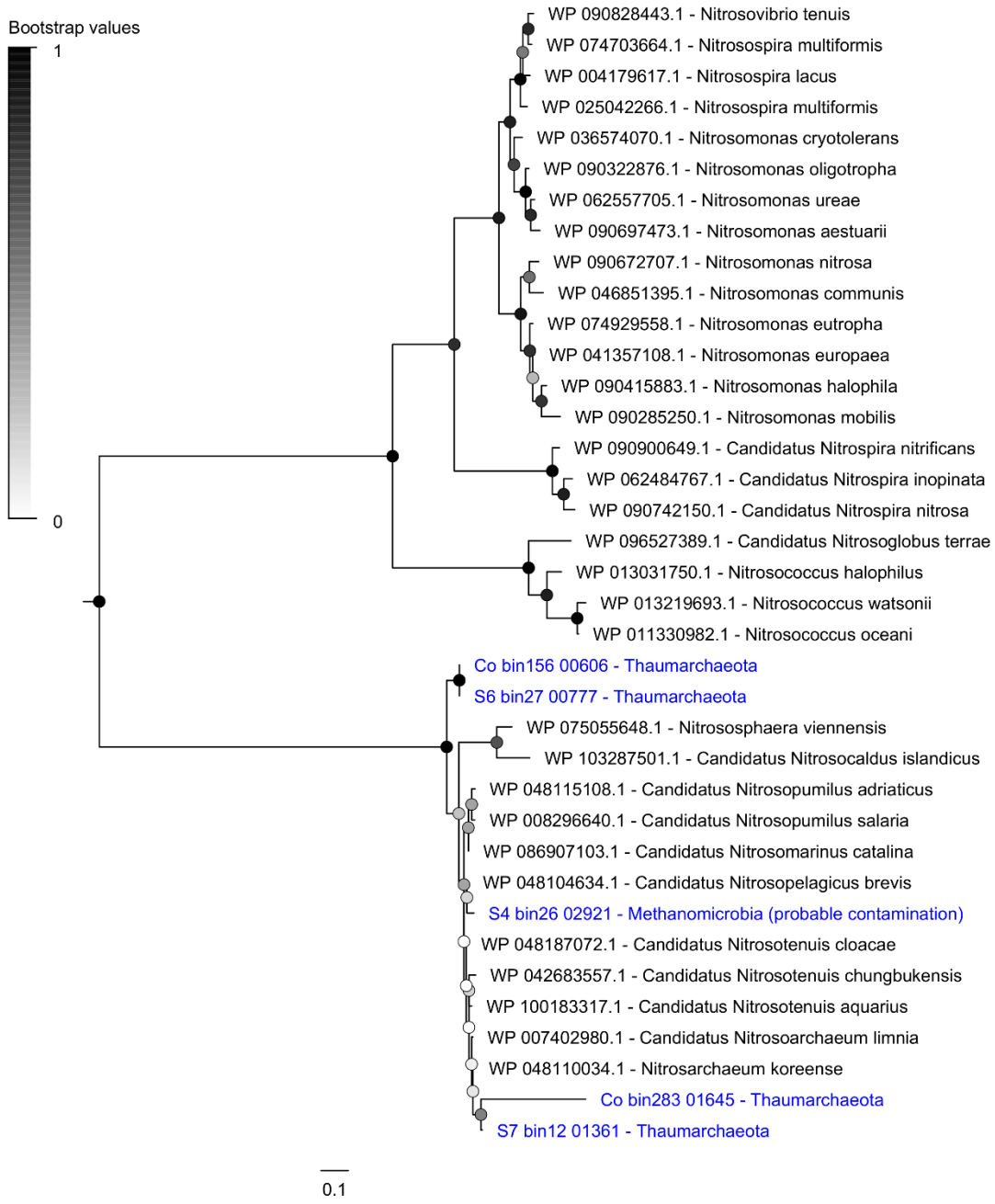
Supplementary Figure 4 Maximum-likelihood tree of amino acid sequences of the sulfide-quinone oxidoreductase (Sqr), a marker for sulfide oxidation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



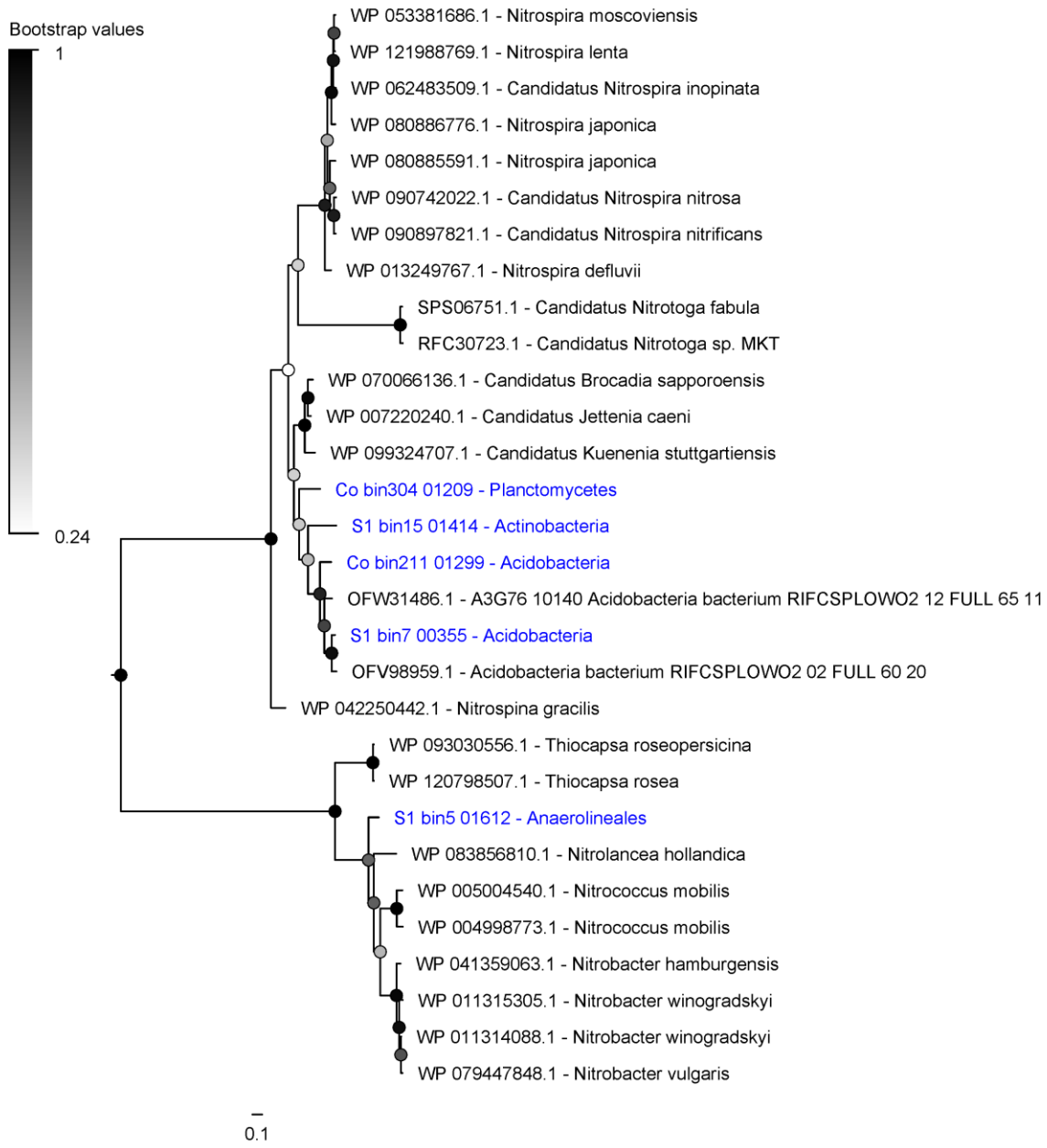
Supplementary Figure 5 Maximum-likelihood tree of amino acid sequences of dissimilatory sulfite reductase A subunit (DsrA). The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). This enzyme is a marker for dissimilatory sulfite reduction (top and bottom major clades; *Deltaproteobacteria*, Zixibacteria, *Dehalococcoidia* bins) and sulfide oxidation (middle clade; *Gammaproteobacteria* and Alphaproteobacteria bins). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



