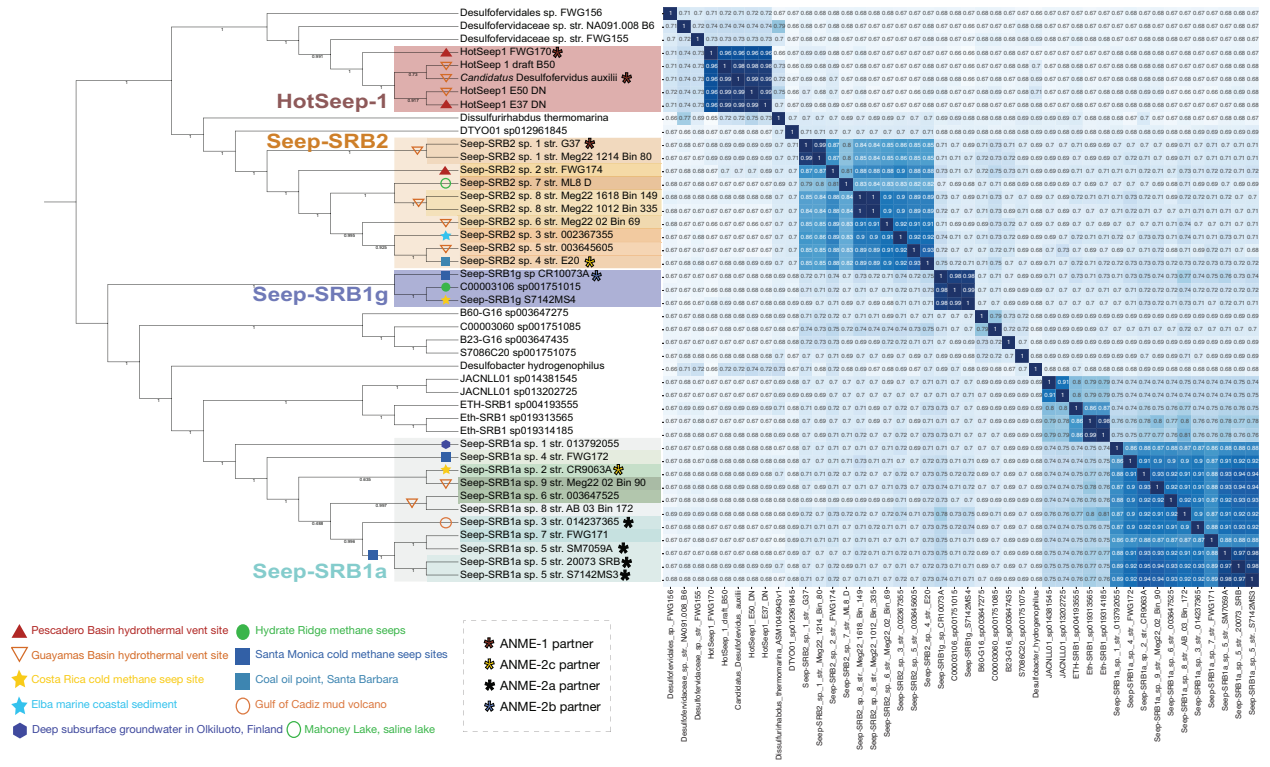


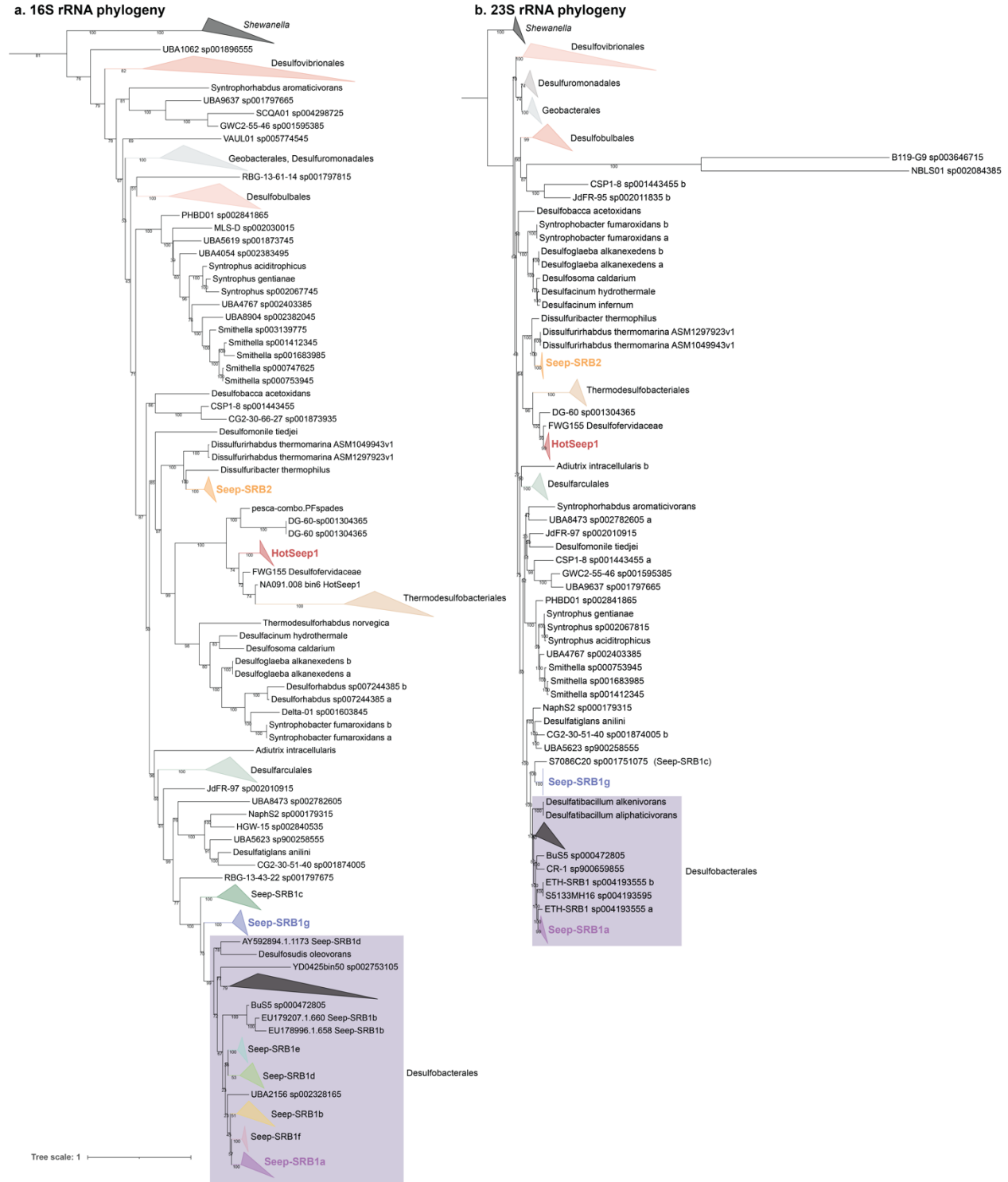
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Supplementary Figure 1. Taxonomic separation of syntrophic sulfate reducing bacteria using average nucleotide identity. The average nucleotide identity of genomes from each clade of the syntrophic sulfate reducing bacteria and some related bacteria were computed using the PyANI program available through Anvi'o. The different clades of syntrophic SRB – HotSeep1, Seep-SRB2, Seep-SRB1a and Seep-SRB1g are colored according to the attached legend. The Seep-SRB1a genomes in particular are differently colored depending on whether they partner