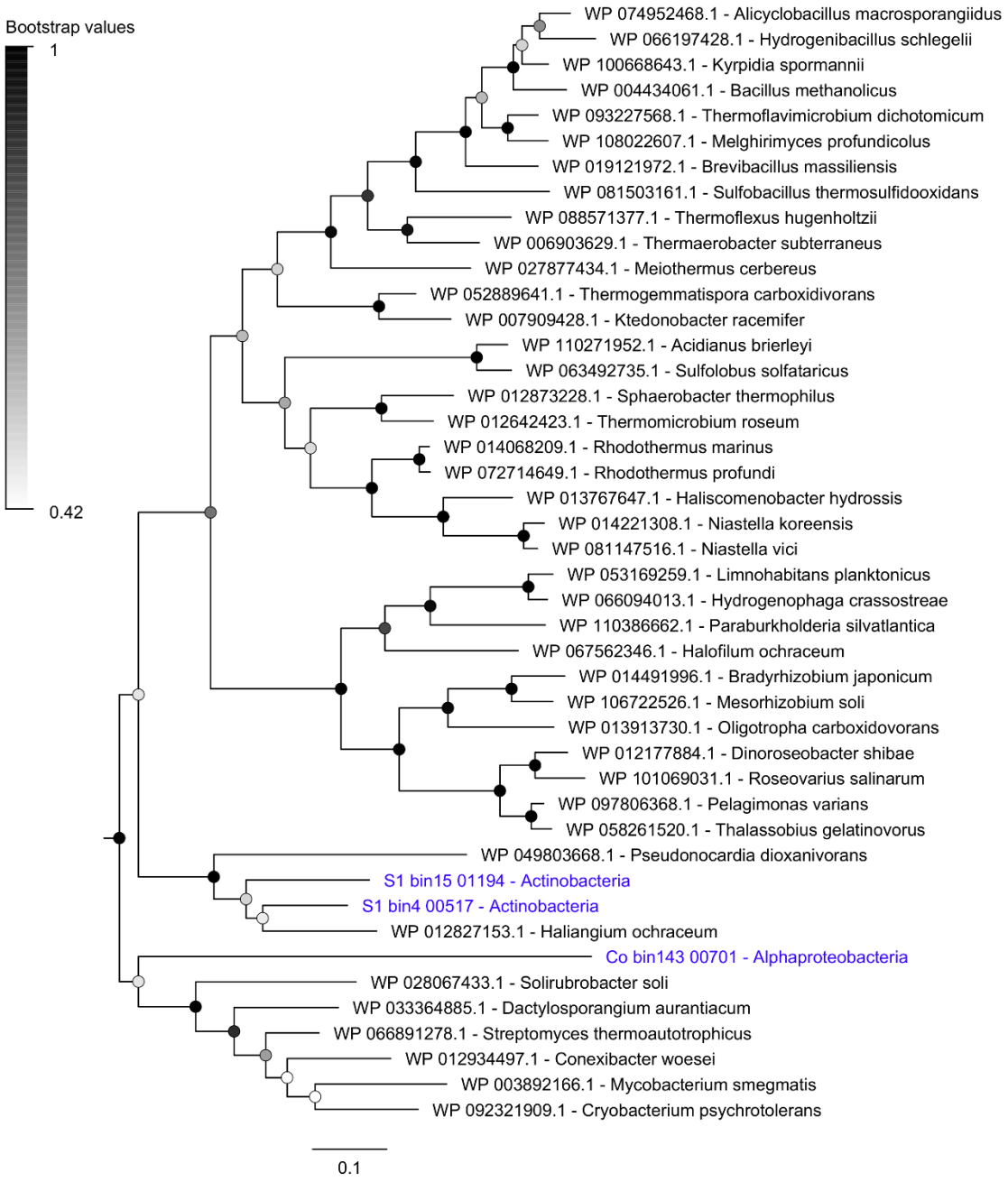
Supplementary Figure 6 Maximum-likelihood tree of amino acid sequences of the thiosulfohydrolase (SoxB), a marker for thiosulfate oxidation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