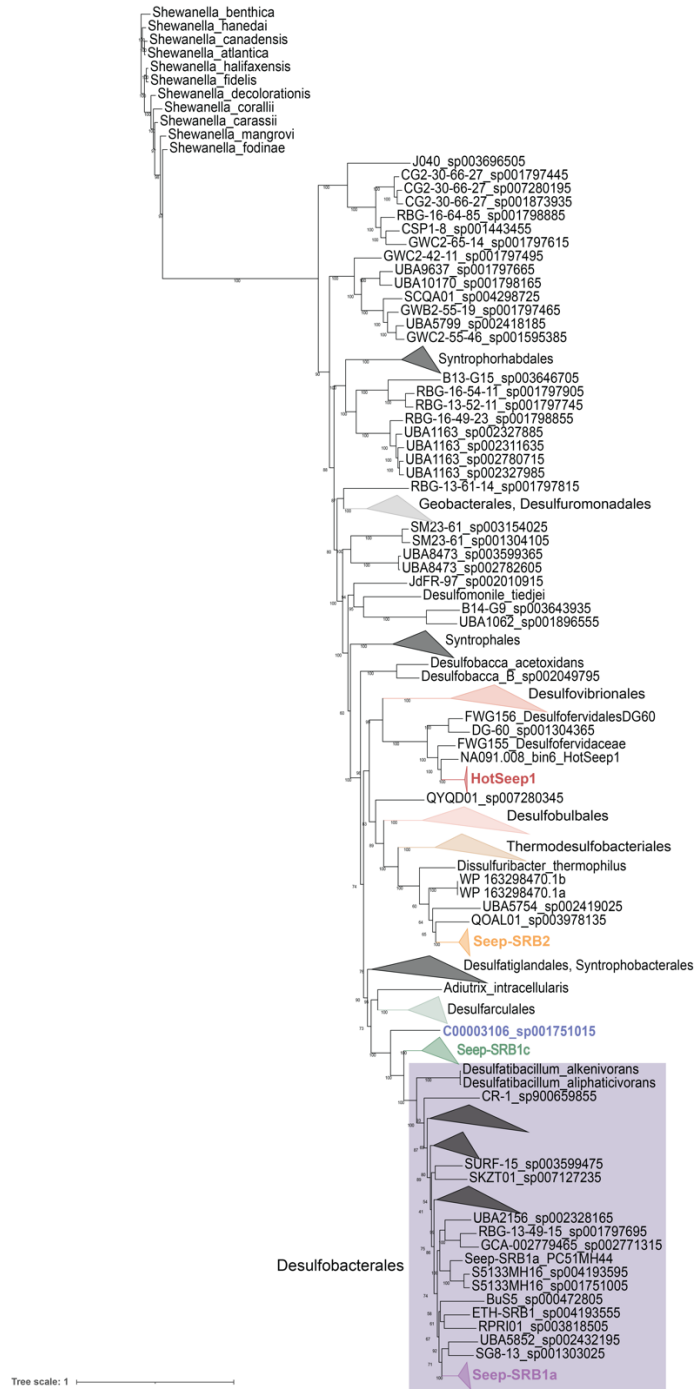
ANME-2a or ANME-2c respectively. The geographic location from which each genome was extracted is indicated on each node or clade in the tree.



Supplementary Figure 2. Comparison of 16S rRNA and 23S rRNA phylogeny of organisms from the phylum Desulfobacterota. 16S rRNA and 23S rRNA sequences were extracted from all organisms from the phylum Desulfobacterota available in GTDB release 95, and from

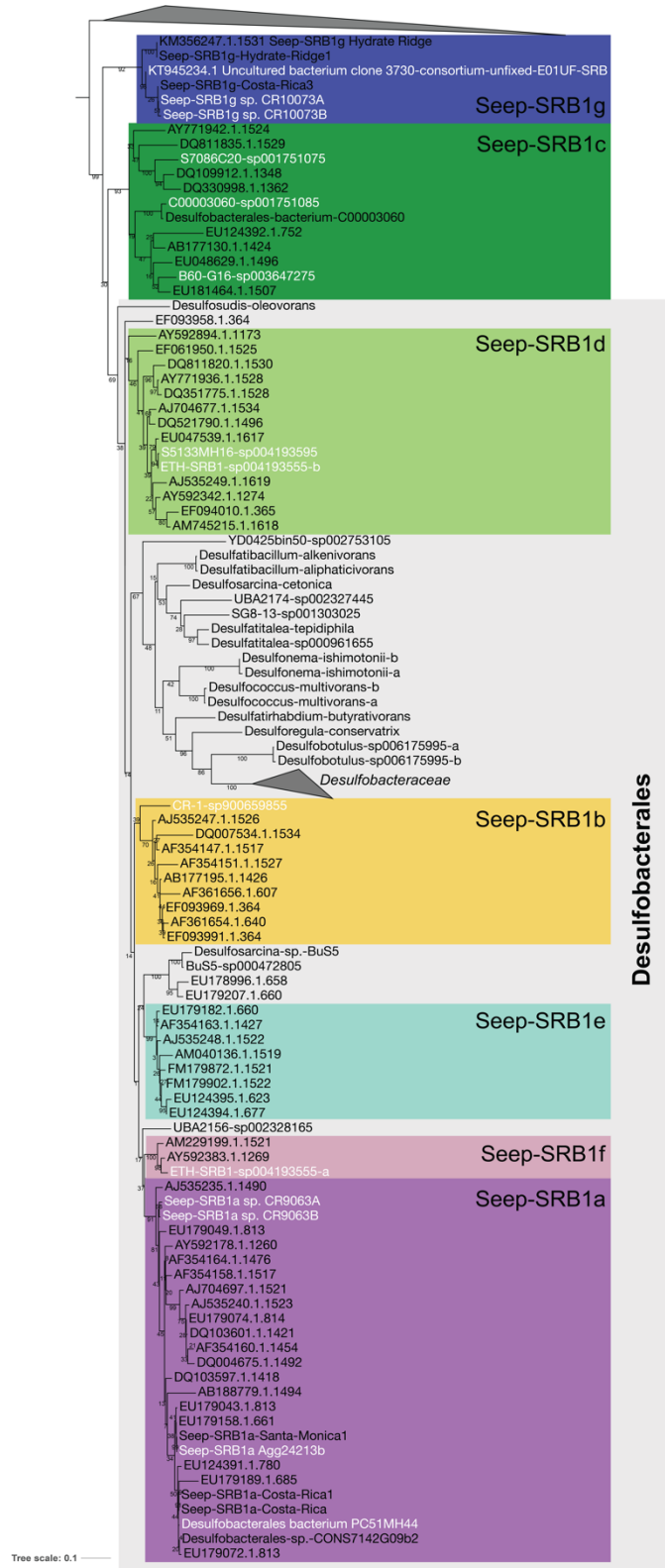
syntrophic SRB. These sequences were aligned using MUSCLE and a tree was inferred using IQTREE2. Hot-Seep1 is placed adjacent to the order Thermodesulfbacteriales in both these trees.

a. RpoB phylogeny

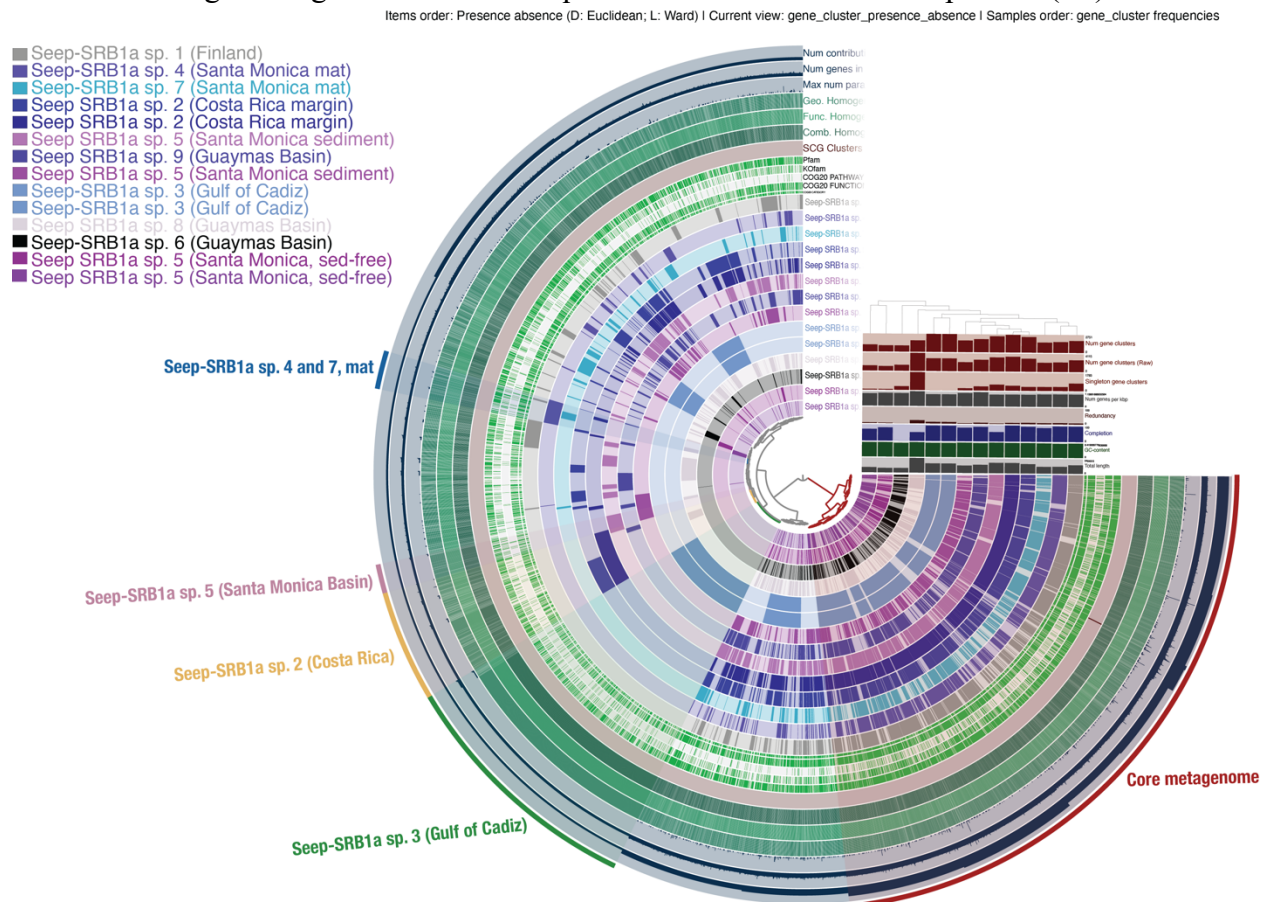


Supplementary Figure 3. Phylogeny of RpoB from organisms within the phylum Desulfobacterota. Sequences of RNA Polymerase, subunit B were extracted from all organisms from the phylum Desulfobacterota available in GTDB release 95, and from syntrophic SRB using BLASTP with an e-value cut-off of e^{-30} and appropriate query sequences. The sequences were confirmed to RpoB by manual inspection of a multiple sequence alignment generated using

MUSCLE and the tree was inferred using IQTREE2. In this tree, Hot-Seep1 is found adjacent to the order Desulfovibrionales.



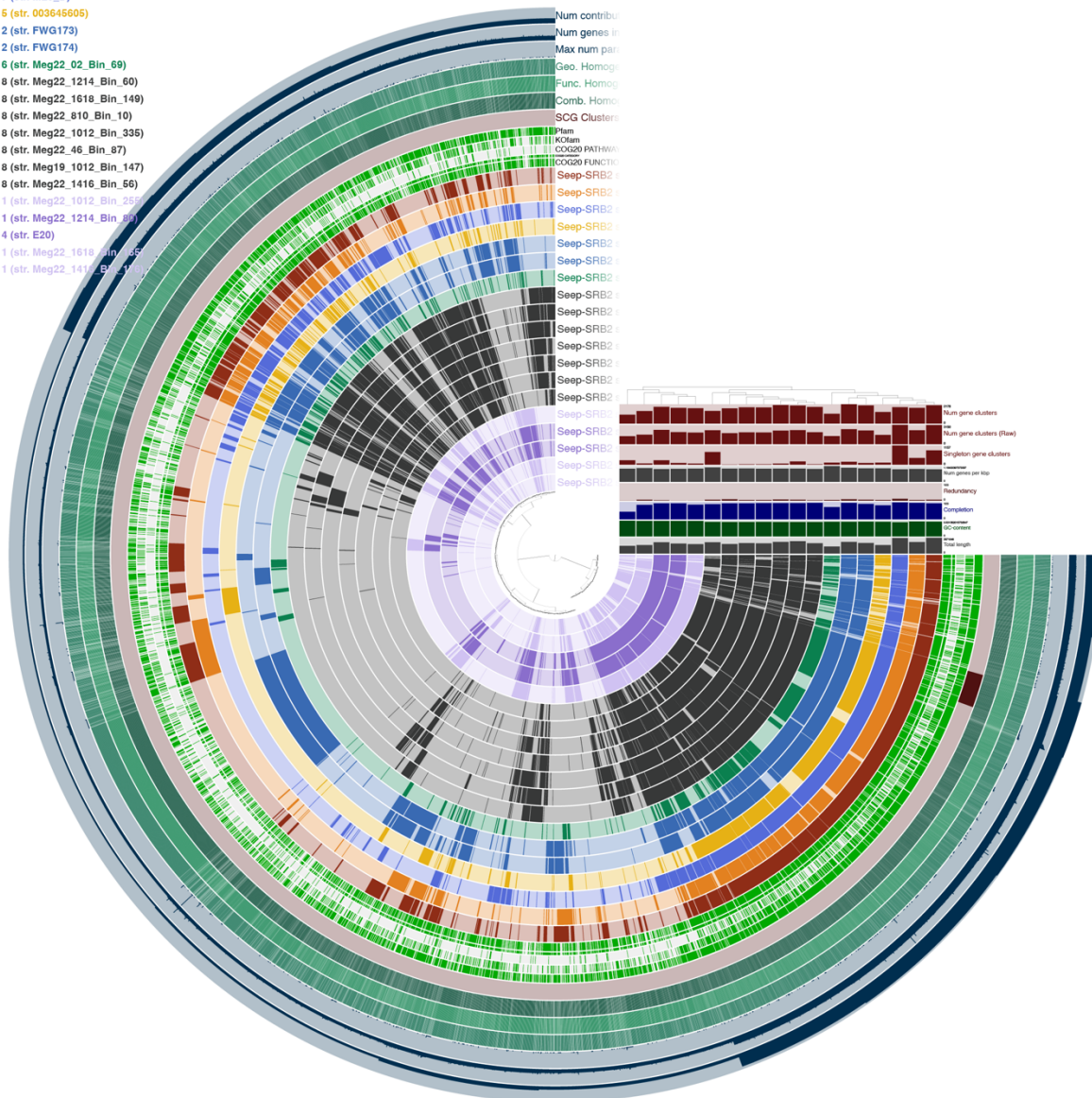
Supplementary Figure 4. Placement of various Seep-SRB1 clades within the phylum Desulfobacterota. A phylogenetic tree of full length 16S sequences from various Seep-SRB1 clades including the original 16S rRNA sequences used to define the Seep-SRB1(a-f) clades.



Supplementary Figure 5. Pan-genome analysis of Seep-SRB1a metagenomes. Fourteen genomes from nine Seep-SRB1a species were analyzed using the Anvi'o pan-genome analysis pipeline. Five gene cluster bins were annotated based on genes that were identified as part of the core metagenome, unique to Seep-SRB1a sp. 2, Seep-SRB1a sp. 3, Seep-SRB1a sp. 5 and from the Seep-SRB1a sp. 4 and 7.

Seep-SRB2 8g

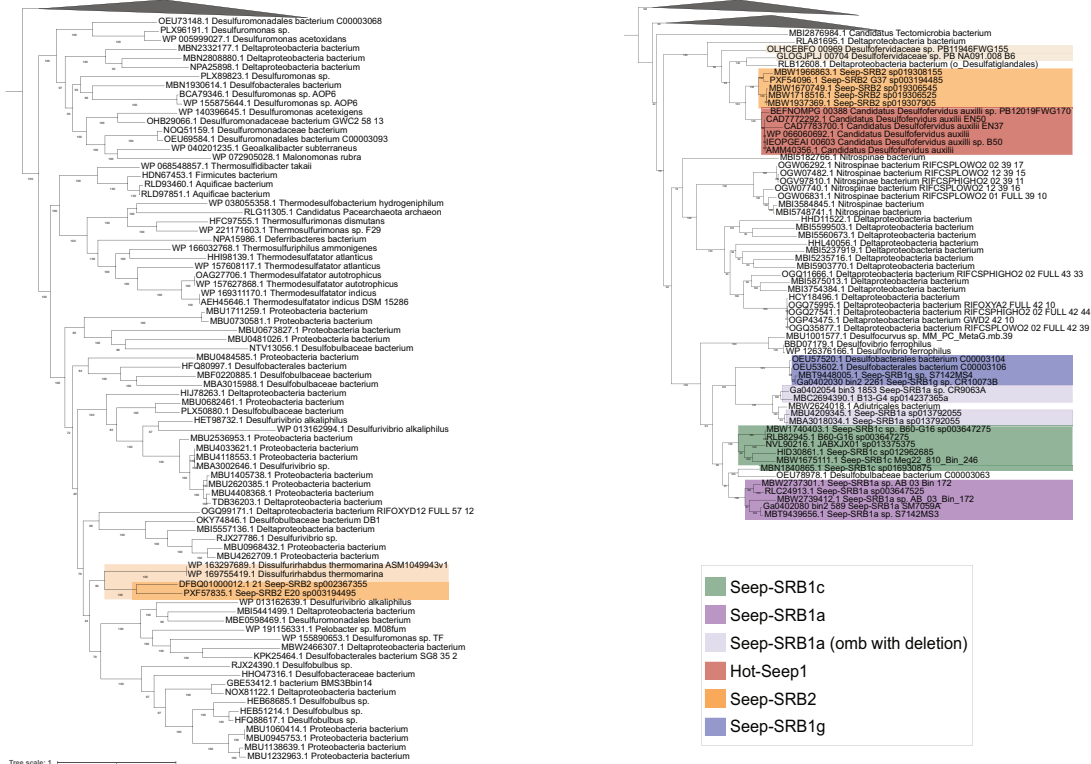
Seep-SRB2 sp. 1 (str. G37)
 Seep-SRB2 sp. 3 (str. 002367355) Items order: Presence absence (D: Euclidean; L: Ward) | Current view: gene_cluster_presence_absence | Samples order: gene_cluster frequencies
 Seep-SRB2 sp. 7 (str. ML8_D)
 Seep-SRB2 sp. 5 (str. 003645605)
 Seep-SRB2 sp. 2 (str. FWG173)
 Seep-SRB2 sp. 2 (str. FWG174)
 Seep-SRB2 sp. 6 (str. Meg22_02_Bin_69)
 Seep-SRB2 sp. 8 (str. Meg22_1214_Bin_60)
 Seep-SRB2 sp. 8 (str. Meg22_1618_Bin_149)
 Seep-SRB2 sp. 8 (str. Meg22_810_Bin_10)
 Seep-SRB2 sp. 8 (str. Meg22_1012_Bin_335)
 Seep-SRB2 sp. 8 (str. Meg22_46_Bin_87)
 Seep-SRB2 sp. 8 (str. Meg19_1012_Bin_147)
 Seep-SRB2 sp. 8 (str. Meg22_1416_Bin_56)
 Seep-SRB2 sp. 1 (str. Meg22_1012_Bin_257)
 Seep-SRB2 sp. 1 (str. Meg22_1214_Bin_98)
 Seep-SRB2 sp. 4 (str. E20)
 Seep-SRB2 sp. 1 (str. Meg22_1618_Bin_165)
 Seep-SRB2 sp. 1 (str. Meg22_1416_Bin_170)