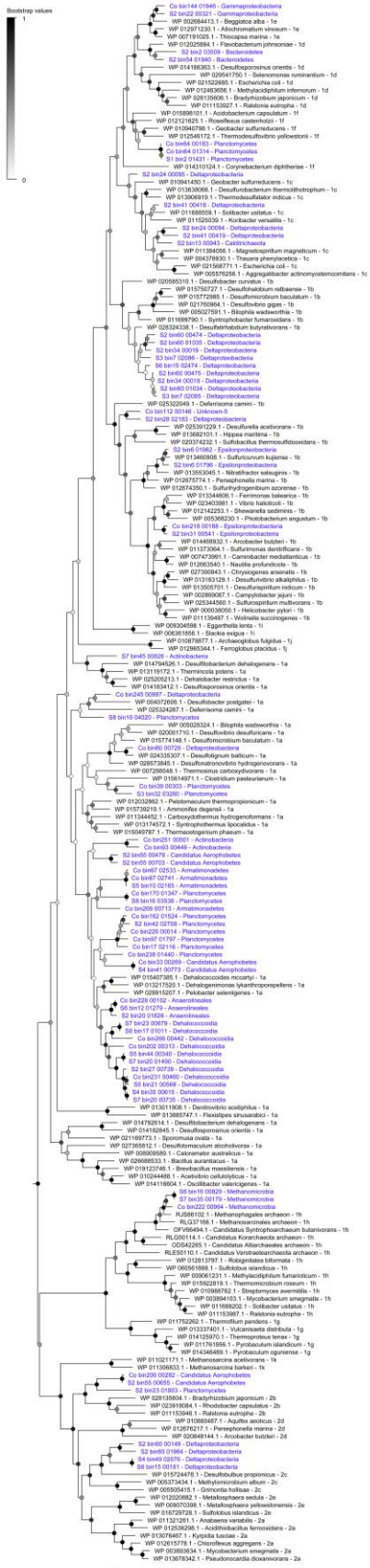
Supplementary Figure 7 Maximum-likelihood tree of amino acid sequences of ammonia monooxygenase A subunit (AmoA), a marker for ammonia oxidation during aerobic nitrification. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



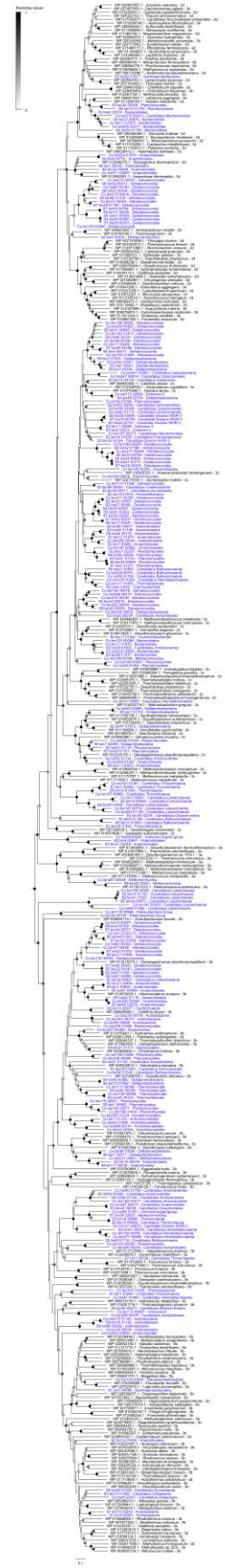
Supplementary Figure 8 Maximum-likelihood tree of amino acid sequences of the nitrite oxidoreductase A subunit (NxrA), a marker for nitrite oxidation during aerobic nitrification. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



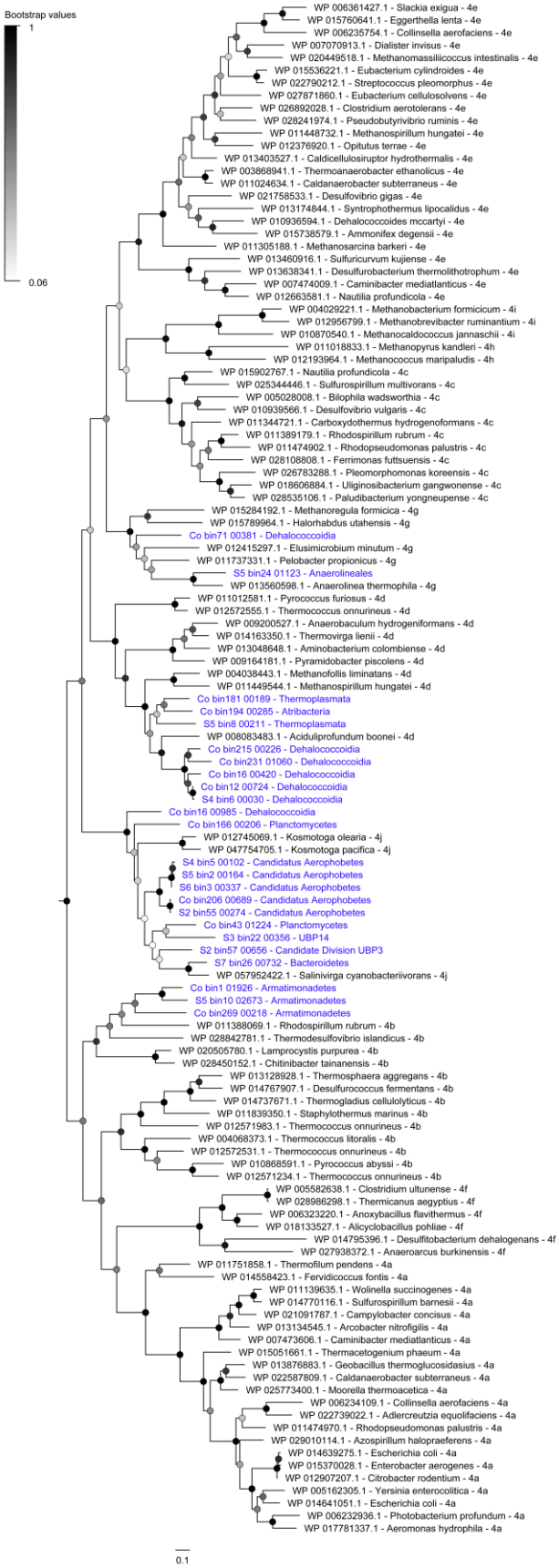
Supplementary Figure 9 Maximum-likelihood tree of amino acid sequences of carbon monoxide dehydrogenase large subunit (CoxL), a marker for aerobic carbon monoxide oxidation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.