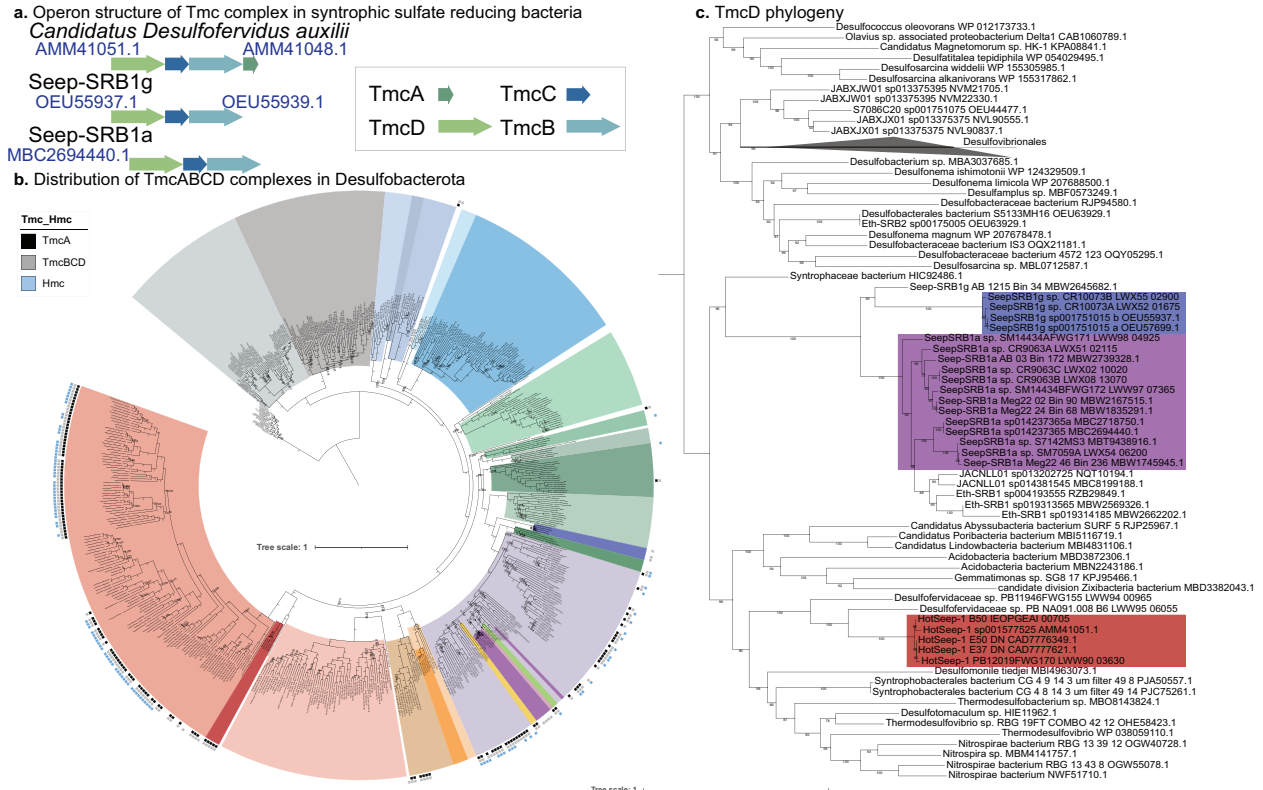
Supplementary Figure 6. Pan-genome analysis of Seep-SRB2 metagenomes. Fourteen genomes from nine Seep-SRB1a species were analyzed using the Anvi'o pan-genome analysis pipeline. Three gene cluster bins were annotated based on genes that were identified as part of the core metagenome, present in Seep-SRB2 sp. 1 and absent in Seep-SRB2 sp.1

a. Phylogenetic placement of the outer membrane beta barrel from Seep-SRB2 (E20)

b. Phylogenetic placement of outer membrane beta barrel, omb from other syntrophic SRB



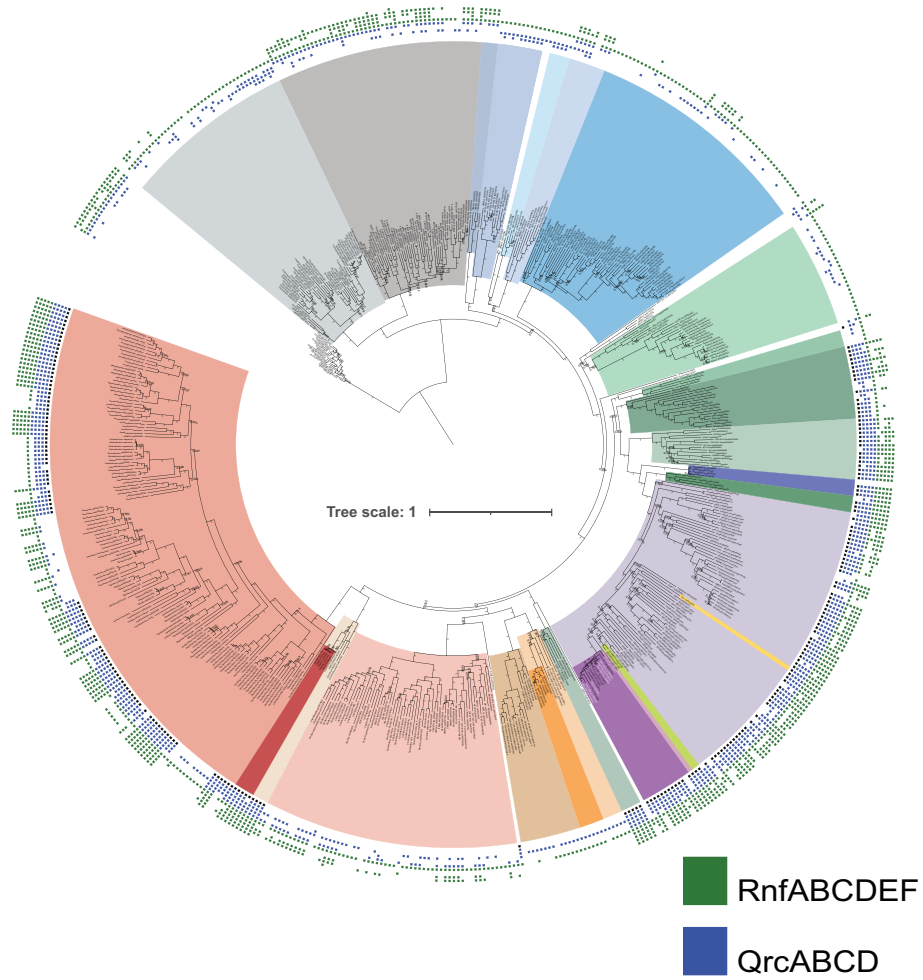
Supplementary Figure 7. Phylogenetic placement of the outer membrane beta barrel, OetI from the putative DIET cluster. A multiple sequence alignment, **Supplementary multiple sequence alignment MSA2** of the OetI protein sequences extracted from the genomes of syntrophic SRB and the NCBI database was generated using MUSCLE. This alignment was used to infer a phylogenetic tree using IQ-Tree2 and visualized on the iTOL web server. a. The phylogenetic placement of OetI from E20 Seep-SRB2 next to OetI from Thermodesulfobacteria and *Dissulfurirhabdus thermomarina* demonstrates that it was possibly vertically acquired from a gene transfer that was ancestral to the Seep-SRB2 and then vertically transferred. b. The phylogenetic placement of Seep-SRB1a and Seep-SRB1g OetI suggests that they are related. Additionally, the placement of OetI from G37 Seep-SRB2 next to OetI from Desulfurobacteriales, suggests that the Seep-SRB2 partner of ANME-1 acquired its DIET cluster from HotSeep1.



Supplementary Figure 8. The Tmc complex in Seep-SRB1a and Seep-SRB1g are divergent.
 A. The operons containing Tmc in *Candidatus Desulfofervidus auxilii* (HotSeep1), Seep-SRB1g and Seep-SRB1a show that TmcA is present in the former operon while it is missing in the latter two. B. The distribution of Tmc is mapped on to the phylum Desulfobacterota, showing that it is common in the order Desulfobacterales and Desulfovibrionales. C. Phylogeny of TmcD demonstrates that the Tmc complex in Seep-SRB1a and Seep-SRB1g cluster together and appear to be different from the Tmc complex in other organisms from the orders Desulfobacterales and C00003060.

Colored ranges

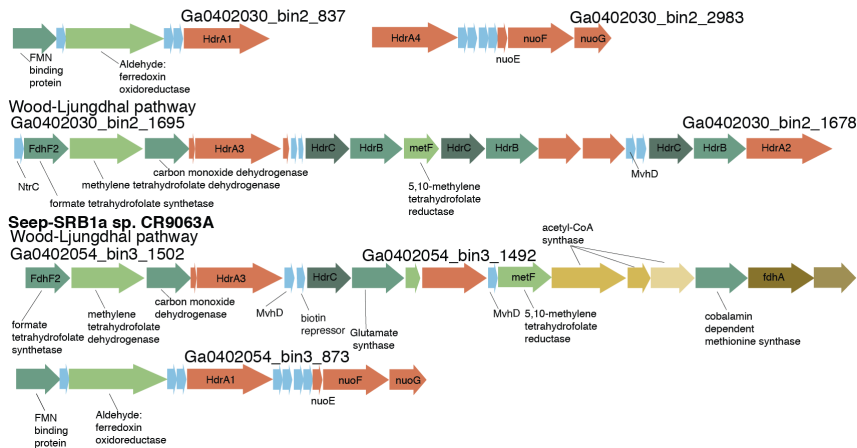
- o_Geobacterales
- o_Desulfuromonadales
- o_UBA1062
- o_MBNT15
- o_GWC2
- o_BSN033
- o_Syntrophales
- o_Syntrophorhabdadales
- o_Desulfobaccales
- o_Syntrophobacterales
- o_Desulfatiglandales
- Seep-SRB1g
- Seep-SRB1c
- o_Desulfobacterales
- Seep-SRB1a
- Seep-SRB1f
- Seep-SRB1d
- Seep-SRB1b
- o_Desulfarculales
- o_Dissulfuribacterales
- Seep-SRB2
- o_Thermodesulfobacterales
- o_Desulfobulbales
- o_Desulfovibrionales
- o_Desulfofervidales
- HotSeep1



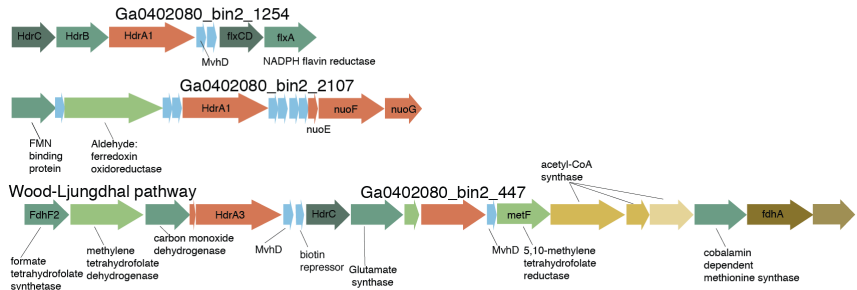
Supplementary Figure 9. Distribution of QrcABCD and RnfABCDEF in Desulfobacterota.

The presence and absence of Qrc and Rnf was demonstrated across the Desulfobacterota using BLASTP searches of different query sequences of these complexes. Both these complexes are absent from the orders Desulfobulbales, Thermodesulfobacterales and Dissulfuribacterales.

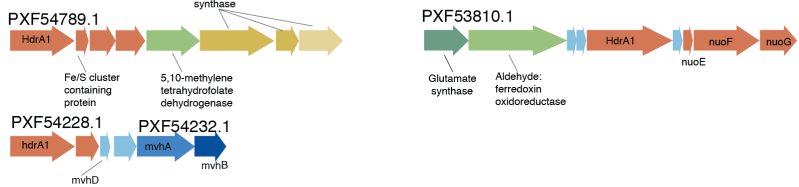
Seep-SRB1g sp. C10073A



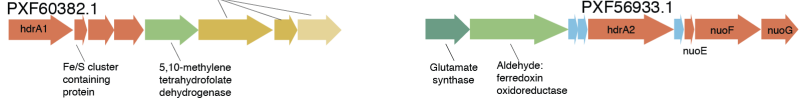
Seep-SRB1a sp. SM7059A



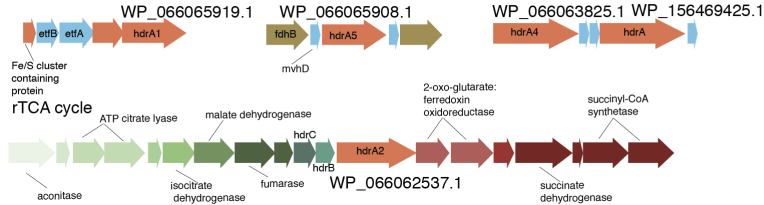
G37 Seep-SRB2



E20 Seep-SRB2



Hot-Seep1

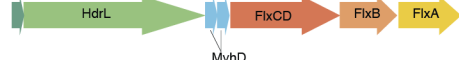


Supplementary Figure 10. Gene neighborhoods of various HdrA containing complexes and carbon fixation pathways in syntrophic SRB. HdrA homologs were identified in the four syntrophic SRB clades with the following gene neighborhoods. Multiple HdrA homologs were identified adjacent to the carbon fixation pathways in syntrophic SRB, specifically Seep-SRB1a and Seep-SRB1g.