Bootstrap values
1
0
Co bin144 01946 - Gammaproteobacteria
S2 bin22 00321 - Gammaproteobacteria
WP 002684413.1 - Beggiatoa alba - 1e
WP 012971230.1 - Allochromatium vinosum - 1e
WP 007191025.1 - Thiocapsa marina - 1e
WP 012025884.1 - Flavobacterium johnsoniae - 1d
S2 bin2 03509 - Bacteroidetes
S2 bin54 01940 - Bacteroidetes
WP 014186363.1 - Desulfosporosinus orientis - 1d
WP 029541750.1 - Selenomonas ruminantium - 1d
WP 021522685.1 - Escherichia coli - 1d
WP 012463656.1 - Methylacidiphilum infernorum - 1d
WP 028135606.1 - Bradyrhizobium japonicum - 1d
WP 011153927.1 - Ralstonia eutropha - 1d
WP 015898101.1 - Acidobacterium capsulatum - 1f
WP 012121625.1 - Roseiflexus castenholzii - 1f
WP 010940798.1 - Geobacter sulfurreducens - 1f
WP 012546172.1 - Thermodesulfovibrio yellowstonii - 1f
Co bin64 00183 - Planctomycetes
Co bin64 01314 - Planctomycetes
S1 bin2 01431 - Planctomycetes
WP 014310124.1 - Corynebacterium diphtheriae - 1f
S2 bin24 00095 - Deltaproteobacteria
WP 010941450.1 - Geobacter sulfurreducens - 1c
WP 013638066.1 - Desulfurobacterium thermolithotrophum - 1c
WP 013906919.1 - Thermodesulfatator indicus - 1c
S2 bin41 00418 - Deltaproteobacteria
WP 011688559.1 - Solibacter usitatus - 1c
WP 011526339.1 - Koribacter versatilis - 1c
S2 bin24 00094 - Deltaproteobacteria
S2 bin41 00419 - Deltaproteobacteria
S2 bin13 00043 - Calditrichaeota
WP 011364056.1 - Magnetospirillum magneticum - 1c
WP 004378930.1 - Thauera phenylacetica - 1c
WP 021568771.1 - Escherichia coli - 1c
WP 005576256.1 - Aggregatibacter actinomycetemcomitans - 1c
WP 020585310.1 - Desulfotalea curvatus - 1b
WP 015750727.1 - Desulfohalobium retbaense - 1b
WP 015772985.1 - Desulfomicrobium baculatum - 1b
WP 021760964.1 - Desulfovibrio gigas - 1b
WP 005027591.1 - Bilophila wadsworthia - 1b
WP 011699790.1 - Syntrophobacter fumaroxidans - 1b
WP 028324338.1 - Desulfotalea psychrophila - 1b
S2 bin60 00474 - Deltaproteobacteria
S2 bin60 01035 - Deltaproteobacteria
S2 bin34 00019 - Deltaproteobacteria
S3 bin7 02086 - Deltaproteobacteria
S6 bin15 02474 - Deltaproteobacteria
S2 bin60 00479 - Deltaproteobacteria
S2 bin34 00018 - Deltaproteobacteria
S2 bin60 01034 - Deltaproteobacteria
S3 bin7 02085 - Deltaproteobacteria
WP 025322049.1 - Deferrisoma camini - 1b
Co bin112 00146 - Unknown 0
S2 bin28 02183 - Deltaproteobacteria
WP 025391229.1 - Desulfurella acetivorans - 1b
WP 013682101.1 - Hippea maritima - 1b
WP 020374232.1 - Sulfobacillus thermosulfidooxidans - 1b
S2 bin6 01982 - Epsilonproteobacteria
WP 013460908.1 - Sulfuricurvum kujiense - 1b
S2 bin6 01796 - Epsilonproteobacteria
WP 013553045.1 - Nitratifractor salsuginis - 1b
WP 012675774.1 - Persephonella marina - 1b
WP 012674350.1 - Sulfurihydrogenibium azorense - 1b
WP 013344606.1 - Fervimonas balearica - 1b
WP 023403981.1 - Vibrio halioticoli - 1b
WP 012142253.1 - Shewanella sediminis - 1b
WP 005368230.1 - Photobacterium angustum - 1b
Co bin218 00188 - Epsilonproteobacteria
S2 bin31 00041 - Epsilonproteobacteria
WP 014468932.1 - Arcobacter butzleri - 1b
WP 011373064.1 - Sulfurimonas denitrificans - 1b
WP 007473991.1 - Caminibacter mediatlanticus - 1b
WP 012863540.1 - Nautilia profundicola - 1b
WP 027390843.1 - Chrysiogenes arsenatis - 1b
WP 013163129.1 - Desulfurivibrio alkaliphilus - 1b
WP 013505701.1 - Desulfurispirillum indicum - 1b
WP 002890087.1 - Campylobacter jejuni - 1b
WP 025344560.1 - Sulfurospirillum multivorans - 1b
WP 000038058.1 - Helicobacter pylori - 1b
WP 011139497.1 - Wolinella succinogenes - 1b
WP 009304598.1 - Eggerthella lenta - 1i
WP 006361856.1 - Slackia exigua - 1i
WP 010878877.1 - Archaeoglobus fulgidus - 1j
WP 012965344.1 - Ferroglobus placidus - 1j
S7 bin45 00828 - Actinobacteria
WP 014794526.1 - Desulfitobacterium dehalogenans - 1a
WP 013119172.1 - Thermincola potens - 1a
WP 025205213.1 - Dehalobacter restrictus - 1a
WP 014183412.1 - Desulfosporosinus orientis - 1a
Co bin245 00997 - Deltaproteobacteria
WP 004072606.1 - Desulfobacter postgatei - 1a
WP 025324267.1 - Deferrisoma camini - 1a
S8 bin16 04020 - Planctomycetes
WP 005028324.1 - Bilophila wadsworthia - 1a
WP 020003710.1 - Desulfovibrio desulfuricans - 1a
WP 015774148.1 - Desulfomicrobium baculatum - 1a
Co bin80 00729 - Deltaproteobacteria
WP 024335307.1 - Desulfotignum balticum - 1a
WP 028573845.1 - Desulfonatronovibrio hydrogenovorans - 1a
WP 007286648.1 - Thermosinus carboxydivorans - 1a
WP 015614971.1 - Clostridium pasteurianum - 1a
Co bin39 00303 - Planctomycetes
S3 bin32 03280 - Planctomycetes
WP 012032862.1 - Pelotomaculum thermopropionicum - 1a
WP 015739219.1 - Ammonifex degensii - 1a
WP 011344452.1 - Carboxydothermus hydrogenoformans - 1a
WP 013174572.1 - Syntrophothermus lipocalidus - 1a
WP 015049787.1 - Thermacetogenium phaeum - 1a
Co bin251 00001 - Actinobacteria
Co bin63 00449 - Actinobacteria
S2 bin55 00478 - Candidatus Aerophobetes
S2 bin55 00703 - Candidatus Aerophobetes
Co bin67 02533 - Armatimonadetes
Co bin87 02741 - Armatimonadetes
S8 bin10 02165 - Armatimonadetes
Co bin170 01347 - Planctomycetes
S8 bin16 03538 - Planctomycetes
Co bin269 00713 - Armatimonadetes
Co bin182 01524 - Planctomycetes
S2 bin42 02708 - Planctomycetes
Co bin226 00014 - Planctomycetes
Co bin97 01797 - Planctomycetes
Co bin17 02116 - Planctomycetes
Co bin238 01440 - Planctomycetes
Co bin33 00269 - Candidatus Aerophobetes
S4 bin41 00773 - Candidatus Aerophobetes
WP 015487385.1 - Dehalococcoides mccartyi - 1a
WP 013217520.1 - Dehalogenimonas lykanthroporepellens - 1a
WP 029915207.1 - Pelobacter seleniigenes - 1a
Co bin228 00102 - Anaerolineales
S6 bin12 01279 - Anaerolineales
S2 bin20 01826 - Anaerolineales
S7 bin23 00879 - Dehalococcoidia
S8 bin17 01011 - Dehalococcoidia
Co bin266 00442 - Dehalococcoidia
Co bin202 00313 - Dehalococcoidia
S5 bin44 00340 - Dehalococcoidia
S7 bin20 01490 - Dehalococcoidia
S2 bin27 00738 - Dehalococcoidia
Co bin231 00490 - Dehalococcoidia
S5 bin21 00568 - Dehalococcoidia
S4 bin26 00615 - Dehalococcoidia
S7 bin20 00735 - Dehalococcoidia
WP 013011608.1 - Desulfovibrio aespoliphilus - 1a
WP 013885747.1 - Flexistipes sinusarabici - 1a
WP 014792614.1 - Desulfitobacterium dehalogenans - 1a
WP 014182845.1 - Desulfosporosinus orientis - 1a
WP 021169773.1 - Sporomusa ovata - 1a
WP 027385812.1 - Desulfotomaculum alcoholivorax - 1a
WP 008909589.1 - Caloramator australicus - 1a
WP 026888533.1 - Bacillus aurantiacus - 1a
WP 019123746.1 - Brevibacillus massiliensis - 1a
WP 010244488.1 - Acetivibrio cellulolyticus - 1a
WP 014116604.1 - Oscillibacter valericigenes - 1a
S6 bin16 00829 - Methanomicrobia
S7 bin35 00179 - Methanomicrobia
Co bin222 00964 - Methanomicrobia
RUS86102.1 - Methanophagales archaeon - 1h
RLG37166.1 - Methanosarcinales archaeon - 1h
OFV66494.1 - Candidatus Syntrophoarchaeum butanivorans - 1h
RLG50114.1 - Candidatus Korarchaeota archaeon - 1h
OD542265.1 - Candidatus Altiarchaeales archaeon - 1h
RLE50110.1 - Candidatus Verstraetearchaeota archaeon - 1h
WP 012813797.1 - Robiginitalea biformata - 1h
WP 080561888.1 - Sulfolobus islandicus - 1h
WP 009061231.1 - Methylacidiphilum fumariolicum - 1h
WP 015922819.1 - Thermomicrobium roseum - 1h
WP 010985762.1 - Streptomyces avermitilis - 1h
WP 003894103.1 - Mycobacterium smegmatis - 1h
WP 011688202.1 - Solibacter usitatus - 1h
WP 011153987.1 - Ralstonia eutropha - 1h
WP 011752262.1 - Thermofilum pendens - 1g
WP 013337401.1 - Vulcanisaeta distributa - 1g
WP 014125970.1 - Thermoproteus tenax - 1g
WP 011761956.1 - Pyrobaculum islandicum - 1g
WP 014346469.1 - Pyrobaculum oguriense - 1g
WP 011021171.1 - Methanosarcina acetivorans - 1k
WP 011306833.1 - Methanosarcina barkeri - 1k
Co bin290 00282 - Candidatus Aerophobetes
S2 bin55 00655 - Candidatus Aerophobetes
S2 bin23 01903 - Planctomycetes
WP 028135804.1 - Bradyrhizobium japonicum - 2b
WP 023819084.1 - Rhodobacter capsulatus - 2b
WP 011153946.1 - Ralstonia eutropha - 2b
WP 010880487.1 - Aquifex aeolicus - 2d
WP 012676217.1 - Persephonella marina - 2d
WP 020481144.1 - Arcobacter butzleri - 2d
S2 bin60 00149 - Deltaproteobacteria
S2 bin50 01964 - Deltaproteobacteria
S4 bin49 02076 - Deltaproteobacteria
S6 bin15 00181 - Deltaproteobacteria
WP 015724478.1 - Desulfobulbus propionicus - 2c
WP 005373434.1 - Methylomicrobium album - 2c
WP 005505415.1 - Gimmonita hollisae - 2c
WP 012020882.1 - Metallosphaera sedula - 2e
WP 009070398.1 - Metallosphaera yellowstonensis - 2e
WP 016729728.1 - Sulfolobus islandicus - 2e
WP 011321261.1 - Arcobacns variabilis - 2a
WP 012536298.1 - Acidithiobacillus ferrooxidans - 2a
WP 013076467.1 - Kytpidia tusciae - 2a
WP 012615778.1 - Chloroflexus aggregans - 2a
WP 003893634.1 - Mycobacterium smegmatis - 2a
WP 013678342.1 - Pseudonocardia dioxanivorans - 2a
0.1