a. Distribution of FlxABCD complexes

Seep-SRB1g sp. C10073A

Ga0402030_bin2_2424 Ga0402030_bin2_2429



Seep-SRB1a sp. C9063A

Ga0402057_bin2_2285 Ga0402057_bin2_2278



Seep-SRB1a sp. C7059A

Ga0402080_bin2_1252 Ga0402080_bin2_1258



Seep-SRB2 G37

PXF53184.1 PXF53179.1



Seep-SRB2 E20

PXF58558.1 PXF58562.1



b. Distribution of fdhA/fdhF2 containing complexes

Seep-SRB1g sp. C10073A

Ga0402030_bin2_1785



Ga0402030_bin2_1229



Ga0402030_bin2_2007



Ga0402030_bin2_694



Fe/S cluster containing protein
NADPH (flavin) reductase

Seep-SRB1a sp. C9063A

Ga0402057_bin2_1209



Ga0402057_bin2_1144



Ga0402057_bin2_930



Fe/S cluster containing protein
NADPH (flavin) reductase

Seep-SRB1a sp. SM7059A

Ga0402080_bin2_2131



Seep-SRB2 G37

PXF55613.1



Fe/S cluster containing protein

Seep-SRB2 E20

PXF60403.1



Fe/S cluster containing protein

PXF60073.1

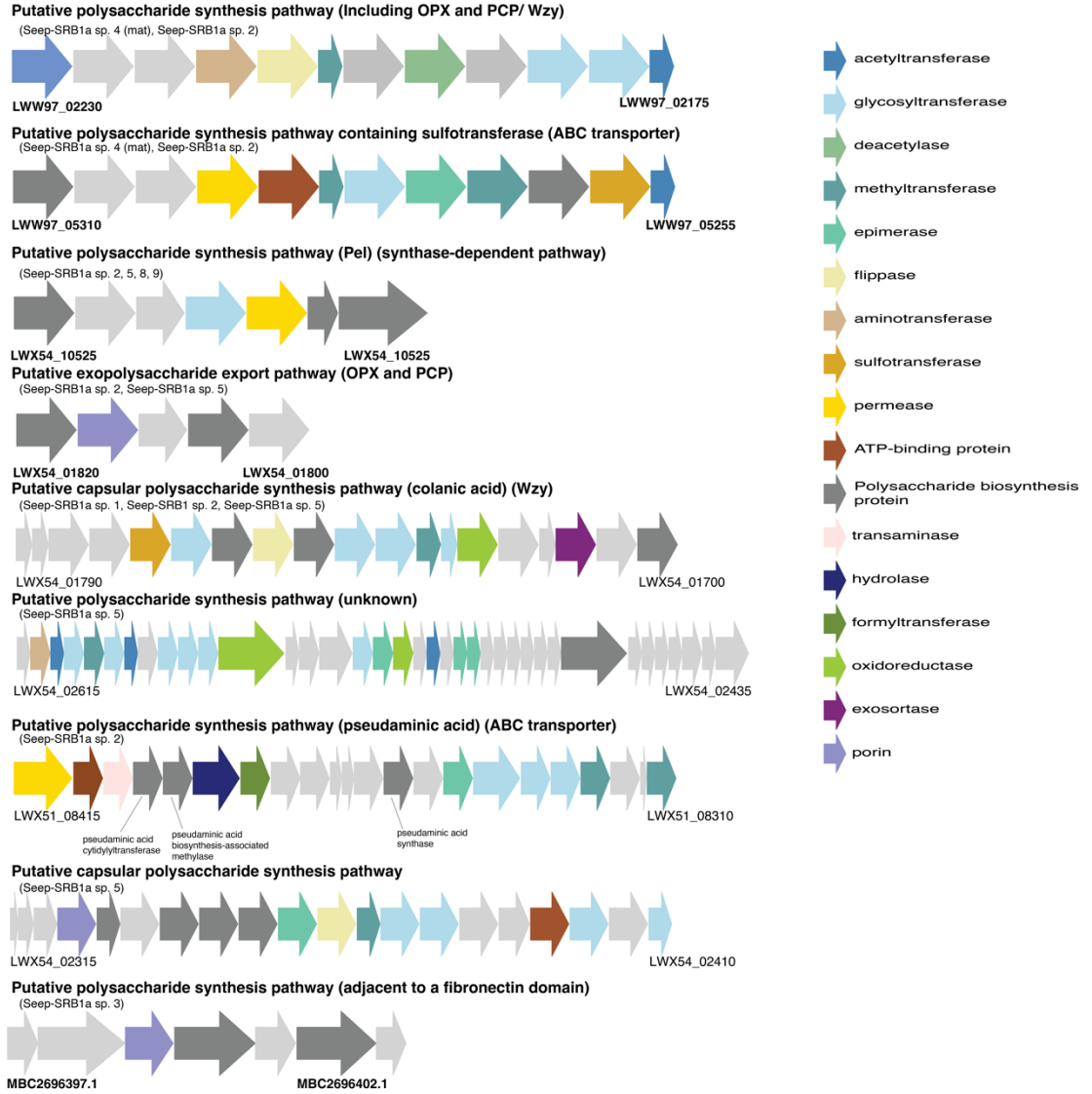


PXF58566.1