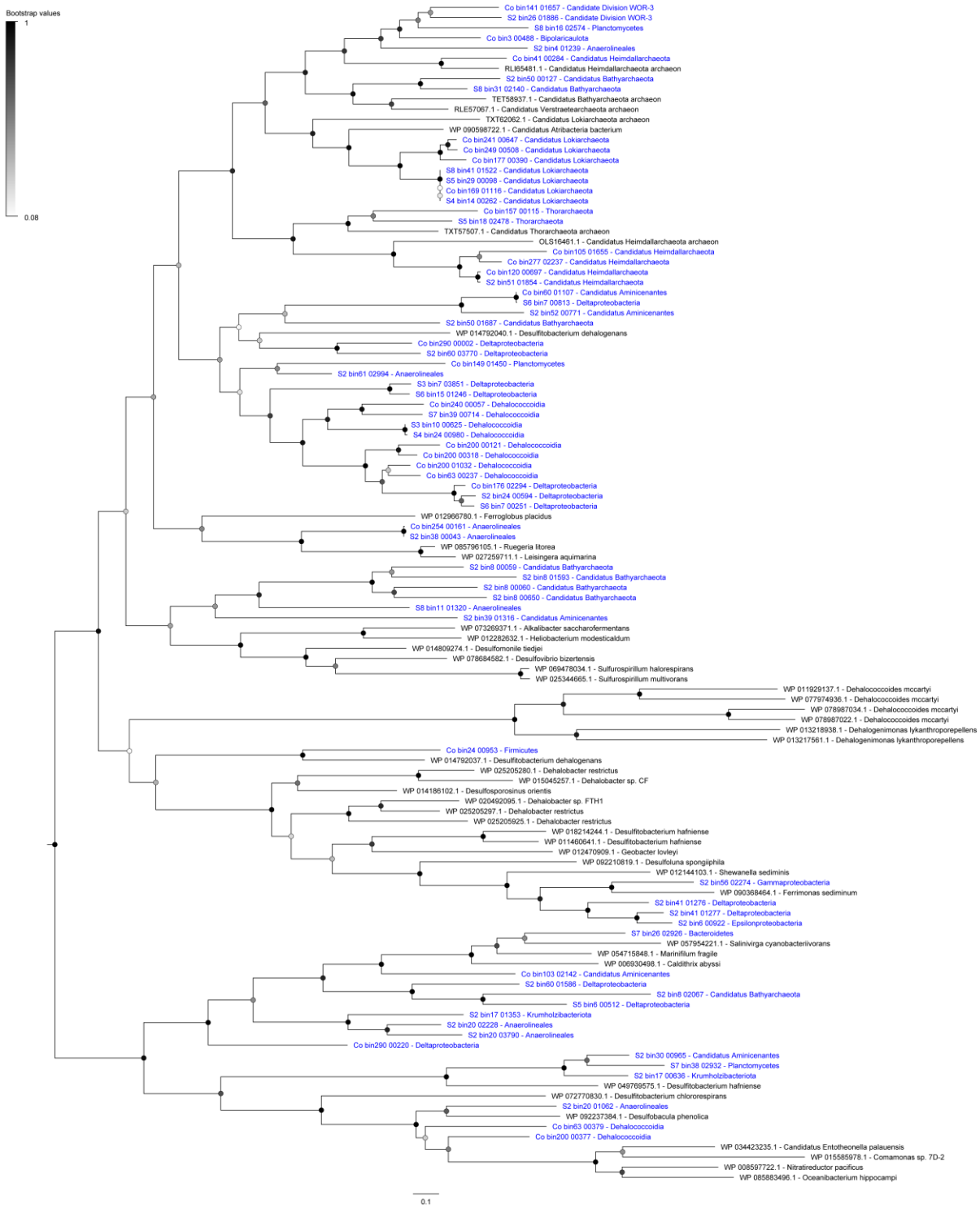
Supplementary Figure 10 Maximum-likelihood tree of amino acid sequences of group 1 and 2 [NiFe]-hydrogenase large subunits, a marker for hydrogen oxidation during respiratory processes. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted according to the HydDB classification scheme. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



Supplementary Figure 11 Neighbour-joining tree of amino acid sequences of the group 3 [NiFe]-hydrogenase large subunit, a marker for bidirectional hydrogen metabolism during various processes. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted according to the HydDB classification scheme. The tree was constructed using the Poisson model with gaps treated with pairwise deletion, was bootstrapped with 50 replicates, and was midpoint-rooted. To enable neighbour-joining, 54 incomplete sequences of the original 298 hits were excluded from this analysis.

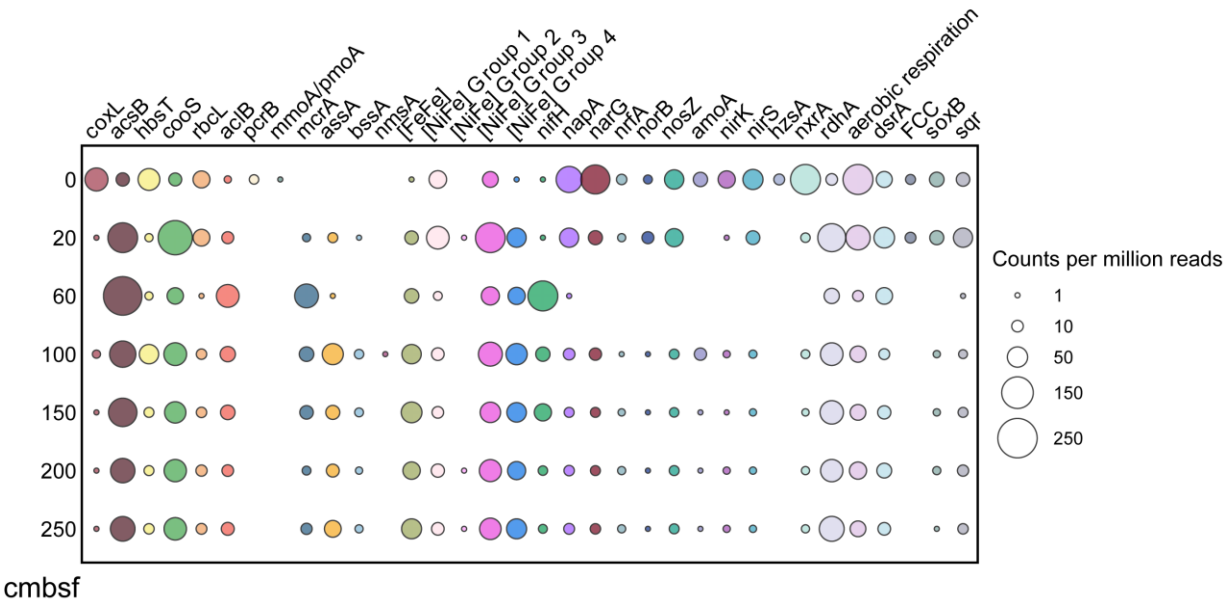

Bootstrap values
1
0.06
WP 006361427.1 - Slackia exigua - 4e
WP 015760641.1 - Eggerthella lenta - 4e
WP 006235754.1 - Collinsella aerofaciens - 4e
WP 007070913.1 - Dialister invisus - 4e
WP 020449518.1 - Methanomassiliicoccus intestinalis - 4e
WP 015536221.1 - Eubacterium cylindroides - 4e
WP 022790212.1 - Streptococcus pleomorphus - 4e
WP 027871860.1 - Eubacterium cellulosolvens - 4e
WP 026892028.1 - Clostridium aerotolerans - 4e
WP 028241974.1 - Pseudobutyrivibrio ruminis - 4e
WP 011448732.1 - Methanospirillum hungatei - 4e
WP 012376920.1 - Opitutus terrae - 4e
WP 013403527.1 - Caldicellulosiruptor hydrothermalis - 4e
WP 003868941.1 - Thermoanaerobacter ethanolicus - 4e
WP 011024634.1 - Caldanaerobacter subterraneus - 4e
WP 021758533.1 - Desulfovibrio gigas - 4e
WP 013174844.1 - Syntrophothermus lipocalidus - 4e
WP 010936594.1 - Dehalococcoides mccartyi - 4e
WP 015738579.1 - Ammonifex degensii - 4e
WP 011305188.1 - Methanosarcina barkeri - 4e
WP 013460916.1 - Sulfuricurvum kujiense - 4e
WP 013638341.1 - Desulfurobacterium thermolithotrophum - 4e
WP 007474009.1 - Caminibacter mediatlanticus - 4e
WP 012663581.1 - Nautilia profundicola - 4e
WP 004029221.1 - Methanobacterium formicicum - 4i
WP 012956799.1 - Methanobrevibacter ruminantium - 4i
WP 010870540.1 - Methanocaldococcus jannaschii - 4i
WP 011018833.1 - Methanopyrus kandleri - 4h
WP 012193964.1 - Methanococcus maripaludis - 4h
WP 015902767.1 - Nautilia profundicola - 4c
WP 025344446.1 - Sulfurospirillum multivorans - 4c
WP 005028008.1 - Bilophila wadsworthia - 4c
WP 010939566.1 - Desulfovibrio vulgaris - 4c
WP 011344721.1 - Carboxydothermus hydrogenoformans - 4c
WP 011389179.1 - Rhodospirillum rubrum - 4c
WP 011474902.1 - Rhodopseudomonas palustris - 4c
WP 028108808.1 - Ferrimonas futtsuensis - 4c
WP 026783288.1 - Pleomorphomonas koreensis - 4c
WP 018606884.1 - Uliginosibacterium gangwonense - 4c
WP 028535106.1 - Paludibacterium yongneupense - 4c
WP 015284192.1 - Methanoregula formicica - 4g
WP 015789964.1 - Halorhabdus utahensis - 4g
Co bin71 00381 - Dehalococcoidia
WP 012415297.1 - Elusimicrobium minutum - 4g
WP 011737331.1 - Pelobacter propionicus - 4g
S5 bin24 01123 - Anaerolineales
WP 013560598.1 - Anaerolinea thermophila - 4g
WP 011012581.1 - Pyrococcus furiosus - 4d
WP 012572555.1 - Thermococcus onnurineus - 4d
WP 009200527.1 - Anaerobaculum hydrogeniformans - 4d
WP 014163350.1 - Thermovirga lienii - 4d
WP 013048648.1 - Aminobacterium colombiense - 4d
WP 009164181.1 - Pyramidobacter piscolens - 4d
WP 004038443.1 - Methanofollis liminatans - 4d
WP 011449544.1 - Methanospirillum hungatei - 4d
Co bin181 00189 - Thermoplasmata
Co bin194 00285 - Atribacteria
S5 bin8 00211 - Thermoplasmata
WP 008083483.1 - Aciduliprofundum boonei - 4d
Co bin215 00226 - Dehalococcoidia
Co bin231 01060 - Dehalococcoidia
Co bin16 00420 - Dehalococcoidia
Co bin12 00724 - Dehalococcoidia
S4 bin6 00030 - Dehalococcoidia
Co bin16 00985 - Dehalococcoidia
Co bin166 00206 - Planctomycetes
WP 012745069.1 - Kosmotoga olearia - 4j
WP 047754705.1 - Kosmotoga pacifica - 4j
S4 bin5 00102 - Candidatus Aerophobetes
S5 bin2 00164 - Candidatus Aerophobetes
S6 bin3 00337 - Candidatus Aerophobetes
Co bin206 00689 - Candidatus Aerophobetes
S2 bin55 00274 - Candidatus Aerophobetes
Co bin43 01224 - Planctomycetes
S3 bin22 00356 - UBP14
S2 bin57 00656 - Candidate Division UBP3
S7 bin26 00732 - Bacteroidetes
WP 057952422.1 - Salinivirga cyanobacteriivorans - 4j
Co bin1 01926 - Armatimonadetes
S5 bin10 02673 - Armatimonadetes
Co bin269 00218 - Armatimonadetes
WP 011388069.1 - Rhodospirillum rubrum - 4b
WP 028842781.1 - Thermodesulfovibrio islandicus - 4b
WP 020505780.1 - Lamprocystis purpurea - 4b
WP 028450152.1 - Chitinibacter tainanensis - 4b
WP 013128928.1 - Thermosphaera aggregans - 4b
WP 014767907.1 - Desulfurococcus fermentans - 4b
WP 014737671.1 - Thermogladius cellulolyticus - 4b
WP 011839350.1 - Staphylothermus marinus - 4b
WP 012571983.1 - Thermococcus onnurineus - 4b
WP 004068373.1 - Thermococcus litoralis - 4b
WP 012572531.1 - Thermococcus onnurineus - 4b
WP 010868591.1 - Pyrococcus abyssi - 4b
WP 012571234.1 - Thermococcus onnurineus - 4b
WP 005582638.1 - Clostridium ultunense - 4f
WP 028986298.1 - Thermicanus aegyptius - 4f
WP 006323220.1 - Anoxybacillus flavithermus - 4f
WP 018133527.1 - Alicyclobacillus pohliae - 4f
WP 014795396.1 - Desulfitobacterium dehalogenans - 4f
WP 027938372.1 - Anaeroarcus burkinensis - 4f
WP 011751858.1 - Thermofilum pendens - 4a
WP 014558423.1 - Fervidicoccus fontis - 4a
WP 011139635.1 - Wolinella succinogenes - 4a
WP 014770116.1 - Sulfurospirillum barnesii - 4a
WP 021091787.1 - Campylobacter concisus - 4a
WP 013134545.1 - Arcobacter nitrofigilis - 4a
WP 007473606.1 - Caminibacter mediatlanticus - 4a
WP 015051661.1 - Thermacetogenium phaeum - 4a
WP 013876883.1 - Geobacillus thermoglucosidasius - 4a
WP 022587809.1 - Caldanaerobacter subterraneus - 4a
WP 025773400.1 - Moorella thermoacetica - 4a
WP 006234109.1 - Collinsella aerofaciens - 4a
WP 022739022.1 - Adlercreutzia equolifaciens - 4a
WP 011474970.1 - Rhodopseudomonas palustris - 4a
WP 029010114.1 - Azospirillum halopraeferens - 4a
WP 014639275.1 - Escherichia coli - 4a
WP 015370028.1 - Enterobacter aerogenes - 4a
WP 012907207.1 - Citrobacter rodentium - 4a
WP 005162305.1 - Yersinia enterocolitica - 4a
WP 014641051.1 - Escherichia coli - 4a
WP 006232936.1 - Photobacterium profundum - 4a
WP 017781337.1 - Aeromonas hydrophila - 4a
0.1