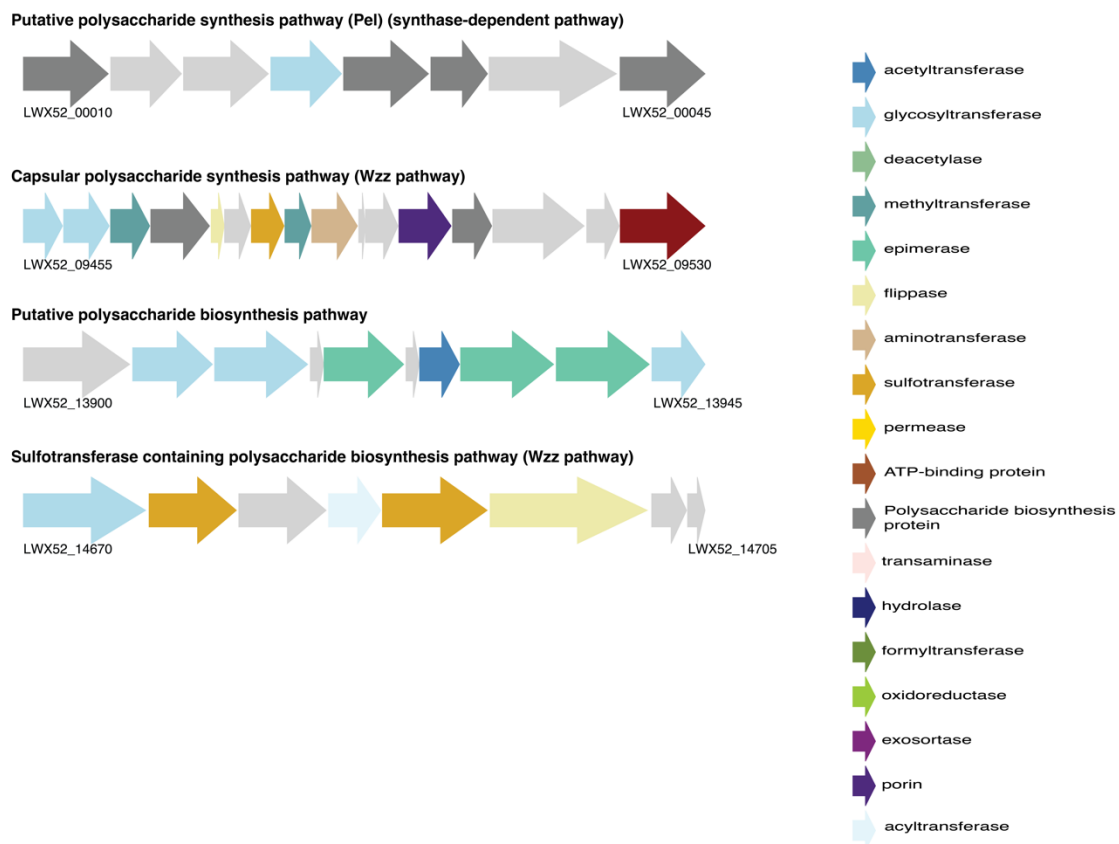


molybdopterin oxidoreductase family protein

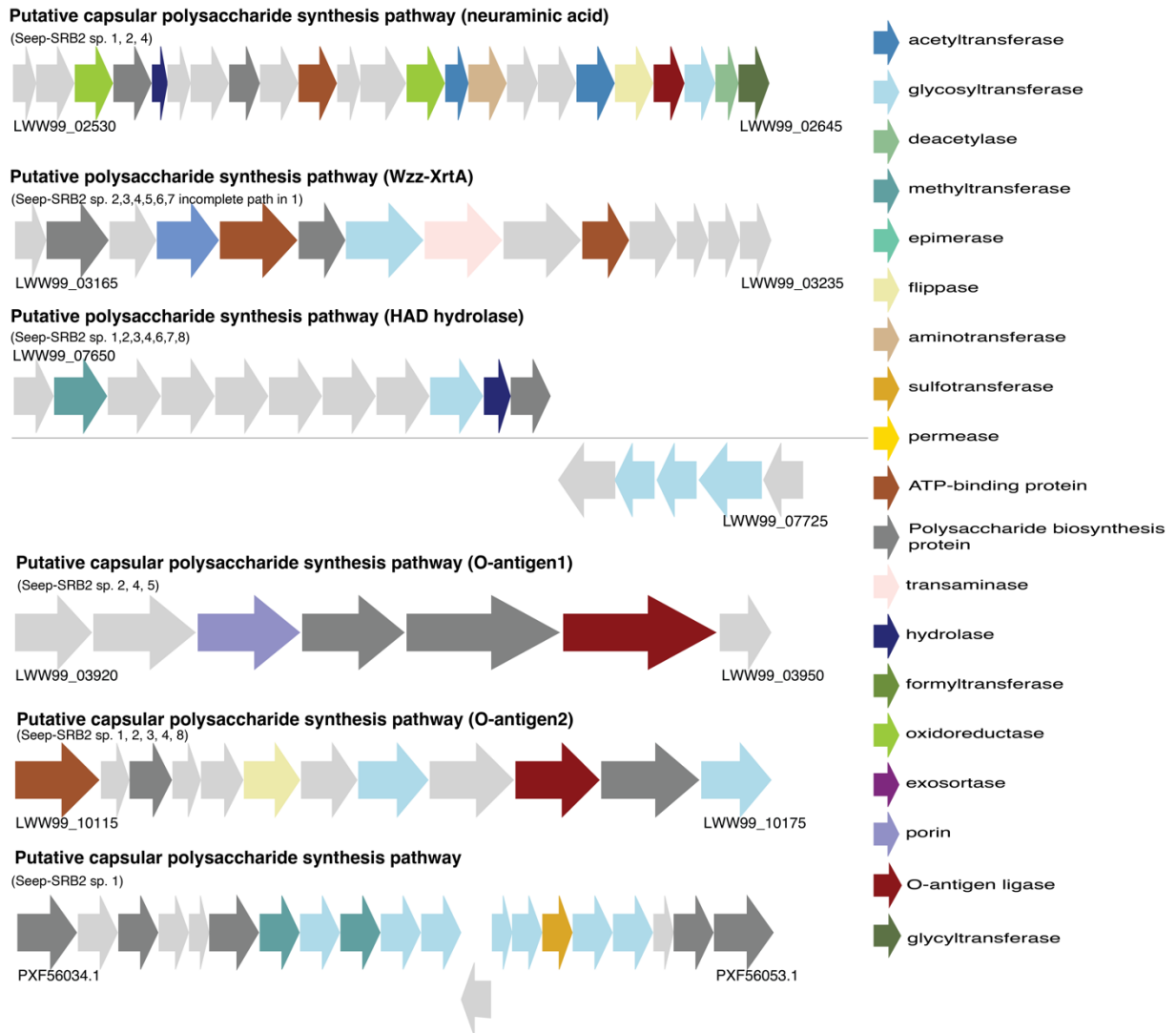
Supplementary Figure 11. Operons of Flx-Hdr complexes and gene neighborhood of putative formate utilizing proteins. a. Flx-Hdr complexes which recycle electrons between NADH, ferredoxins and DsrC are found in Seep-SRB1g, Seep-SRB1a and Seep-SRB2. b. Many putative formate utilizing proteins are found in Seep-SRB1g, Seep-SRB1a and Seep-SRB2. In Seep-SRB1g and Seep-SRB1a, periplasmic formate dehydrogenases (fdhAB) are found. fdhA domains as identified here are typically found in the periplasm and have a respiratory function while fdhF2 are typically cytoplasmic.



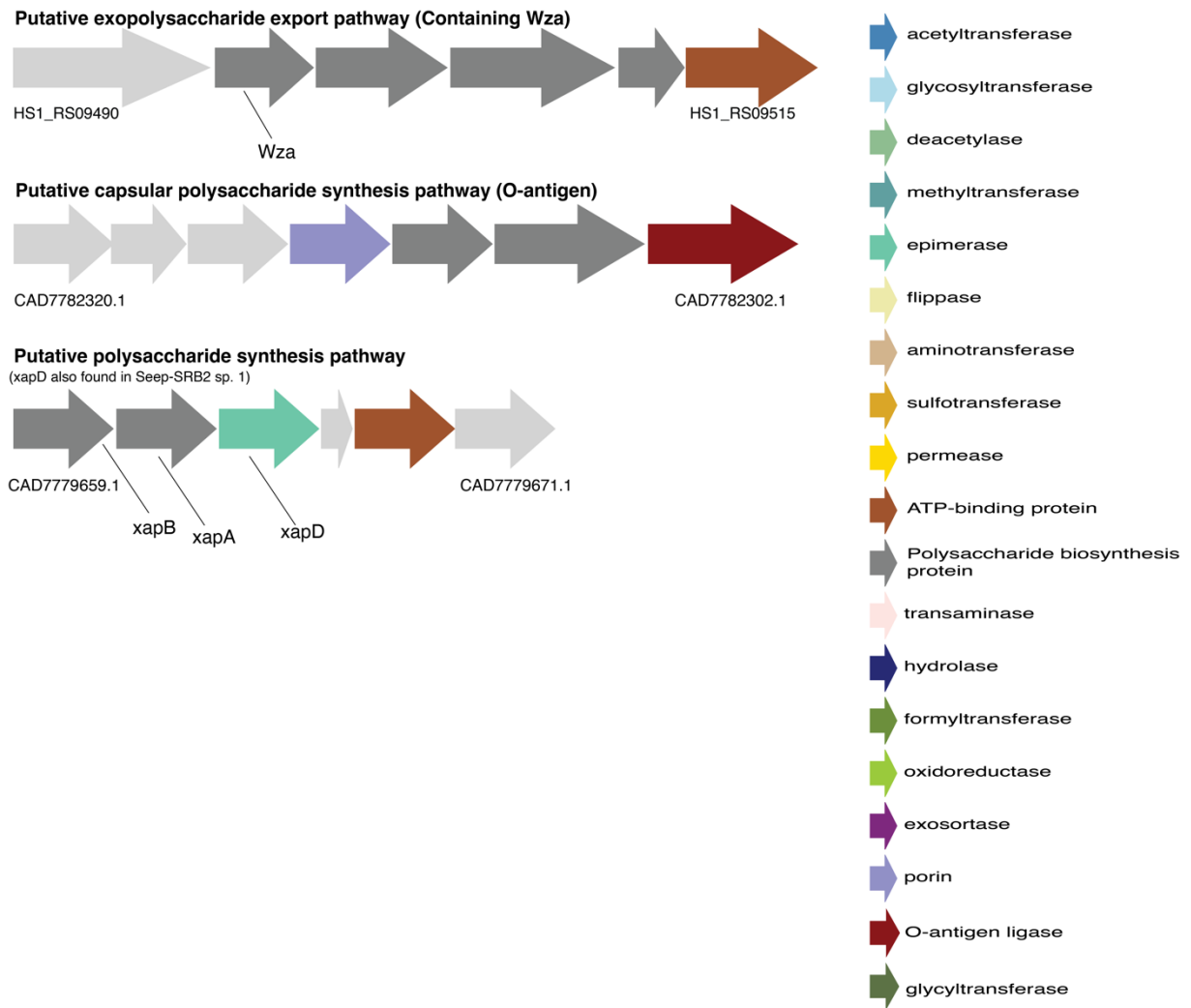
Supplementary Figure 12. Putative polysaccharide biosynthesis pathways in Seep-SRB1a.



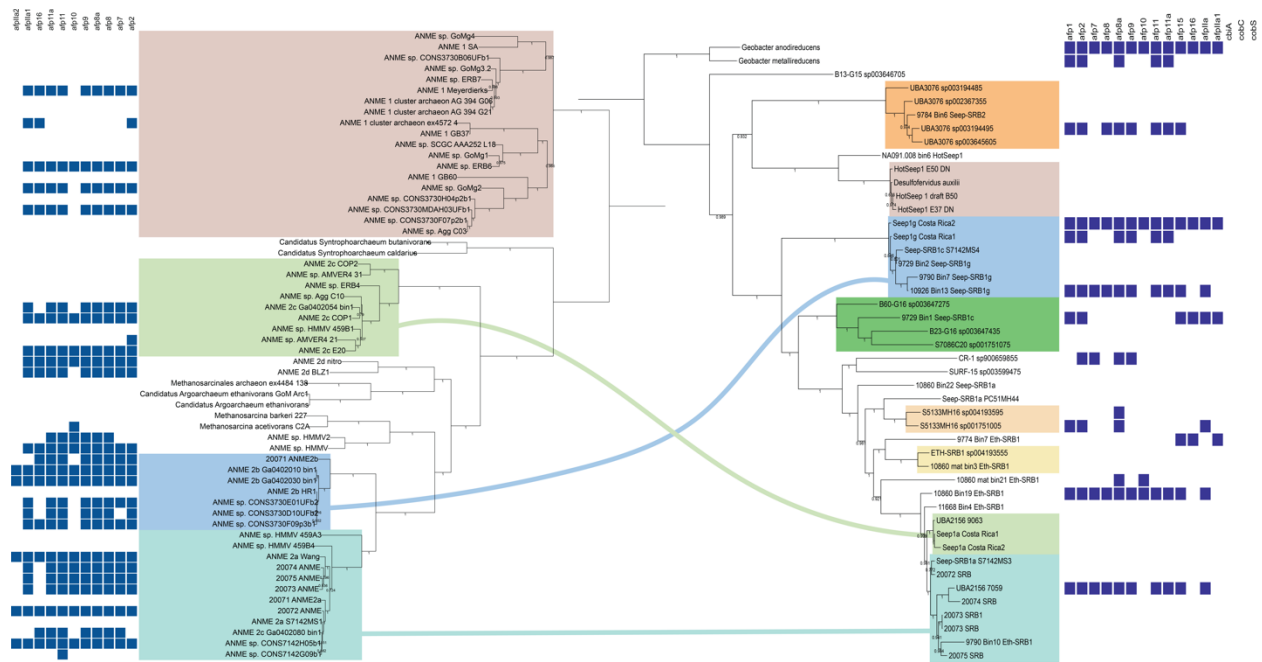
Supplementary Figure 13. Putative polysaccharide biosynthesis pathways in Seep-SRB1g.