Supplementary Figure 12 Maximum-likelihood tree of amino acid sequences of the group 4 [NiFe]-hydrogenase large subunit, a marker primarily for hydrogen production during fermentative and respiratory processes. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted according to the HydDB classification scheme. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.

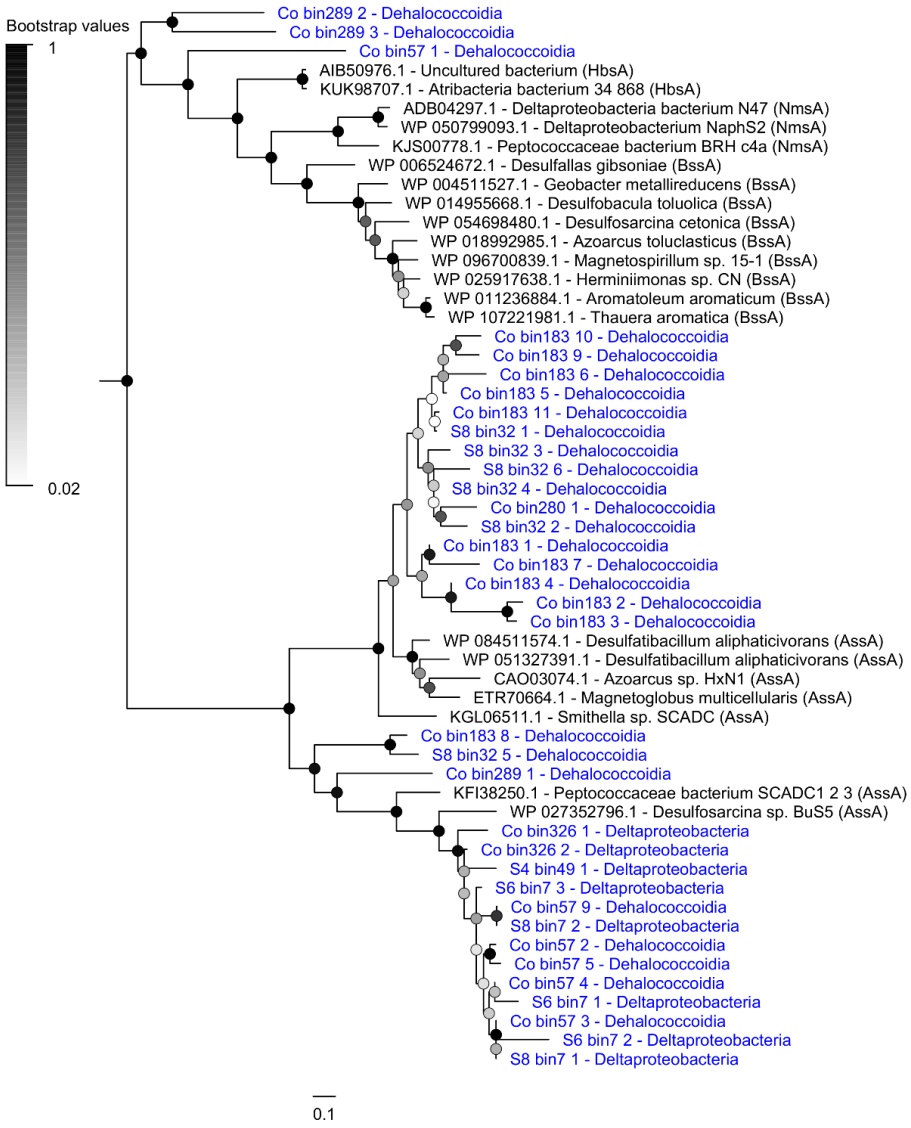


Supplementary Figure 13 Maximum-likelihood tree of amino acid sequences of the reductive dehalogenase A subunit (RdhA), a marker for reductive dehalogenation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this

study (blue) alongside representative reference sequences (black). The subgroup of each reference sequence is denoted. The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.



Supplementary Figure 14 Depth-differentiated metabolic capacity based on normalized short-read counts of genes encoding the catalytic subunits of key metabolic enzymes in unassembled quality-filtered reads. Genes are marked by different colors. Details on the annotation of the enzymes are presented in **Supplementary Data 5**.



Supplementary Figure 15 Maximum-likelihood tree of amino acid sequences of catalytic subunits of canonical fumarate-adding enzymes, which are markers for anaerobic hydrocarbon degradation. The tree shows sequences from cold seep metagenome-assembled genomes obtained in this study (blue) alongside representative reference sequences (black). Different clades correspond to alkylsuccinate synthases (AssA), benzylsuccinate synthases (BssA), naphthylmethylsuccinate synthases (NmsA), and hydroxybenzylsuccinate synthases (HbsA). The tree was constructed using the JTT matrix-based model, used all sites, and was bootstrapped with 50 replicates and midpoint-rooted.

Supplementary references

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