Supplementary Figure 14. Putative Polysaccharide biosynthesis pathways in Seep-SRB2.

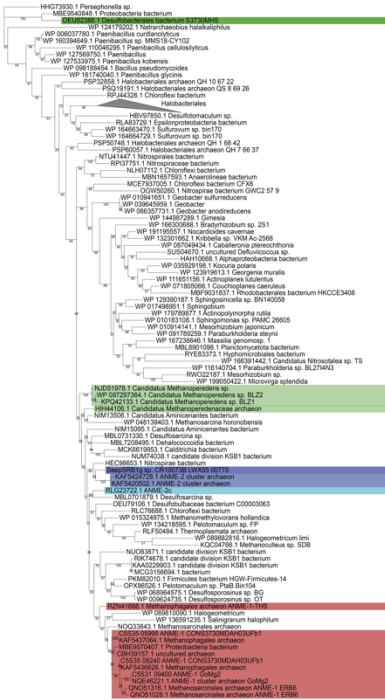


Supplementary Figure 15. Putative polysaccharide biosynthesis pathways in HotSeep-1.

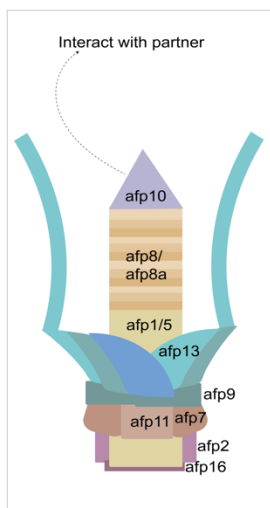
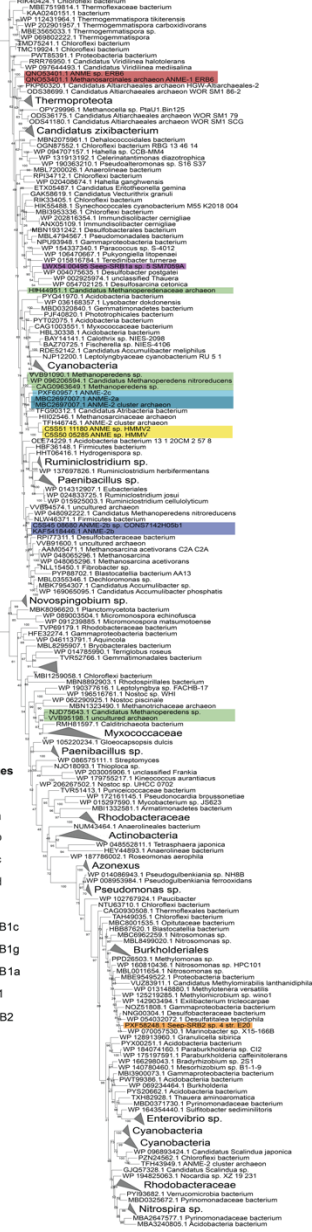


Supplementary Figure 16. Presence of extracellular contractile injection systems (eCIS) in ANME and SRB. The presence of eCIS conduits in ANME and SRB was identified using BLASTP and dbcIS [cite Chen et al. Cell Reports]. While the eCIS clusters are widely distributed in ANME-2a and ANME-2b, they are only sparsely distributed in ANME-1. They are only present in the Seep-SRB1g species found in Costa Rica and one of the Seep-SRB1a species from Santa Monica Basin.

a. *afp10* phylogeny, Type IIa



b. *afp10* phylogeny, Type IIc



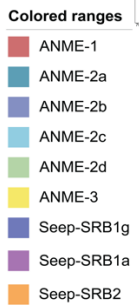
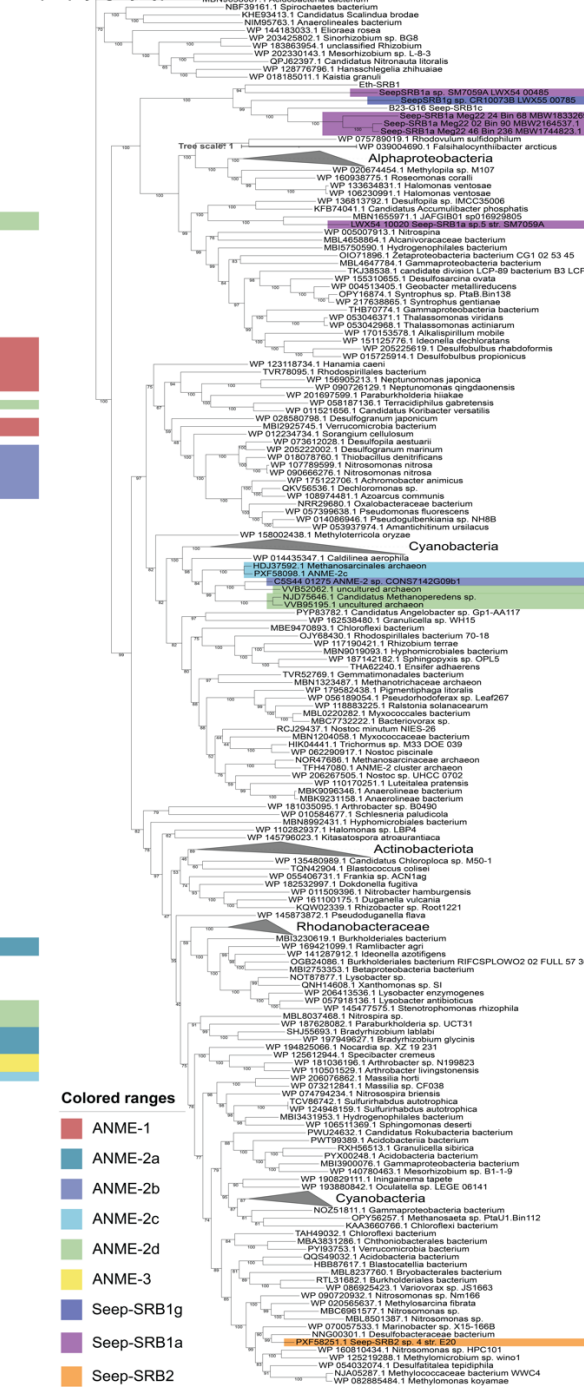
- Colored ranges
- ANME-1
 - ANME-2a
 - ANME-2b
 - ANME-2c
 - ANME-2d
 - ANME-3
 - Seep-SRB1c
 - Seep-SRB1g
 - Seep-SRB1a
 - Eth-SRB1
 - Seep-SRB2

Supplementary Figure 17. Phylogeny of *afp10*, the spike protein from the extracellular contractile injection system. Afp10 is the PAAR-domain containing protein that typically interacts with the target organism of the eCIS. Afp10 sequences were extracted from ANME and SRB using BLASTP and dbEClS. These sequences were then used as queries to repeatedly search and identify the closest homologs from the NCBI database. A sequence alignment was then made using MUSCLE, manually inspected and filtered, and the tree was inferred using RAxML. Seep-SRB1a sequences are related to other eCIS sequences from Desulfobacterales while Seep-SRB2 *afp10* and Seep-SRB1g *afp10* sequences do not cluster with evolutionarily related bacteria.

a. *afp11* phylogeny, Type Ia



b. *afp11* phylogeny, Type IIc



Supplementary Figure 18. Phylogeny of *afp11*, a base plate protein from the extracellular contractile injection system. *Afp11* belongs to the baseplate of the eCIS and does not interact directly with the target organism. *Afp11* sequences were extracted from ANME and SRB using BLASTP and dbeCIS. These sequences were then used as queries to repeatedly search and identify the closest homologs from the NCBI database. A sequence alignment was then made using MUSCLE, manually inspected and filtered, and the tree was inferred using RAxML. Seep-SRB1a sequences are related to other eCIS sequences from Desulfobacteriales while Seep-SRB2 *afp10* and Seep-SRB1g *afp10* sequences do not cluster with evolutionarily related bacteria.